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The Effects of Exercise on Dose and Dose Distribution of Inhaled Automotive Pollutants

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Includes the Commentary of the Institute's Health Review Committee

Research Report Number 45

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HEALTH REVIEW COMMITTEE'S COMMENTARY Health Effects Institute

The Effects of Exercise on Dose and Dose Distribution of Inhaled Automotive Pollutants

Michael T. Kleinman[1] and William J. Mautz

ABSTRACT

The purpose of this study was to determine how changes in ventilation rate and in the entry route of air pollutants into the respiratory tract (nose versus mouth breathing) affected the respiratory tract uptake and penetration of inhaled gaseous and particulate pollutants associated with automobile emissions. Experiments were performed with female beagle dogs exposed while standing at rest or while exercising on a treadmill at 5 km/hour and a 7.5 percent grade. Dogs were exposed to nitrogen dioxide at concentrations of 1 and 5 parts per million[2] (ppm), to formaldehyde at 2 and 10 ppm, and to an aerosol of ammonium nitrate particles (0.3 μm mass median aerodynamic diameter) at 1 mg/m^3.

Total respiratory system uptake and effects on breath time, expired tidal volume, fractional expiration time, minute ventilation, respiratory gas exchange, ventilation equivalents for oxygen and carbon dioxide, and dynamic pulmonary resistance and compliance were measured in exercising and resting dogs exposed for two hours to 5 ppm nitrogen dioxide and 10 ppm formaldehyde in combination with 1 mg/m^3 of ammonium nitrate particles. Regional penetration of pollutants through oral and nasal airways and pollutant uptake in the lung were measured in a separate group of six tracheostomized dogs standing at rest while being exposed to nitrogen dioxide, formaldehyde, and ammonium nitrate particles. Hypercapnic stimulation was used to modify ventilation rates in the tracheostomized dogs while pollutant penetration and uptake were measured.

Dogs exposed to 5 ppm of nitrogen dioxide at rest tended to breathe more rapidly ($p < 0.05$) and more shallowly (a nonsignificant trend) than dogs exposed to purified air. The changes observed were similar in direction, but of smaller magnitude, to changes observed when the same dogs were exposed during exercise to ozone at 0.6 ppm in a separate study. Rapid-shallow breathing was not observed when the dogs were exposed during exercise to 5 ppm nitrogen dioxide.

Dogs exposed to a mixture of 10 ppm formaldehyde and 1 mg/m^3 ammonium nitrate particles during exercise showed a shift to larger tidal volume breathing, but the response was much less pronounced than the slow-deep breathing pattern response observed in a separate study of dogs exposed to 10 ppm formaldehyde alone. The total respiratory system uptake of formaldehyde from the formaldehyde and ammonium nitrate mixture was larger than that measured for 10 ppm of formaldehyde alone in another exercise and exposure study.

In tracheostomized dogs exposed at rest, formaldehyde, a highly water-soluble gas, was rapidly removed from inspired air by the moist surfaces of the upper airways, and only a small fraction penetrated to the trachea (less than 10 percent), whether breathing was by nose or mouth. Nitrogen dioxide, a much less water-soluble gas, penetrated the upper airways more readily (greater than 50 percent). For both gases, penetration was greater during mouth breathing than during nose breathing, and penetration increased with increased ventilation. Fractional penetration of ammonium nitrate particles was highly variable, and there were no significant differences between measurements of penetration obtained during oral or nasal inhalation.

INTRODUCTION

Removing contaminants from inspired air by "scrubbing" in the upper respiratory tract (URT) is a critical part of the lungs' defense against infectious agents and toxic pollutants in ambient and workplace atmospheres. For many airborne substances with potentially toxic effects on the lung, the fraction of inspired material that actually penetrates the airways of the head to reach target sites in the lung is relatively small. Because biological response is a direct function of the dose reaching these target sites, it is important to understand how the chemical and physical characteristics of the pollutants and the anatomical and ventilatory characteristics of the airways modify the quantity of pollutant that penetrates the airways to reach the lung.

The nasal and oral airways and the pharyngeal regions of the head, which constitute the URT, present a convoluted, liquid-covered surface to incoming particles and gases. The primary purpose of this tissue is the humidification and heating of inspired air. During inspiration, gases are absorbed and dissolved into the liquid lining and particles are

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[2] A list of abbreviations appears at the end of this report for your reference.

deposited on the surfaces by a combination of impaction, gravitational settling, and diffusion. The distance that a gas molecule or a particle can traverse (or penetrate) along the airway depends on the physical and chemical properties of the material and the speed of travel, which is a function of the rate and depth of respiration. Respiration patterns are related to metabolic gas exchange rate and the amounts of physical work being performed. This is an important concept because it implies that the dosimetry of a toxic agent can be modified by work-related exercise, as well as by the physical and chemical properties of the inhaled agent.

The processes by which inhaled gases or vapors are scrubbed, or absorbed, in the URT have been described by Morgan and Frank (1977) and by Aharonson and coworkers (1974). The most important factors influencing these processes are: the partitioning of the inhaled compound between the gas phase and the liquid phase of the mucous layer lining the respiratory airway, which is related to the water solubility of the chemical (Henderson and Haggard 1943); the residence time of the gas in an airway segment, which is a function of ventilation frequency and volume (Aharonson et al. 1974; Morgan and Frank 1977); the degree of turbulence in the airstream (Brain 1970); and the area of absorbing surface in the airway (LaBelle et al. 1955; Morgan and Frank 1977). Other physical and chemical properties that affect pulmonary penetration of inhaled gases or vapors include the diffusivity of the gas and the presence of particles that may adsorb gases and act as carriers that promote deeper respiratory tract transport (McJilton et al. 1976). Finally, physiological changes such as modifying the rate of production of mucus, altering the chemical and physical properties of the mucous layer, and changing airway caliber due to airway dilation or constriction in response to the inhalation of an irritant substance may play a role in modifying the dose distribution of toxic materials in the respiratory system.

Theoretical relationships between penetration and these chemical, physical, and physiological factors are useful for designing experiments in which the effects of specific experimental variables on dosimetry can be evaluated. Earlier work in our laboratory (Kleinman 1984) built upon the results previously published by Morgan and Frank (1977), Speizer and Frank (1966), and Yokayama and Frank (1972) to express uptake in terms that are useful for extrapolating among different experimental conditions. When an airborne contaminant is inhaled, it is absorbed into the liquid lining of the airways. The mass per unit of time(dm/dt) of the contaminant removed from the air and transported across the contact surface is proportional to the surface area (A) and to the local driving force, which is the difference between the partial pressures of the solute in the gas phase, p_g, and in the liquid phase, p_l, as shown in Equation 1 (LaBelle et al. 1955).

$$\frac{dm}{dt} = -rA\,(p_g - p_l). \tag{1}$$

The proportionality constant, r, is a mass transfer coefficient and depends on physical properties of the gas, such as solubility and diffusivity. The fractional penetration (P) of a contaminant is expressed in Equation 2 (obtained by the integration of Equation 1) as a function of ventilation rate ($\dot{V}$), airway surface area (A), and the mass transfer coefficient, r.

$$P = \frac{m}{m_0} = \exp[-r\frac{RT}{M}\frac{A}{\dot{V}}]. \tag{2}$$

In this equation, m is the mass of contaminant that penetrates an airway surface area, A; m_0 is the mass of contaminant inhaled; r is the mass transfer coefficient of the compound; and R, T, and M are, respectively, the gas law constant, temperature in degrees Kelvin, and molecular weight of the compound. Fractional uptake is defined as $1 - P$. The theoretical model provides a general form for the relationship between P, A, and $\dot{V}$.

This study was designed to provide a data set that could be used to validate and develop models to study the effects of exercise on pulmonary deposition of inhaled materials. The influences of pollutant concentration, changes in ventilation rate, and interaction between contaminants on respiratory system dose were considered, as well as the intrinsic chemical and physical properties of the agent.

The study design included exposures of normal dogs at rest and during exercise to determine relationships between total respiratory system uptake and observed pollutant-induced physiological changes. To measure separately upper and lower respiratory tract pollutant uptake, dogs were prepared with a permanent tracheostomy. Three air pollutant compounds with very different chemical characteristics were selected for study: formaldehyde (HCHO), a highly water-soluble upper airway irritant gas; nitrogen dioxide (NO_2), a less water-soluble pulmonary irritant gas; and an aerosol of ammonium nitrate (AMN) particles. Nitrogen dioxide and HCHO are primary pollutants from automotive emissions and represent compounds with very different solubility characteristics. Ammonium nitrate particles are a secondary pollutant aerosol, formed mainly in ambient air by NO_2 reactions that yield nitric acid vapor, which is subsequently neutralized by atmospheric ammonia (Kleinman et al. 1979). Ammonium nitrate particles constitute a major fraction of ambient fine particles and may modify the transport of inhaled gases in the respiratory system. The use of substances with different chemical and

physical characteristics, and the use of different levels of ventilation during exposure, permitted an assessment of the adequacy of the theoretical model to predict respiratory tract uptake under various conditions relevant to exposures of human populations in polluted cities.

SPECIFIC AIMS

This project examined how factors such as pollutant concentration and composition, anatomical features of airways, changes in the rate of ventilation, and interactions between toxicants influenced the doses of automobile-related pollutants that reached respiratory tract target sites, and how those doses related to observed physiological responses. The goal was to determine how well a theoretical model could predict dosimetric changes when relevant factors such as pollutant characteristics or exercise rates were changed. The pollutants selected for study were HCHO, NO_2, and an aerosol of AMN particles, all compounds related to automotive emissions that have very different physical and chemical properties. The following hypotheses were tested: (1) penetration is greater during oral breathing than during nasal breathing; (2) penetration is greater during exercise than during rest; (3) penetration of insoluble gases is greater than that of soluble gases; (4) penetration of a water-soluble gas can be altered in the presence of a droplet aerosol; and (5) greater uptake results in increased physiological effects.

Penetration of inhaled pollutants through the URT and uptake in the lower respiratory tract were measured in tracheostomized dogs. Penetration and uptake were altered by changes in ventilation rate, tidal volume, and nasal versus oral routes of entry. The ventilation rates were appropriate for activities ranging from rest to moderately heavy exercise. The regional penetration and uptake data obtained from the tracheostomized dogs were used to predict total respiratory system uptake for resting and exercising dogs.

Total respiratory system uptake and respiratory physiological functions were measured in single exposures of dogs at rest and during exercise. The predictions of total respiratory system uptake based on the regional penetration data were compared with the values of total respiratory system uptake measured in the intact dogs.

METHODS

EXPERIMENTAL DESIGN

Fourteen female beagle dogs were used in these experiments; their ages and weights are given in Table 1. Six of the 14 dogs were enrolled in exercise (treadmill) studies

Table 1. Participation of Dogs in Exposure Studies

			Whole Respiratory Tract Exposures at Rest or During Exercise			Regional Respiratory Tract Exposures at Rest		
Dog[a]	Age[b] (years)	Body Mass (kg)	NO_2 During Exercise	NO_2 at Rest	HCHO + AMN During Exercise	NO_2	HCHO	AMN
1	9	11.3	X	X	X			
2	4	10.6	X	X	X			
3	3	10.8	X	X				
4	3	11.4	X					
5	4	10.4	X	X	X			
6	3	10.1			X			
7	3	10.3			X			
8	3	13.7			X			
9	3	10.3	X	X		X	X	X
10	9	11.1				X		
11	3	9.8				X	X	X
12	3	10.0				X	X	X
13	10	10.0				X	X	X
14	2	9.4				X	X	X

[a] All dogs were female.

[b] Age on entry to study.

and were trained to run on a treadmill while wearing exposure masks. Another six dogs were surgically prepared with permanent tracheostomies for uptake studies and were trained to stand quietly with minimal restraint and breathe through tracheostomy tubes. They were also trained to tolerate breathing through their mouths with their noses plugged, and breathing through their noses while an investigator held their mouths closed. Table 1 shows the dogs' participation in the different phases of the study. The group size for each exposure experiment was initially six. However, data from certain dogs could not be used because of behavioral factors (no. 12 would not stand still in some experiments) or chronic illness (no. 10 developed mammary cancer and chronic cough). In both of these cases, the data obtained were greatly different from those of the other dogs in their respective groups and were not included in the statistical analyses. With the exception of one dog used in the tracheostomy studies (no. 9), there was no overlap between groups. This dog participated in two of the exercise studies, but had to be retired because of arthritis, which interfered with her running ability; however, she was otherwise healthy and, therefore, was tracheostomized and used in nonexercise uptake studies.

All of the protocols used were evaluated and approved by the Animal Research Committee of the University of California at Irvine. All of the animals were housed in facilities approved by the campus veterinarian, accredited by the American Association for the Accreditation of Laboratory Animal Care, and inspected by the U.S. Food and Drug Administration.

Whole respiratory tract exposures to pollutants with measurements of pulmonary function responses to pollutant exposures at rest or during exercise were performed one to two days after a full protocol control run with purified air. These exposures were scheduled to provide a minimum of five months between exposure of the same dogs to different pollutant exposures. Regional penetration and uptake measurements with tracheostomized dogs were performed in sessions of one to two hours, usually with several days intervening between measurements made from the same dog exposed to the same pollutant. In some instances, measurements were made on successive days, but dogs were not exposed more than once in a day. In regional penetration and uptake exposures, 1 to 8 percent carbon dioxide (CO_2) exposure was used to increase minute ventilation. Minute ventilation, breath frequency, and tidal volume were measured to characterize dose factors affecting uptake and penetration. Because pollutant exposure duration and intermittent use of CO_2 exposure varied, pulmonary function responses to pollutant exposures in regional penetration and uptake measurements were not evaluated.

PROCEDURES

Atmosphere Generation and Characterization

Nitrogen dioxide was metered into purified, humidified (85 percent relative humidity) dilution air from a 1 percent mixture in nitrogen to achieve concentrations in the exposure atmosphere of either 1 or 5 ppm, depending on the desired exposure level. The concentrations of NO_2 in inspired and expired air samples were determined using a chemiluminescence detector (Monitor Labs, Model 8840, Lear Siegler Measurement Controls, Englewood, CO).

Formaldehyde was generated by vaporizing paraformaldehyde and diluting it in purified air. Concentrations of HCHO in inspired and expired air were measured by first collecting breathing-zone air samples in a midget impinger. The concentration of dissolved HCHO was than determined by colorimetric measurement of a chromotropic acid derivative (Altshuller et al. 1962). A 20-m pathlength gas infrared spectrometer (Miran 1B, Foxboro Laboratories, Foxboro, MA) was used to monitor continuously the HCHO concentration in the pollutant exposure delivery system.

Ammonium nitrate aerosol was generated from a dilute solution of the salt using a Collison nebulizer (BGI Inc., Waltham, MA). The static charge on the aerosol was reduced to Boltzmann equilibrium levels using a ^{85}Kr static discharger (Model 3012, ThermoSystems, Inc., St. Paul, MN). The particle mass concentration was measured by collecting inspired and expired air on membrane filters, extracting the deposited particles into distilled water, and analyzing the extracts for nitrate ions using ion exchange chromatography. Particle samples classified by size were collected using an eight-stage cascade impactor (Sierra Model 210, Andersen 2000 Inc., Peachtree City, GA); the stages were extracted and analyzed for nitrate ions with the method described above. These data were used to compute the mass median aerodynamic diameter (MMAD) and to estimate the geometric standard deviation (GSD) of the AMN aerosol.

Animal Housing

The purebred female beagle dogs used in these studies either were born and raised at the Air Pollution Health Effects Laboratory at the University of California at Irvine, or were obtained from licensed breeders (Laboratory Research Enterprises, Kalamazoo, MI; Marshall Farms USA, North Rose, NY; Laboratory for Energy Related Health Research, University of California at Davis). The dogs were housed in Dexotex-floored kennel runs that allowed contact between the animals. The kennels were supplied with purified air (22 air changes per hour) and maintained at 72°F, with 30 to 70 percent relative humidity. The air was purified using

alumina impregnated with potassium permanganate (Purafil II, Atlanta, GA) and high-efficiency particulate air (HEPA) filters. Light cycles were set to reflect normal seasonal changes. Dogs were fed a daily individual ration (Hills Science Diet, Colgate Palmolive Co., Topeka, KA) with water ad libitum. The dogs were placed in an adjoining exercise area when the runs were cleaned and sanitized each day. The dogs resided in these kennels for at least six weeks before exposure experiments and throughout the periods of experimental measurements.

Exercising Dog Exposures

Exercising exposures were performed in a refrigerated treadmill exposure system. Refrigeration was controlled by computer to stabilize the dogs' rectal temperatures, so that they could exercise without requiring panting respiration for thermoregulation (Stavert et al. 1982a). Dogs were trained initially to run on the treadmill, and then to run while wearing a low-deadspace (30 cm^3) respiratory mask. This latex mask was made from a mold shaped like a dog muzzle. It was outfitted with two-way respiratory valves (Hans-Rudolph, Kansas City, MO) to isolate inspired and expired air (Goldberg et al. 1981; Stavert et al. 1982b). Flow resistance of the mask and valve assembly was less than 1 cm H_2O·sec/L of air. Respiratory gases were sampled continuously from a port in the mask centered at the mouth, and were analyzed with a mass spectrometer (Perkin Elmer Corp., Pomona, CA; model 1100). Inspiratory and expiratory flow rates were measured with two Fleisch no. 2 pneumotachographs (Dynasciences, Blue Bell, PA) and pressure transducers (Validyne Engineering Corp., Northridge, CA). The temperature of the inspiratory pneumotachograph and its attached tubing was electrically controlled at 23°C to prevent inspiratory air from cooling in the refrigerated treadmill, and the temperature of the expiratory tubing and pneumotachograph was controlled at 39°C to prevent water vapor condensation.

An esophageal balloon catheter was placed in the dog for transpulmonary pressure measurements before the mask was positioned. The balloon (Youngs Rubber Co., Trenton, NJ) was attached to 1.5 m of polyethelyne (PE) 200 tubing which was attached to a pressure transducer (Validyne Engineering Corp., model MP45) referenced to the mask at the dog's mouth through another 1.5 m of PE 200 tubing. The balloon was approximately 11 cm long when prepared with the tubing and was passed to a position in the lower thoracic esophagus (45 cm from the dog's lips). The balloon was evacuated with a syringe and then filled with 2 mL of air by inflating it with 10 mL of air and withdrawing 8 mL of air.

Dynamic pulmonary resistance and compliance were measured by the method of Reischl and Beaucage (1982), and the data obtained with this system were consistent with values reported from our own and other studies with beagle dogs (Dubin 1970; Pickrell et al. 1971; Muggenburg and Mauderly 1974; Reischl and Beaucage 1982; Mautz et al. 1985). Inspiratory flow, expiratory flow, fractional concentration of CO_2 and oxygen (O_2), transpulmonary pressure, rectal temperature, and skin temperature were continuously monitored and recorded on an eight-channel strip chart recorder (Gould-Brush, Model 2800, Gould Inc., Cleveland, OH) coupled to a PDP11 computer (Digital Equipment Corp., Costa Mesa, CA). Breath-by-breath analysis of the pulmonary flow and pressure signals yielded measures of breath time, inspiratory and expiratory times, expired tidal volume, expired minute ventilation, O_2 consumption, CO_2 production, pulmonary resistance, and dynamic compliance (Wessel et al. 1979; Reischl and Beaucage 1982; Mautz et al. 1985).

Exercise exposure experiments lasted 180 minutes and breath-by-breath data were recorded for two minutes at the end of each 20-minute interval. There were a total of nine data collection periods, each consisting of approximately 100 breaths. During collection periods 1 and 9, the animal stood at rest. During collection period 2, the animal performed warm-up exercise in clean air at 5 km/hour and 7.5 percent grade. The test pollutant atmosphere was introduced after data acquisition period 2, and the animal continued to exercise for data collection periods 3 through 8. Total exercise time was 140 minutes (periods 2 through 8); pollutant exposure occurred during 120 minutes of exercise (periods 3 through 8) and 20 minutes of rest (period 9).

Continuous resting exposures were conducted with the dog standing on a stationary treadmill. The protocol time was shortened to 80 minutes to accommodate the patience level of the standing dogs. Data were recorded for four minutes in the latter portion of each 10-minute interval. During data collection periods 1 and 2, the dog breathed purified air; data from data collection periods 3 through 8 were recorded while the dog breathed the test atmosphere. A full protocol clean air control run was performed one to two days before each pollutant exposure run. Following each exposure, the dogs were returned to clean air kennels.

Pulmonary function data were analyzed using a two-factor repeated measures analysis of variance (ANOVA) (Snedecor and Cochran 1980). The two factors were time during exposure (data collection periods 3 through 8) and the atmosphere (pollutant or purified air). Significant effects ($p < 0.05$) of pollutant atmospheres on group mean pulmonary function values were revealed as direct pollutant effects or as pollutant–exposure time interactions.

Measurement of Upper Respiratory Tract Pollutant Penetration

Beagle dogs were surgically prepared with a permanent tracheostomy in surgical facilities approved by the University. The technique described by Ritter (1984) was used to prepare a tracheostomy with skin folds that normally closed the stoma, so that the dogs ventilated via the upper airways. After a four- to six-week recovery period, each dog was trained to accept the insertion of caudally and cephalically directed endotracheal tubes and to stand in a sling with its head in a head-only exposure chamber for periods of up to 2 hours. The head-only exposure chamber was designed as a glove box with a window allowing the trainer to observe, encourage, and manipulate the dog. The trainer allowed the dog to either breathe orally with the nose plugged, or nasally, with the mouth and lips held closed. Because of the proximity of equipment and technicians, the trainer was often aware when exposures were taking place. However, the trainer's knowledge of the exposures was not regarded as a problem, because characterization of pulmonary physiological responses to the exposures was not the object of this phase of the project.

Initially, we attempted to ventilate the URT with test pollutants, using a mechanical ventilator attached to the upper tracheostomy tube, while the dog breathed room air through the lower tracheostomy tube. However, the upper airways would not open in response to mechanically driven airflow independent of the natural lung breathing cycle. This approach was then abandoned in favor of using the dogs' inspiratory airflow to ventilate the upper airways via

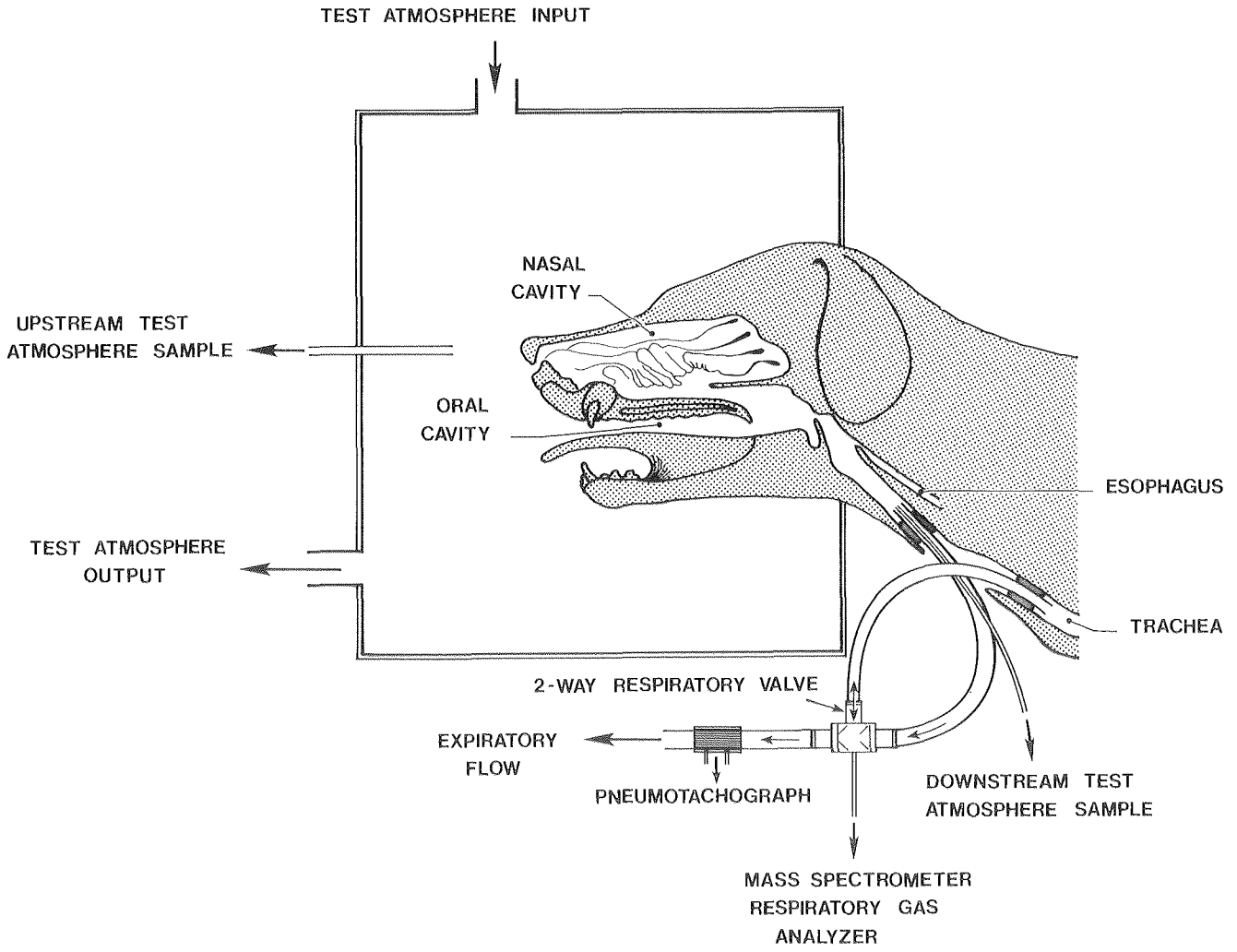

Figure 1. Experimental setup for measuring regional penetration and uptake of inhaled particles and gases in the upper respiratory tracts of awake, tracheostomized dogs.

a Hans-Rudolph two-way respiratory valve connecting the tracheostomy tubes (Figure 1). Flow through the upper airways was in unidirectional inspiratory pulses. The respiratory dead space of the apparatus, in which bidirectional flow occurred, was confined to the lower limb of the loop and the two-way respiratory valve. This volume, 23 mL, was measured by independently filling this segment of the apparatus with water, and was approximately 12 percent of the 200-mL resting tidal volume. The apparatus respiratory dead space volume was not simply additive to the total anatomical dead space of the dogs before intubation because, with the apparatus in place, the upper airways had unidirectional flow and no longer contributed to anatomical dead space. The two-way valve and expiratory flow tubing were wrapped and heated with an electric element fabric tape to prevent condensation of water vapor. Respiratory gas concentrations through the inspiratory and expiratory phase were measured at the two-way respiratory valve, and the expired breath frequency, tidal volume, and minute ventilation were measured with a pneumotachograph on the expiratory flow line and were corrected for the sample flow (60 mL/min) to the respiratory mass spectrometer during the expiratory phase. Respiratory ventilation in the tracheostomized dogs was modified by adding 0 to 8 percent CO_2 to the inspired air to alter respiratory drive. Ventilation rates were varied from resting values of 1 to 2 L/min in air up to 14 L/min in air mixed with CO_2. These ventilation rates bracketed the normal range of rates observed in dogs at rest and during moderate exercise. Measurements of penetration and uptake of pollutant compounds were made at each ventilation rate during periods of one to five minutes and were accepted when dogs exhibited consistent breathing patterns during the measurement period.

Air was sampled within the chamber to determine the concentration of the pollutant inhaled (*Ci*), and from the trachea at a point just downstream of the larynx at the tip of the endotracheal tube, to determine the concentration of the pollutant in the air after transit through the URT (*Ct*)(Figure 1). The sampled air was diluted 50 percent with dry, purified air to prevent condensation in the sampling lines. The fraction of inhaled pollutant that was absorbed after passage through the nasal or oral airways was computed from the equation:

$$\text{Fractional uptake} = \frac{Ci - Ct}{Ci}. \quad (3)$$

These pollutant uptake measurements were representative of unidirectional, pulsatile flow in the head.

Fractional uptake of pollutants in the sampling apparatus was independently measured, and total fractional uptake (*UT*) measurements were corrected for sampling apparatus uptake (*US*) to yield respiratory system uptake (*UR*) using the following equation:

$$UR = \frac{UT - US}{1 - US}. \quad (4)$$

This correction is equivalent to the system loss correction used by Gerrity and coworkers (1988). The addition of water vapor to the air passing through the respiratory system was a source of error in uptake determinations, but the diluting effect of water vapor on *Ct* was very small. In the worst possible case, in which inspired air would be humidified from 85 percent relative humidity at 22°C to 100 percent relative humidity at 37°C, *Ct* would be reduced by 5 percent due to dilution by water vapor. The effect of underestimating *Ct* by 5 percent on the determination of fractional uptake would depend on the value of the fractional uptake; fractional uptake of 0.9 would be underestimated by 0.6 percent, and fractional uptake of 0.6 would be underestimated by 3.5 percent.

Measurement of Pollutant Uptake in the Lower Respiratory Tract (Lung)

A caudally directed endotracheal tube was inserted into the tracheostomy and connected to a two-way respiratory valve that isolated inspired and expired air. Atmosphere was inhaled directly via the endotracheal tube, and the concentrations of pollutant in inspired (*Ci*) and expired (*Ce*) air were measured. Ventilation was modified using 0 to 8 percent CO_2 as described for URT penetration measurements. Measurements of lower respiratory tract uptake were made using 1 and 5 ppm NO_2, 2 ppm HCHO, and 1 mg/m^3 AMN particles. In initial attempts to expose the lower respiratory tract to 10 ppm HCHO, dogs were visibly uncomfortable. Therefore, the concentration was reduced to 2 ppm HCHO.

The valve and tracheostomy tube apparatus had a respiratory dead space volume of 23 mL. The effect of this dead space on uptake measurements was twofold. First, the pollutant concentration in the air inspired by the dog was slightly lower than that measured in the inspired air sample because at the end of the previous expiration the dead space contained "scrubbed" expired air that slightly diluted the air inhaled by the dogs. Second, the concentration of the expired air increased slightly because at the end of inspiration the dead space contained "unscrubbed" atmosphere from the previous inspiration that then mixed with the expired air. Equation 5 was used to compute the lower respiratory tract uptake from the measured concentrations of inspired and expired pollutant, and to correct for the apparatus dead space effect on inspired and expired concentrations.

$$\text{Uptake} = \frac{V_T(CeV_T - CiV_D)}{V_D(CeV_T - CiV_D) + Ci(V_T - V_D)^2}, \quad (5)$$

Where: V_T = Tidal Volume
V_D = Dead Space Volume

The dead-space volume of the artificial loop was not involved in the fractional uptake calculations because when the loop was used, air flow through the head and the loop was unidirectional inhalation; therefore, uptake measurements were made only for gases passing through the head to the tip of the loop. Uptake measurements for the lower respiratory tract were made with a short tracheostomy tube and a two-way respiratory valve with a total dead space of 23 mL. Ventilation of this apparatus was tidal and, in this case, the correction for the apparatus dead space was made using Equation 5.

Pollutant losses to the sampling apparatus for upper respiratory tract uptake measurements included losses to the tip of the upper tracheostomy tube and losses to the gas sample line that opened at the tip of the tracheostomy tube. The body of the artificial loop was not part of the path over which uptake in the head was measured. For lower respiratory tract uptake measurements the short tracheostomy tube and two-way valve were the relevant sites of loss to the apparatus. Experimental error in gas concentration measurements and the assumptions about the behavior of apparatus uptake and dead space washout described in Equations 4 and 5 are the sources of uncertainty in our uptake estimates. In the case of formaldehyde, which is extremely soluble and has an uptake expected to be near 1.0, this uncertainty led to occasional estimates with values exceeding 1.0.

Ventilatory increases induced by CO_2 inhalation and exercise do not represent identical pulmonary physiological responses and may involve differences in breathing pattern (frequency and tidal volume), airway caliber, and alveolar recruitment. In measuring respiratory uptake, differences in breathing patterns were probably the most important variables possibly affecting dose distribution and uptake. Breathing patterns during regional uptake measurements from tracheostomized dogs under CO_2 stimulation varied at a given minute ventilation, but the means were similar to breathing patterns during exercise exposures. For example, mean minute volume of expired air ($\dot{V}_E$) during exercise exposure data collection periods ranged from 9.4 to 10.8 L/min, with corresponding tidal volumes ranging from 0.25 to 0.30 L and frequencies ranging from 35 to 38/min (Appendix A, Tables A.1, A.2, A.3, A.21, A.22, and A.23). The selection of lung uptake measurements with CO_2 stimulation that produced $\dot{V}_E$ ranging from 8 to 12 L/min (n = 14; Appendix B, Tables B.1, B.2, B.5, and B.6) had tidal volumes averaging 0.29 L (range 0.17 to 0.44 L) and frequencies averaging 39/min (range 22 to 63/min).

Total Respiratory System Uptake in Exercising Dogs

Dogs were trained intensively to wear a low-dead-space (30 cm^3) respiratory mask (Stavert et al. 1982b) and to exercise on a refrigerated treadmill that eliminated thermoregulatory panting (Stavert et al. 1982a). Air was sampled from points immediately upstream of the inspiratory valve and immediately downstream from the expiratory valve. Concentrations of pollutants in inspired and expired air were determined and Equation 5 was used to compute pollutant uptake values corrected for the effect of mask dead space. Ventilation rates during these measurements ranged from 1.5 L/min (at rest) to about 10 L/min, the equivalent of a moderate exercise workload (Phalen et al. 1988).

STATISTICAL METHODS

The uptake and penetration data for the upper and lower respiratory tracts were analyzed separately for each pollutant regimen in the studies of resting and exercising dogs. Linear least squares fits of log-transformed penetration data versus the inverse of ventilation during oral and nasal breathing were performed using data aggregated within ventilation limits, as described in the Results section. The pulmonary function data for exercising and resting studies were analyzed using a two-factor ANOVA.

RESULTS

PULMONARY FUNCTION OF DOGS EXPOSED DURING EXERCISE AND REST TO 5 PPM NITROGEN DIOXIDE

Five dogs were exposed to NO_2 (5.1 ± 0.2 ppm, mean ± SD) in both resting and exercising exposure protocols. Values of pulmonary function variables measured during the exposures are shown in Appendix A, Tables A.1 through A.20. Data were analyzed using a repeated measures ANOVA. Effects of atmosphere, duration of exposure (time), and atmosphere-time interactions were evaluated. Exercise increased the metabolic gas exchange ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$) and minute ventilation by a factor of 2.2 over resting rates. Exercising dogs exposed to NO_2 did not show alterations in breathing patterns (Table 2). The only statistically significant effect observed in the exercising exposures was a depression in ventilation equivalent for O_2. Ventilation equivalent for CO_2 showed a trend toward lower values dur-

Table 2. Analysis of Variance with Repeated Measures: Pulmonary Function Values for Dogs Exposed to 5 ppm of Nitrogen Dioxide During Exercise

Pulmonary Function	Average Values During Exercise Exposure (Mean ± SD)			ANOVA Significance Level ($p < 0.05$)	
	Clean Air	Atmosphere	n	A[a]	A × T[b]
Breath time (seconds)	1.55 ± 0.07	1.57 ± 0.05	6	NS[c]	NS
Expired tidal volume (L, BTPS[d])	0.27 ± 0.08	0.27 ± 0.01	6	NS	NS
Minute ventilation (L/min, BTPS)	10.57 ± 0.29	10.48 ± 0.19	6	NS	NS
O_2 consumption (L/min, STPD[e])	0.36 ± 0.02	0.38 ± 0.01	6	NS	NS
CO_2 production (L/min, STPD)	0.28 ± 0.01	0.30 ± 0.01	6	NS	NS
Ventilation equivalent, O_2 (L/mmol, BTPS)	0.67 ± 0.03	0.62 ± 0.01	6	0.045	NS
Ventilation equivalent, CO_2 (L/mmol, BTPS)	0.86 ± 0.04	0.79 ± 0.02	6	NS	NS
Pulmonary resistance (cm H_2O·min/L, BTPS)	0.25 ± 0.01	0.26 ± 0.01	6	NS	NS
Dynamic compliance (L/cm H_2O, BTPS)	0.032 ± 0.001	0.033 ± 0.001	6	NS	NS

[a] Atmosphere effect.

[b] Effect of interaction between atmosphere and exposure time.

[c] NS = not significant.

[d] BTPS = body temperature and pressure, saturated with water vapor.

[e] STPD = standard temperature and pressure, dry.

Table 3. Analysis of Variance with Repeated Measures: Pulmonary Function Values for Dogs Exposed to 5 ppm Nitrogen Dioxide at Rest

Pulmonary Function	Average Values During Resting Exposure (Mean ± SD)			ANOVA Significance Level ($p < 0.05$)	
	Clean Air	Atmosphere	n	A[a]	A × T[b]
Breath time (seconds)	3.17 ± 0.12	2.84 ± 0.14	5	NS[c]	0.043
Expired tidal volume (L, BTPS[d])	0.18 ± 0.01	0.17 ± 0.00	5	NS	NS
Minute ventilation (L/min, BTPS)	3.57 ± 0.14	3.78 ± 0.13	5	NS	NS
O_2 consumption (L/min, STPD[e])	0.087 ± 0.004	0.094 ± 0.004	5	NS	NS
CO_2 production (L/min, STPD)	0.076 ± 0.003	0.079 ± 0.003	5	NS	NS
Ventilation equivalent, O_2 (L/mmol, BTPS)	0.92 ± 0.03	0.90 ± 0.04	5	NS	NS
Ventilation equivalent, CO_2 (L/mmol, BTPS)	1.07 ± 0.03	1.08 ± 0.04	5	NS	NS
Pulmonary resistance (cm H_2O·min/L, BTPS)	0.37 ± 0.04	0.35 ± 0.03	4[f]	NS	NS
Dynamic Compliance (L/cm H_2O, BTPS)	0.051 ± 0.003	0.051 ± 0.003	4[f]	NS	NS

[a] Atmosphere effect.

[b] Effect of interaction between atmosphere and exposure time.

[c] NS = not significant.

[d] BTPS = body temperature and pressure, saturated with water vapor.

[e] STPD = standard temperature and pressure, dry.

[f] The esophageal balloon malfunctioned in one dog and decreased this n value.

ing NO_2 exposure; however, the difference was not significant ($p > 0.05$). In resting exposures to NO_2 (5.1 ± 0.2 ppm, mean ± SD), dogs developed shorter breath time, revealed as a significant interaction between exposure to NO_2 and duration of exposure (Table 3). Although there were trends toward increased $\dot{V}_E$ and $\dot{V}_{O_2}$, neither of these responses was statistically significant.

PULMONARY FUNCTION OF DOGS EXPOSED DURING EXERCISE AND REST TO FORMALDEHYDE PLUS AMMONIUM NITRATE PARTICLES

Dogs exposed to HCHO (9.8 ± 2.0 ppm, mean ± SD) plus AMN particles (1.4 ± 0.1 mg/m^3; 0.29 ± 0.13 μm MMAD; 2.96 ± 0.68 GSD, means ± SD of measurements made on

each exposure day) during exercise showed a shift toward a deep-breathing pattern (Table 4; Appendix A, Tables A.21 through A.31, for mean values of pulmonary function variables). Breath time showed a nonsignificant trend toward shorter breaths, but expired tidal volume was 9 percent greater at the end of exposure than the expired tidal volumes for dogs exposed to clean air. Volume of expired air per minute increased significantly, and ventilation equivalents for both O_2 and CO_2 increased during the exposure, although not significantly.

RESPIRATORY UPTAKE

The data base of URT uptake measurements is given in Appendix B. Penetration of NO_2 inhaled through the airways of the head to the trachea was measured in spontaneously breathing awake dogs during both oral and nasal breathing, and ventilation rates were varied by increasing the inspired CO_2 concentration to values up to 8 percent. Observed ventilation rates ranged from 1.6 to 25 L/min; most of the values were between 2 and 8 L/min. Measurements were made using NO_2 concentrations of 1.05 ± 0.10 ppm and 5.14 ± 0.20 ppm (mean ± SD). The differences in penetration during mouth breathing or for lung uptake were not dependent on concentration. However, there was significantly greater URT penetration of 5 ppm NO_2 than of 1 ppm NO_2 during nasal breathing. The ventilation trends were consistent, although there was considerable overlap among the data distributions due to experimental variation. The 1- and 5-ppm data were combined for subsequent analyses of relationships between ventilation and URT penetration during mouth breathing and for lung uptake. Only the 1-ppm data were used in developing the equation for penetration during nose breathing.

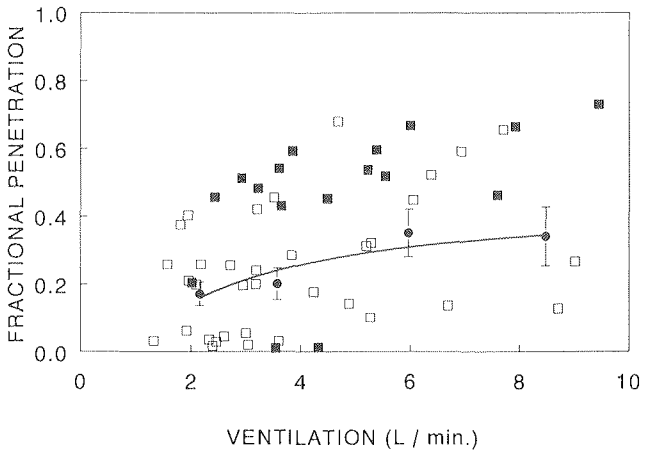

Figure 2. Fractional penetration in the upper respiratory tracts of dogs exposed to 1 and 5 ppm NO_2 during nasal breathing. The line represents a least squares fit to the 1 ppm data only. A □ indicates data from an individual dog exposed to 1 ppm NO_2; a ▩ indicates data from an individual dog exposed to 5 ppm NO_2; and a ⧳ indicates a group average ± SE for those exposed to 1 ppm NO_2.

The oral and nasal penetration data were grouped into $\dot{V}_E$ ranges 1 to 2.9, 3 to 4.9, 5 to 6.9, and 7 or more L/min; the average penetration fraction and the average ventilation rate in each range was calculated and plotted for nasal breathing in Figure 2 and for oral breathing in Figure 3. The error bars represent the SE of the grouped penetration frac-

Table 4. Analysis of Variance with Repeated Measures: Pulmonary Function Values for Dogs Exposed to 9.8 ppm Formaldehyde Plus 1.4 mg/m^3 Ammonium Nitrate Aerosol During Exercise

Pulmonary Function[a]	Average Values During Exercise Exposure (Mean ± SD)			ANOVA Significance Level ($p < 0.05$)	
	Clean Air	Atmosphere	n	A[b]	A × T[c]
Breath time (seconds)	1.69 ± 0.04	1.65 ± 0.04	6	NS[d]	NS
Expired tidal volume (L, BTPS[e])	0.27 ± 0.22	0.29 ± 0.01	6	0.031	NS
Minute ventilation (L/min, BTPS)	9.74 ± 0.22	10.53 ± 0.18	6	0.003	NS
O_2 consumption (L/min, STPD[f])	0.34 ± 0.01	0.33 ± 0.01	6	NS	NS
CO_2 production (L/min, STPD)	0.26 ± 0.01	0.25 ± 0.01	6	NS	NS
Ventilation equivalent, O_2 (L/mmol, BTPS)	0.68 ± 0.01	0.78 ± 0.05	6	NS	NS
Ventilation equivalent, CO_2 (L/mmol, BTPS)	0.88 ± 0.02	0.99 ± 0.06	6	NS	NS

[a] Pulmonary resistance and dynamic compliance were not measured.

[b] Atmosphere effect.

[c] Effect of interaction between atmosphere and exposure time.

[d] NS = not significant.

[e] BTPS = body temperature and pressure, saturated with water vapor.

[f] STPD = standard temperature and pressure, dry.

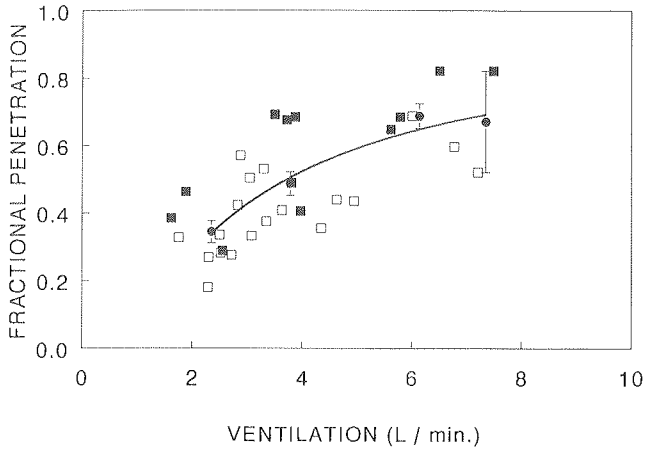

Figure 3. Fractional penetration in the upper respiratory tracts of dogs exposed to 1 and 5 ppm NO_2 during oral breathing. The line represents a least squares fit to the combined data. A □ indicates data from an individual dog exposed to 1 ppm NO_2; a ■ indicates data from an individual dog exposed to 5 ppm NO_2; and a ⧳ indicates a group average ± SE.

tions, and the lines represent least squares fits of the data to Equation 2. The individual data points are also shown in the figures. The data vary considerably, partly because of inter-individual differences and partly because of experimental variability. The grouped data, however, allow clear patterns to be discerned.

At rest, NO_2 penetration during nose breathing was about half that found during mouth breathing. As ventilation increased, penetration increased in both breathing modes. Nose breathing remained the more efficacious filter of NO_2. At ventilation rates approximately three times those of resting levels, which can be considered equivalent to moderate exercise, the oral breathing and nasal breathing group mean penetrations were not significantly different statistically, although the nasal penetration curve remained lower than the oral curve.

Uptake of NO_2 by the lung (ventilation via the tracheostomy) was measured as a function of ventilation (Figure 4). On the average, about 90 percent of the NO_2 entering the lung was removed; the uptake was approximately constant for ventilation rates ranging from 3 to 16 L/min, but appeared to be higher at lower ventilation rates. Clearly, uptake fractions greater than 1.0 are not possible; the higher values between 1 and 2 L/min probably represent an experimental error occurring in the dead space correction equation (Equation 5) for low tidal volumes in which respiratory valve dead space was greater than 8 percent of tidal volume.

Penetration of HCHO through the upper airways is shown in Figure 5. The mean ± SD of the exposure concentrations was 7.3 ± 1.6 ppm of HCHO. Chemical analyses of HCHO concentration required much longer sample times than those required for NO_2 analyses, fewer measurements were available, and the exposure concentrations were more variable than for NO_2. Like the NO_2 data, the HCHO data were grouped into ventilation rate classes. The data were fit to Equation 2, and the least squares curve was plotted. The URT removed HCHO more readily than NO_2, and there was less penetration with nose breathing than with mouth breathing. However, even during oral breathing at high ventilation rates, the upper airways removed about 85 percent of the inspired HCHO. Lower respiratory tract uptake of

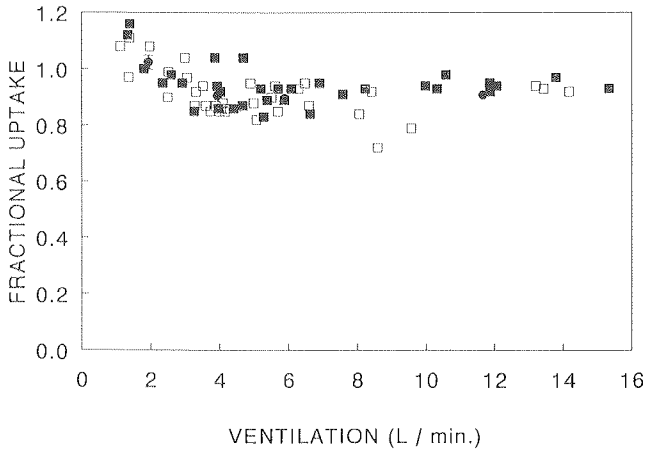

Figure 4. Fractional uptake in the lower respiratory tracts of dogs exposed to 1 and 5 ppm NO_2. A □ indicates data from an individual dog exposed to 1 ppm NO_2; a ■ indicates data from an individual dog exposed to 5 ppm NO_2; and a ⧳ indicates a group average ± SE.

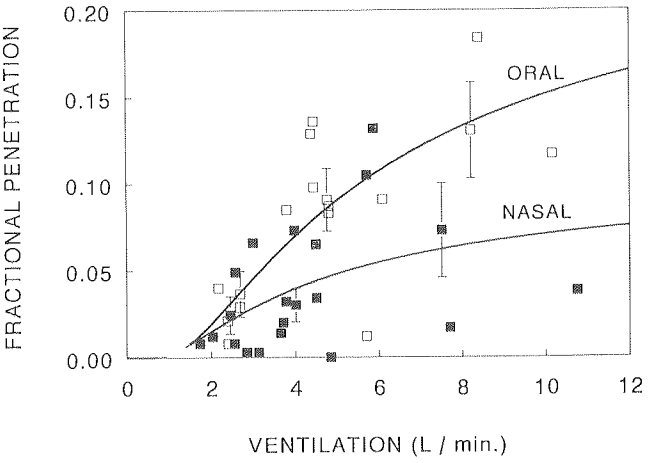

Figure 5. Fractional penetration in the upper respiratory tracts of dogs exposed to 7 ppm HCHO during oral and nasal breathing. A □ indicates data from an individual dog breathing orally; a ⧳ indicates a group average ± SE of data from dogs breathing orally; a ■ indicates data from an individual dog breathing nasally; and a ⧳ indicates a group average ± SE of data from dogs breathing nasally.

HCHO (2.2 ± 0.4 ppm HCHO) is shown in Figure 6. Approximately 90 percent of the HCHO entering the trachea was retained in the lower respiratory tract.

Penetration of AMN particles (1.4 ± 0.1 mg/m^3, 0.2 ± 0.1 μm MMAD, 3.8 ± 1.5 GSD, means ± SD of measurements made on three different exposure days) was measured during oral and nasal breathing. These measurements were difficult to perform because they required periods of approximately 15 minutes of stable breathing to obtain aerosol samples from the tracheal airway. The oral and nasal penetration data, plotted in Figure 7, showed a large scatter, and no significant trend with ventilation. The average fractional penetration of all ventilation rates was greater during nasal breathing than during oral breathing (0.60 ± 0.06 SE, n = 10 vs. 0.39 ± 0.05 SE, n = 10). The deposition of particles smaller than approximately 0.4 μm diameter in airways is predominantly dependent upon the mechanism of diffusion. The particles used in this study had an MMAD of approximately 0.2 μm, in which range diffusion deposition occurs. Therefore, to provide an indication of possible trends, we used a least squares fit to an equation of the same form that was used for penetration of the gases discussed above. Particle deposition in the lung, which is shown in Figure 8, decreased with increasing ventilation.

Total respiratory system uptake of NO_2 measured in nontracheostomized dogs during exercise and while standing at rest on the treadmill is shown in Figure 9. Fractional uptake of 5 ppm NO_2 during the exercise exposures was 0.94 ± 0.02 SE (n = 6 dogs); during continuous resting exposures the fractional uptake was 0.78 ± 0.03 SE (n = 5 dogs). Nitrogen dioxide fractional uptake measured during rest at the end of exercise exposures (not shown in Figure 9) was 0.77 ± 0.07 SE, n = 6. Total respiratory system fractional uptake of HCHO was 0.98 ± 0.05 SE (n = 6 dogs) during exercise exposures to a mixture consisting of 10 ppm HCHO and 1 mg/m^3 AMN particles (Figure 10), and was 1.02 ± 0.09 SE (n = 6 dogs) during rest at the end of exercise exposures (not shown in Figure 10). Essentially all HCHO measured downstream was accounted for after correction for the effects of respiratory mask dead space, and respiratory system uptake was 1.0.

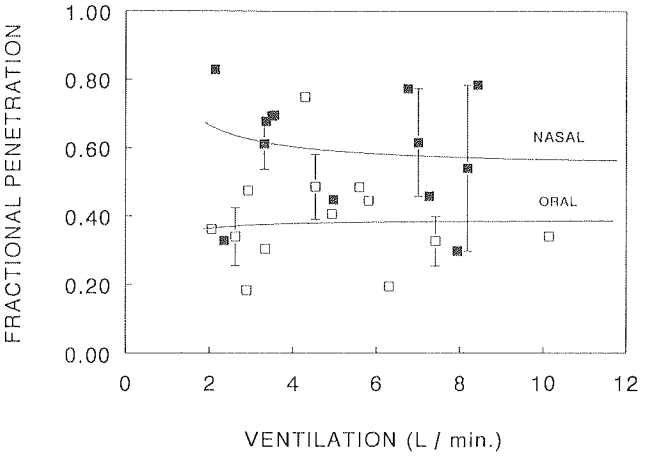

Figure 7. Fractional penetration in the upper respiratory tracts of dogs exposed to AMN particles (1.4 mg/m^3; 0.2 μm MMAD) during oral and nasal breathing. A □ indicates data from an individual dog breathing orally; a ⊕ indicates a group average ± SE of data from dogs breathing orally; a ■ indicates data from an individual dog breathing nasally; and a ⊕ indicates a group average ± SE of data from dogs breathing nasally.

DISCUSSION

This project addressed three factors important to understanding the delivery of toxic compounds to respiratory

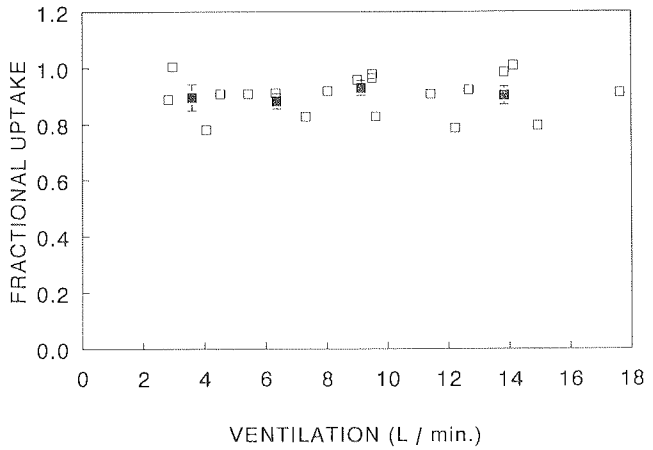

Figure 6. Fractional uptake in the lower respiratory tracts of dogs exposed to HCHO at 2 ppm. A □ indicates data from an individual dog; and a ⊕ indicates a group average ± SE.

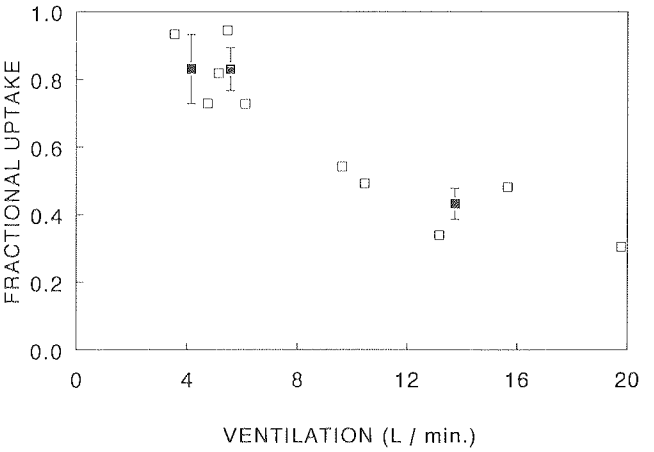

Figure 8. Fractional uptake in the lower respiratory tracts of dogs exposed to AMN particles (1.4 mg/m^3; 0.2 μm MMAD). A □ indicates data from an individual dog; and a ⊕ indicates a group average ± SE.

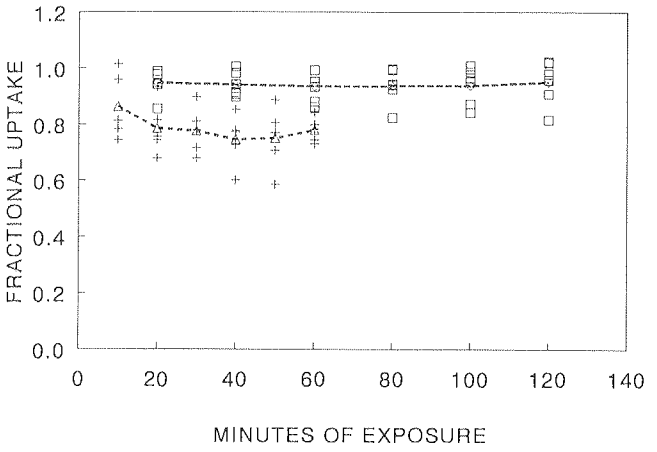

Figure 9. Fractional uptake in the total respiratory systems of dogs exposed to 5 ppm NO_2 at rest and during exercise. A □ indicates data from an individual dog during exercise; a + indicates data from an individual dog at rest; a —◇— indicates a group average of data for dogs during exercise; and a —△— indicates a group average of data for dogs at rest.

tract targets: (1) alterations in penetration produced by changes in ventilation rate and route of entry; (2) alterations of breathing patterns produced by inhaled pollutants and how they might modify dose; and (3) the extent to which differences in penetration may be explained by anatomical differences. The first and third factors were examined in tracheostomized dogs so that inhalation via the nose or mouth could be studied. The second factor was examined in intact dogs exposed at rest or during exercise, so that breathing pattern changes could be observed.

The effect of pollutant concentration on URT uptake was addressed in experiments with NO_2 at 1 and 5 ppm. In oral breathing there was no difference in uptake between these concentration levels. In nasal breathing, however, uptake was significantly greater at 1 ppm of inspired NO_2. These results are similar to findings of Morris and Cavanagh (1987), who studied the URT uptake of acetone and ethanol in the guinea pig and rat. In general, they found that fractional uptake was reduced when the delivery rate of the compound was increased. At a given flow, Morris and Cavanagh (1987) showed that uptake decreased as concentration increased; however, this was apparent only in measurements made at elevated ventilation rates. They also showed that at a given concentration, URT uptake decreased as ventilation increased.

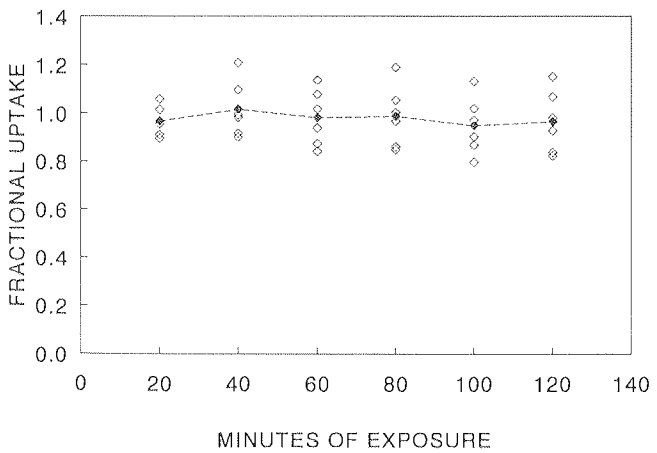

Figure 10. Fractional uptake of HCHO in the total respiratory systems of dogs exposed to a mixture of 9.8 ppm HCHO and AMN particles (1.4 mg/m^3; 0.3 μm MMAD) during exercise. A ◇ indicates data from an individual dog during exercise, and —◆— indicates a group average of data for dogs during exercise.

Vaughan and coworkers (1969) showed that ozone (O_3) penetration increased with increasing O_3 concentration at relatively high concentrations, and this was confirmed by Yokayama and Frank (1972). These results are consistent with the hypothesis that at high concentrations or ventilation delivery rates, the uptake rate begins to be affected by the accumulation of oxidants in the fluid and epithelial lining of the URT. With higher concentrations of reactive oxidants at the surface of the fluid layer, the compounds penetrate further into the layer, increasing the effective distance for diffusive flux even if consumptive reaction rates remain directly proportional to local concentration in the layer. As this effective distance increases, diffusive flux will not be directly proportional to inhaled concentration, and fractional uptake is expected to decline with increasing inhaled concentration. On the other hand, Frank and associates (1969) demonstrated that penetration of sulfur dioxide (SO_2) decreased with increasing inhaled concentrations. In this case, fractional penetration at all concentrations was less than 0.001, due to the high solubility of SO_2. At concentrations of 10 to 50 ppm used in the study by Frank and coworkers (1969), it is possible that irritation of respiratory tract tissue may have caused increased production of mucus, leading to an increase in the amount of absorbent fluid in the presence of the higher concentrations of SO_2 (Brain 1970). Gerrity and associates (1988) showed no difference in O_3 URT uptake in humans at concentrations of 0.1, 0.2, and 0.4 ppm, concentrations an order of magnitude less than the range at which we observed concentration-dependent uptake for NO_2.

Upper respiratory tract uptake of 10 ppm HCHO was much greater than that for NO_2, but showed a similar dependence on the route of breathing. The total respiratory system uptake results for HCHO were consistent with reported literature values for highly water-soluble gases, such as SO_2 (Frank et al. 1969). The URT uptake values, which at higher ventilation rates were in the 85 to 90 per-

cent range, were not as high as the uptake values reported for SO_2 (Frank et al. 1969). This result may be due to differences in experimental design. Frank and colleagues (1969) used continuous flows through the head, whereas our preparation flows were pulsatile, although not bidirectional. The highest continuous inspiratory flow used by Frank and colleagues (1969) was 35 L/min; in our preparation, peak inspiratory flows at the higher minute ventilation rates ranged from 30 to 60 L/min.

Total respiratory uptake of HCHO alone was measured previously in exercising dogs, using the same exercise protocol as for the present study (Crocker 1984). In that study, HCHO fractional uptake during exercise was 0.86 ± 0.01 SE (n = 5), which is lower than HCHO fractional uptake (0.98) in the presence of AMN particles in the present study (Figure 10). Breathing pattern responses also differed between the two studies. With HCHO alone, the expired tidal volume increase (to 0.29 ± 0.03 L [mean ± SE]) was similar to that with HCHO plus AMN particles. However, with exposure to HCHO alone, breathing frequency declined by 22 percent to 30.0 ± 1.2 breaths/min (mean ± SE) leading to a significant decline (10 percent) in $\dot{V}_E$ to 9.8 ± 1.0 L/min. Because both the breathing pattern and $\dot{V}_E$ were different in the two exposures, it is not certain that the difference in HCHO uptake was due only to the presence of AMN particles. However, the differences in breathing pattern and $\dot{V}_E$ were not as large as might be necessary to produce such large differences in uptake (Table 5), and the lower frequency and lower $\dot{V}_E$ in HCHO alone is expected to result in larger, rather than smaller, fractional uptake.

The data for aerosol uptake in the lung (Figure 8) show that for particles of 0.3 μm MMAD, fractional deposition decreased with increasing ventilation rate; in this size range, particle removal is dominated by diffusion, a time-dependent mechanism, and these findings are, therefore, consistent with expectations. The magnitude of deposition both in upper and lower respiratory tracts was, however, much greater than expected. For example, Bennett and Smaldone (1987) showed that for deposition of similarly sized particles in excised lungs at a ventilation rate equivalent to 5 L/min, fractional uptake (or deposition) was between 0.1 and 0.2; in our preparation, fractional uptake was approximately 0.7. Total respiratory system fractional uptake rates for nonhygroscopic particles of 0.25 μm MMAD have been reported in the range of 0.2 to 0.4, as summarized by Stuart (1984). Data summarized by Yu and associates (1981) and modeling by Yu and Xu (1987) for deposition of diesel particles in the heads of humans and rats (particle aerodynamic diameters ranging from 0.2 to 23 μm) suggest that fractional deposition rates for nose breathing should be on the order of 0.03 at 5 L/min if the particle size distribution does not change due to hygroscopic growth.

Ammonium nitrate is a hygroscopic aerosol, and the greater URT deposition observed for it in our study may be due in part to particle growth. At 5 L/min, residence time in the head would be approximately 100 msec (Yu and Xu 1987). Even in that short interval, humidity conditioning within the nasal cavity approaches maximum levels (Hanna and Scherer 1986), which means that relative humidity is approximately 90 to 95 percent. An approximate twofold particle growth for a hygroscopic aerosol in a humidity regime that increases from 80 or 85 percent (relative humid-

Table 5. Comparison of Predicted and Measured Total Respiratory System Uptake[a]

		Regional Uptake		Total Respiratory Uptake		
				Predicted		
Exposure	Mean $\dot{V}_E$	Head	Lung	Case 1	Case 2	Measured
NO_2						
Exercise	10.5	0.51	0.90	0.95	0.98	0.94
Rest after exercise	5.0	0.58	0.90	0.95	0.98	0.77
Rest	3.8	0.63	0.90	0.96	0.99	0.78
HCHO in AMN						
Exercise	10.5	0.95[b]	0.90[b]	0.99	1.00	0.98
Rest after exercise	5.3	0.96[b]	0.90[b]	1.00	1.00	1.00
HCHO alone						
Exercise	9.8	0.95	0.90	0.99	1.00	0.86
Rest after exercise	5.3	0.96	0.90	1.00	1.00	0.92

[a] Regional uptake values were estimated from data in Figures 2, 3, 4, 5, and 6. Measured values for total respiratory uptake of HCHO alone are from Crocker (1984).

[b] Estimated from data for HCHO alone in the present study.

ity range in inspired air) to 90 or 95 percent is a reasonable expectation (Martonen and Clark 1983). Some vaporization of AMN to ammonia and nitric acid vapor at physiological temperature is also possible (Tang 1980). Hygroscopic growth and vaporization losses may partially explain our high deposition measurements for AMN particles in both the URT and lung. Losses in the sampling system may have also contributed to increased deposition. As part of the sampling protocol, we measured losses of AMN particles in the sampling system. These measurements were performed at 85 percent relative humidity and not at 90 to 95 percent; thus, sampling line losses in the expiratory limb of the sampling system may have been underestimated. Finally, the dog's URT may be a more efficient aerosol collector than other mammals used in modeling and excised lung measurements. Given the multiple factors that can affect aerosol deposition, our results provide an upper limit for aerosol deposition in the respiratory tract.

The total respiratory system fractional uptake for NO_2 and HCHO approached 1.0 in exercising dogs. Changes in uptake related to changes in breathing patterns during exercise exposure were not large enough to be distinguished. Therefore, we were unable to confirm our earlier findings with ozone (Kleinman et al. 1983) that as breathing shifts to a rapid and shallow pattern, the fraction of O_3 expired increases, which suggests a protective mechanism for the breathing pattern shift.

In principal, total respiratory system uptake can be predicted from a set of regional measurements of uptake using the relationship of total uptake to uptake in series (such as Equation 4 for the combination of sampling apparatus uptake and respiratory tract uptake). The relationship of total uptake to component uptakes in series is:

$$UT = U_1 + U_2 - U_1U_2 \qquad (6)$$

where UT is total fractional uptake and U_1 and U_2 are component fractional uptakes in series.

Table 5 shows a comparison between the total respiratory system uptake predicted from tracheostomized dog uptake measurements for the URT and lung and the total uptake measured from dogs exercising or standing on the treadmill. Regional uptake measurements were estimated from Figures 2, 3, 4, 5, and 6 as a function of minute ventilation during whole respiratory tract exposures at rest and during exercise (Appendix A, Tables A.3, A.13, and A.23) (Crocker 1984). There are several important assumptions in this analysis. The volume of expired air was modified differently in whole respiratory tract and in regional uptake measurements (exercise vs. CO_2 inhalation), and the analysis assumes that uptake at a given $\dot{V}_E$ was similar in the two treatments (see the Methods section). In this study, total respiratory uptake for HCHO was measured in the presence of AMN particles, whereas regional uptake measurements were performed with HCHO alone. Table 5 shows the estimates for total respiratory system uptake for two sets of uptakes in series representing extreme cases. In case 1, total uptake is the sum of URT uptake in the head followed by uptake in the lung, and any additional uptake or addition of pollutant to expired air passing through the URT is ignored. In case 2, total uptake is a series of three uptakes: uptake by the head on inspiration, followed by uptake by the lung, and, finally, uptake by the head on expiration, which is assumed to be equivalent to inspiratory uptake by the head.

Given the above assumptions, predicted total respiratory system uptake was nearly 1.0 for NO_2 both at rest and during exercise. These predictions agreed with measured values of total uptake during exercise, but not at rest. Uptake measured both during continuous rest and at rest following exercise exposure was much lower than predicted. A lower-than-expected NO_2 uptake could result from one or both of two conditions: (1) significant quantities of NO_2 absorbed by the URT on inspiration are released back into the tidal volume during expiration through the URT; and (2) NO_2 in the respiratory dead space volume in the head at the end of inspiration is not absorbed as effectively as it is from air inspired completely through the head and lungs, and on expiration, head respiratory dead space contributes significant NO_2 to total expired tidal volume. The fact that the disparity between expected and measured total uptake occurred for measurements at rest but not during exercise exposures favors the second possibility. Exercise exposure at a higher $\dot{V}_E$ passes a larger quantity of NO_2 through the URT per unit of time. Because lung fractional uptake was 0.9 both during exercise and at rest (Table 5), saturation of URT surfaces with NO_2 and desorption on expiration should have been more prominent during exercise $\dot{V}_E$ than at resting $\dot{V}_E$, if the first condition were dominant. Resting tidal volumes were relatively small (0.17 L in continuous resting exposures, compared to 0.27 L during exercise). The respiratory dead space of the head was thus a larger fraction of tidal volume at rest than during exercise and played a larger role in affecting expired concentrations of NO_2. This suggests that the second condition is of greater importance.

Formaldehyde, on the other hand, showed greater total respiratory tract uptake at rest than during exercise exposure, which, by the above arguments, is expected for two reasons: (1) HCHO is much more soluble than NO_2, and less of the HCHO deposited in the URT on inspiration will be desorbed during the expiratory phase; and (2) tidal volumes in resting exposures to HCHO and HCHO plus AMN particles were larger than in resting exposures to NO_2, so URT dead space was a smaller fraction of tidal volume. The total

respiratory fractional uptake of HCHO predicted from regional uptake of HCHO alone was approximately 1.0 and agreed with values measured in exposures to the mixture of HCHO and AMN particles (Table 5). However, using the same protocol as that used for HCHO plus AMN particles in this study, total respiratory system uptake measured for HCHO alone in an earlier study (Crocker 1984) was lower than predicted. Presumably, total respiratory tract fractional uptakes of less than 1.0 were due either to expired HCHO from URT desorption or from URT dead space, as discussed above. It is possible that AMN particles may act to shift the inhaled dose distribution of HCHO to the lower respiratory tract, which would result in a greater total respiratory tract uptake than that for HCHO alone. The mechanism by which AMN might shift the dose distribution is unknown, but two possibilities are breathing pattern differences and the absorption of HCHO into liquid AMN particles. As described above, HCHO and HCHO plus AMN particles induced different breathing pattern responses. However, the lower frequency of respiration and lower $\dot{V}_E$ in response to HCHO alone (tidal volumes were similar) compared with the response to HCHO plus AMN particles were expected to increase total uptake rather than to produce the observed decrease. Absorption of HCHO into a liquid phase of AMN particles could have enhanced the transport of HCHO into the lung, shifting respiratory distribution and increasing HCHO uptake in the mixture. Although HCHO was not observed in our analyses of AMN particles collected on filter samples during URT exposures, HCHO in solution would have been lost during the sampling process.

These data can be applied to understanding URT penetration and uptake in humans. We assumed in this study that the ratio of airway surface area to ventilation ($A:\dot{V}$) was a controlling parameter (Equation 2) for determining URT penetration. Among mammalian species, ventilation scales with body mass to the 0.75 power (Stahl 1967), and if anatomical surface area scales with mass to the 0.67 power, the $A:\dot{V}$ ratio is expected to decline only slightly with increasing mass (that is, scale with mass to the -0.08 power). However, the URTs of dogs and humans are specialized structures that do not exhibit the similarities implicit in the simple power functions of allometric scaling. Despite large differences in body mass, the URT surface areas for humans and beagle dogs are similar (Snyder et al. 1975; Griscom and Wohl 1985). Using estimates of URT surface area in beagle dogs and humans, based upon reported data of Snyder and coworkers (1975) and Schreider and Raabe (1981), an 11-kg beagle dog has an estimated $\dot{V}_E$ of 2.6 L/min (Stahl 1967), a nasal passage surface area of 205 cm^2, and an $A:\dot{V}$ ratio of 78.8; a 73-kg human has an estimated $\dot{V}_E$ of 11.7 L/min, a nasal passage surface area of 181 cm^2, and an $A:\dot{V}$ ratio of 15.5. The dog $A:\dot{V}$ ratio exceeds that of a human by approximately a factor of five. It can be predicted from Equation 2 that dogs will have higher URT fractional uptake than humans. There are as yet no URT uptake data for humans exposed to NO_2; however, Gerrity and associates (1988) measured URT fractional uptake for O_3 of 0.40. Nitrogen dioxide and O_3 are similar in diffusivity and solubility, and our measurements for dogs breathing nasally at rest ($\dot{V}_E$ approximately 2 L/min) (Figure 2) show a fractional uptake for NO_2 of approximately 0.76. As expected from the large difference in $A:\dot{V}$ ratios at rest, the URT uptake is higher for dogs than for humans. Furthermore, if the beagle dog were breathing with an $A:\dot{V}$ ratio similar to the resting human ratio of 15.5, that would require a minute ventilation of 13.3 L/min, and the fractional uptake for NO_2 (Figure 2) would approach the values measured for O_3 in humans at rest (Gerrity et al. 1988). Although the absence of data on identical compounds makes this analysis only suggestive, the large differences in $A:\dot{V}$ ratios correspond to the expected direction of difference in fractional uptake of these oxidants in humans and dogs. This suggests that these kinds of comparisons should be explored further for different pollutant compounds in different mammalian species.

CONCLUSIONS

The results of this study provide quantitative information on penetration and deposition of air pollutants in the respiratory system. These data are also a base for the further development and validation of models to relate pollutant exposure under various levels of respiratory ventilation to pollutant deposition in the respiratory tract. These models, in turn, will improve assessments of human doses and the relationships of those doses to observed health effects. The following conclusions can be drawn:

1. Pollutant penetration of inhaled gases is greater during mouth breathing than during nose breathing, and penetration increases with increasing ventilation rate. Penetration is dependent upon the water solubility of the gas; less soluble gases penetrate more readily. The model proposed by Kleinman (1984) for SO_2 penetration applied reasonably well to data for HCHO and NO_2.
2. Total respiratory system uptake during exercise was predicted reasonably well from the regional uptake data. Total uptake measurements during rest exposure were less well predicted and suggest that uptake models should consider the fate of the inhaled material within the anatomical dead space of the URT.
3. Total respiratory system uptake of HCHO increased in the presence of AMN particles. However, the increase in

uptake was not accompanied by an increase in physiological response, as was found in HCHO exposures alone. A shift in the distribution of inhaled HCHO from the upper to the lower respiratory tract may have accounted for the observed differences in uptake and response.

4. The dogs used in the study were similar to one another in size and provided a relatively homogeneous sample population, which was necessary for obtaining adequate determinations of regional penetration and respiratory system uptake. Respiratory physiological responses to the pollutant were small and consistent among individuals, and the differences in uptake observed among individuals were not greater than the variation due to other experimental factors. We were, therefore, unable to prove whether or not individual differences in physiological responses to inhaled pollutants in our study were related to differences in regional pollutant distribution and uptake.

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APPENDIX A. Summary Tables of Pulmonary Function in Dogs Exposed to Pollutant Atmospheres at Rest and During Exercise

Table A.1. Breath Time[a] in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	2.41	1.44	6	Clean air	1.95	0.10	5
2	Run 5 km/hour 7.5% grade	Clean air	1.45	0.34	6	Clean air	1.57	0.36	6
3	Run 5 km/hour 7.5% grade	Clean air	1.48	0.34	6	NO_2	1.53	0.36	6
4	Run 5 km/hour 7.5% grade	Clean air	1.50	0.42	6	NO_2	1.60	0.36	6
5	Run 5 km/hour 7.5% grade	Clean air	1.62	0.36	6	NO_2	1.61	0.41	6
6	Run 5 km/hour 7.5% grade	Clean air	1.64	0.43	6	NO_2	1.60	0.51	6
7	Run 5 km/hour 7.5% grade	Clean air	1.58	0.42	6	NO_2	1.59	0.44	6
8	Run 5 km/hour 7.5% grade	Clean air	1.50	0.36	6	NO_2	1.49	0.34	6
9	Rest	Clean air	2.28	1.14	6	NO_2	2.36	1.13	6

[a] Data are given in seconds.

Table A.2. Expired Tidal Volume in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.17	0.05	6	Clean air	0.16	0.04	5
2	Run 5 km/hour 7.5% grade	Clean air	0.26	0.05	6	Clean air	0.28	0.05	6
3	Run 5 km/hour 7.5% grade	Clean air	0.26	0.04	6	NO_2	0.27	0.05	6
4	Run 5 km/hour 7.5% grade	Clean air	0.26	0.05	6	NO_2	0.28	0.05	6
5	Run 5 km/hour 7.5% grade	Clean air	0.28	0.04	6	NO_2	0.28	0.05	6
6	Run 5 km/hour 7.5% grade	Clean air	0.27	0.05	6	NO_2	0.27	0.06	6
7	Run 5 km/hour 7.5% grade	Clean air	0.27	0.05	6	NO_2	0.26	0.04	6
8	Run 5 km/hour 7.5% grade	Clean air	0.27	0.06	6	NO_2	0.25	0.03	6
9	Rest	Clean air	0.19	0.04	6	NO_2	0.19	0.03	6

[a] Values are given in liters under BTPS conditions.

Table A.3. Minute Ventilation in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	5.62	2.81	6	Clean air	5.81	2.81	5
2	Run 5 km/hour 7.5% grade	Clean air	10.61	0.70	6	Clean air	10.88	0.98	6
3	Run 5 km/hour 7.5% grade	Clean air	10.71	1.66	6	NO_2	10.73	1.08	6
4	Run 5 km/hour 7.5% grade	Clean air	10.83	2.04	6	NO_2	10.69	0.66	6
5	Run 5 km/hour 7.5% grade	Clean air	10.39	1.09	6	NO_2	10.45	1.06	6
6	Run 5 km/hour 7.5% grade	Clean air	10.10	1.02	6	NO_2	10.43	1.38	6
7	Run 5 km/hour 7.5% grade	Clean air	10.56	1.45	6	NO_2	10.25	1.53	6
8	Run 5 km/hour 7.5% grade	Clean air	10.84	1.79	6	NO_2	10.32	1.36	6
9	Rest	Clean air	5.57	1.59	6	NO_2	5.04	1.34	6

[a] Values are given in liters per minute under BTPS conditions.

Table A.4. Fractional Expiration Time[a] in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.489	0.043	6	Clean air	0.485	0.052	5
2	Run 5 km/hour 7.5% grade	Clean air	0.461	0.078	6	Clean air	0.458	0.065	6
3	Run 5 km/hour 7.5% grade	Clean air	0.463	0.071	6	NO_2	0.460	0.052	6
4	Run 5 km/hour 7.5% grade	Clean air	0.450	0.070	6	NO_2	0.455	0.069	6
5	Run 5 km/hour 7.5% grade	Clean air	0.464	0.075	6	NO_2	0.463	0.068	6
6	Run 5 km/hour 7.5% grade	Clean air	0.469	0.070	6	NO_2	0.464	0.071	6
7	Run 5 km/hour 7.5% grade	Clean air	0.455	0.071	6	NO_2	0.468	0.065	6
8	Run 5 km/hour 7.5% grade	Clean air	0.454	0.058	6	NO_2	0.462	0.056	6
9	Rest	Clean air	0.507	0.043	6	NO_2	0.492	0.018	6

[a] Fractional expiration time is the ratio of expiration time to total breath time.

Table A.5. Oxygen Consumption in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.14	0.03	6	Clean air	0.15	0.04	5
2	Run 5 km/hour 7.5% grade	Clean air	0.33	0.04	6	Clean air	0.39	0.02	6
3	Run 5 km/hour 7.5% grade	Clean air	0.36	0.05	6	NO_2	0.40	0.02	6
4	Run 5 km/hour 7.5% grade	Clean air	0.40	0.09	6	NO_2	0.39	0.03	6
5	Run 5 km/hour 7.5% grade	Clean air	0.37	0.04	6	NO_2	0.39	0.03	6
6	Run 5 km/hour 7.5% grade	Clean air	0.35	0.06	6	NO_2	0.38	0.05	6
7	Run 5 km/hour 7.5% grade	Clean air	0.36	0.07	6	NO_2	0.37	0.04	6
8	Run 5 km/hour 7.5% grade	Clean air	0.37	0.09	5	NO_2	0.37	0.04	6
9	Rest	Clean air	0.16	0.03	6	NO_2	0.17	0.04	6

[a] Values are given in liters per minute under STPD conditions.

Table A.6. Carbon Dioxide Production in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.12	0.03	6	Clean air	0.13	0.04	5
2	Run 5 km/hour 7.5% grade	Clean air	0.28	0.04	6	Clean air	0.32	0.02	6
3	Run 5 km/hour 7.5% grade	Clean air	0.29	0.04	6	NO_2	0.32	0.02	6
4	Run 5 km/hour 7.5% grade	Clean air	0.27	0.07	6	NO_2	0.31	0.02	6
5	Run 5 km/hour 7.5% grade	Clean air	0.29	0.04	6	NO_2	0.30	0.03	6
6	Run 5 km/hour 7.5% grade	Clean air	0.27	0.04	6	NO_2	0.29	0.04	6
7	Run 5 km/hour 7.5% grade	Clean air	0.28	0.05	6	NO_2	0.29	0.04	6
8	Run 5 km/hour 7.5% grade	Clean air	0.29	0.07	5	NO_2	0.28	0.03	6
9	Rest	Clean air	0.12	0.02	6	NO_2	0.12	0.03	6

[a] Values are given in liters per minute under STPD conditions.

Table A.7. Ventilation Equivalent for Oxygen in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.88	0.30	6	Clean air	0.87	0.17	5
2	Run 5 km/hour 7.5% grade	Clean air	0.73	0.12	6	Clean air	0.63	0.03	6
3	Run 5 km/hour 7.5% grade	Clean air	0.68	0.09	6	NO_2	0.61	0.04	6
4	Run 5 km/hour 7.5% grade	Clean air	0.72	0.20	6	NO_2	0.61	0.05	6
5	Run 5 km/hour 7.5% grade	Clean air	0.63	0.08	6	NO_2	0.61	0.03	6
6	Run 5 km/hour 7.5% grade	Clean air	0.66	0.09	6	NO_2	0.62	0.04	6
7	Run 5 km/hour 7.5% grade	Clean air	0.67	0.09	6	NO_2	0.62	0.03	6
8	Run 5 km/hour 7.5% grade	Clean air	0.66	0.08	5	NO_2	0.63	0.03	6
9	Rest	Clean air	0.78	0.15	6	NO_2	0.73	0.15	6

[a] Values are expressed as the ratio of air expired per minute to oxygen consumed per minute in liters per millimole under BTPS conditions.

Table A.8. Ventilation Equivalent for Carbon Dioxide in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	1.04	0.32	6	Clean air	1.01	0.22	5
2	Run 5 km/hour 7.5% grade	Clean air	0.89	0.17	6	Clean air	0.77	0.03	6
3	Run 5 km/hour 7.5% grade	Clean air	0.84	0.10	6	NO_2	0.76	0.03	6
4	Run 5 km/hour 7.5% grade	Clean air	0.94	0.28	6	NO_2	0.78	0.06	6
5	Run 5 km/hour 7.5% grade	Clean air	0.82	0.11	6	NO_2	0.78	0.05	6
6	Run 5 km/hour 7.5% grade	Clean air	0.85	0.12	6	NO_2	0.80	0.06	6
7	Run 5 km/hour 7.5% grade	Clean air	0.87	0.10	6	NO_2	0.81	0.04	6
8	Run 5 km/hour 7.5% grade	Clean air	0.86	0.11	5	NO_2	0.82	0.03	6
9	Rest	Clean air	1.02	0.17	6	NO_2	0.78	0.18	6

[a] Values are expressed as the ratio of air expired per minute to carbon dioxide produced per minute in liters per millimole of CO_2 under BTPS conditions.

Table A.9. Pulmonary Resistance in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.37	0.27	4	Clean air	0.29	0.05	3
2	Run 5 km/hour 7.5% grade	Clean air	0.24	0.09	5	Clean air	0.28	0.10	5
3	Run 5 km/hour 7.5% grade	Clean air	0.24	0.08	5	NO_2	0.27	0.10	5
4	Run 5 km/hour 7.5% grade	Clean air	0.25	0.09	5	NO_2	0.27	0.11	5
5	Run 5 km/hour 7.5% grade	Clean air	0.24	0.08	5	NO_2	0.27	0.12	5
6	Run 5 km/hour 7.5% grade	Clean air	0.24	0.10	5	NO_2	0.25	0.11	5
7	Run 5 km/hour 7.5% grade	Clean air	0.26	0.12	4	NO_2	0.25	0.12	5
8	Run 5 km/hour 7.5% grade	Clean air	0.26	0.11	4	NO_2	0.26	0.10	5
9	Rest	Clean air	0.19	0.08	5	NO_2	0.23	0.08	5

[a] Values are given in centimeters of water × minutes per liter under BTPS conditions.

Table A.10. Dynamic Compliance in Exercising Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Exercise Condition	Clean Air Control Day				NO_2 Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.039	0.005	4	Clean air	0.031	0.008	3
2	Run 5 km/hour 7.5% grade	Clean air	0.034	0.011	5	Clean air	0.035	0.009	5
3	Run 5 km/hour 7.5% grade	Clean air	0.034	0.011	5	NO_2	0.035	0.010	5
4	Run 5 km/hour 7.5% grade	Clean air	0.032	0.010	5	NO_2	0.034	0.009	5
5	Run 5 km/hour 7.5% grade	Clean air	0.031	0.010	5	NO_2	0.034	0.007	5
6	Run 5 km/hour 7.5% grade	Clean air	0.031	0.010	5	NO_2	0.033	0.008	5
7	Run 5 km/hour 7.5% grade	Clean air	0.030	0.008	5	NO_2	0.032	0.007	5
8	Run 5 km/hour 7.5% grade	Clean air	0.034	0.009	4	NO_2	0.032	0.007	5
9	Rest	Clean air	0.048	0.014	4	NO_2	0.046	0.017	5

[a] Values are given in liters per centimeter of water under BTPS conditions.

Table A.11. Breath Time[a] in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	2.89	1.05	5	Clean air	2.72	0.65	5
2	Clean air	2.91	0.86	5	Clean air	2.75	0.77	5
3	Clean air	3.07	0.92	5	NO_2	2.82	0.83	5
4	Clean air	3.10	0.72	5	NO_2	2.74	0.89	5
5	Clean air	3.22	0.99	5	NO_2	2.67	0.82	5
6	Clean air	3.29	0.80	5	NO_2	2.86	0.58	5
7	Clean air	3.29	0.73	5	NO_2	2.87	1.01	5
8	Clean air	3.02	0.75	5	NO_2	3.09	1.07	5

[a] Values are given in seconds.

Table A.12. Expired Tidal Volume in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.17	0.02	5	Clean air	0.16	0.01	5
2	Clean air	0.16	0.01	5	Clean air	0.16	0.01	5
3	Clean air	0.17	0.02	5	NO_2	0.17	0.02	5
4	Clean air	0.18	0.02	5	NO_2	0.17	0.01	5
5	Clean air	0.19	0.04	5	NO_2	0.17	0.02	5
6	Clean air	0.17	0.01	5	NO_2	0.17	0.02	5
7	Clean air	0.18	0.02	5	NO_2	0.16	0.01	5
8	Clean air	0.18	0.01	5	NO_2	0.17	0.02	5

[a] Values are given in liters under BTPS conditions.

Table A.13. Minute Ventilation in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	3.77	1.02	5	Clean air	3.67	0.83	5
2	Clean air	3.60	0.93	5	Clean air	3.76	0.99	5
3	Clean air	3.61	0.79	5	NO_2	3.80	1.04	5
4	Clean air	3.61	0.69	5	NO_2	3.91	0.98	5
5	Clean air	3.69	0.84	5	NO_2	3.95	0.85	5
6	Clean air	3.39	0.72	5	NO_2	3.64	0.81	5
7	Clean air	3.39	0.75	5	NO_2	3.75	1.08	5
8	Clean air	3.71	0.85	5	NO_2	3.65	0.90	5

[a] Values are given in liters per minute under BTPS conditions.

Table A.14. Fractional Expiration Time[a] in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.491	0.062	5	Clean air	0.480	0.041	5
2	Clean air	0.483	0.063	5	Clean air	0.492	0.049	5
3	Clean air	0.482	0.057	5	NO_2	0.488	0.038	5
4	Clean air	0.494	0.037	5	NO_2	0.487	0.035	5
5	Clean air	0.494	0.050	5	NO_2	0.518	0.046	5
6	Clean air	0.455	0.057	5	NO_2	0.478	0.027	5
7	Clean air	0.458	0.041	5	NO_2	0.485	0.054	5
8	Clean air	0.490	0.049	5	NO_2	0.465	0.080	5

[a] Fractional expiration time is the ratio of expiration time to total breath time.

Table A.15. Oxygen Consumption in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.088	0.022	5	Clean air	0.091	0.018	5
2	Clean air	0.087	0.016	5	Clean air	0.093	0.020	5
3	Clean air	0.087	0.015	5	NO_2	0.099	0.027	5
4	Clean air	0.092	0.011	5	NO_2	0.096	0.017	5
5	Clean air	0.090	0.020	5	NO_2	0.092	0.017	5
6	Clean air	0.080	0.022	5	NO_2	0.087	0.015	5
7	Clean air	0.084	0.017	5	NO_2	0.097	0.024	5
8	Clean air	0.092	0.019	5	NO_2	0.094	0.020	5

[a] Values are given in liters per minute under STPD conditions.

Table A.16. Carbon Dioxide Production in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.077	0.021	5	Clean air	0.077	0.019	5
2	Clean air	0.077	0.018	5	Clean air	0.078	0.018	5
3	Clean air	0.076	0.016	5	NO_2	0.082	0.025	5
4	Clean air	0.080	0.013	5	NO_2	0.082	0.017	5
5	Clean air	0.079	0.022	5	NO_2	0.077	0.015	5
6	Clean air	0.071	0.022	5	NO_2	0.074	0.015	5
7	Clean air	0.073	0.020	5	NO_2	0.080	0.022	5
8	Clean air	0.080	0.020	5	NO_2	0.077	0.018	5

[a] Values are given in liters per minute under STPD conditions.

Table A.17. Ventilation Equivalent for Oxygen in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.96	0.12	5	Clean air	0.90	0.04	5
2	Clean air	0.92	0.09	5	Clean air	0.90	0.11	5
3	Clean air	0.93	0.17	5	NO_2	0.87	0.14	5
4	Clean air	0.88	0.09	5	NO_2	0.90	0.11	5
5	Clean air	0.92	0.13	5	NO_2	0.96	0.08	5
6	Clean air	0.96	0.08	5	NO_2	0.93	0.07	5
7	Clean air	0.91	0.12	5	NO_2	0.86	0.12	5
8	Clean air	0.91	0.17	5	NO_2	0.87	0.10	5

[a] Values are given in liters per millimole of O_2 under BTPS conditions.

Table A.18. Ventilation Equivalent for Carbon Dioxide in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	1.11	0.13	5	Clean air	1.07	0.04	5
2	Clean air	1.04	0.09	5	Clean air	1.08	0.10	5
3	Clean air	1.08	0.17	5	NO_2	1.04	0.16	5
4	Clean air	1.01	0.10	5	NO_2	1.07	0.10	5
5	Clean air	1.07	0.13	5	NO_2	1.15	0.08	5
6	Clean air	1.11	0.16	5	NO_2	1.11	0.06	5
7	Clean air	1.06	0.16	5	NO_2	1.05	0.12	5
8	Clean air	1.06	0.21	5	NO_2	1.06	0.11	5

[a] Values are given in liters per millimole of CO_2 under BTPS conditions.

Table A.19. Pulmonary Resistance in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.30	0.17	4	Clean air	0.28	0.15	4
2	Clean air	0.30	0.15	4	Clean air	0.30	0.15	4
3	Clean air	0.32	0.13	4	NO_2	0.32	0.17	4
4	Clean air	0.32	0.13	4	NO_2	0.35	0.23	4
5	Clean air	0.36	0.19	4	NO_2	0.33	0.19	4
6	Clean air	0.37	0.17	4	NO_2	0.37	0.21	4
7	Clean air	0.41	0.21	4	NO_2	0.40	0.26	4
8	Clean air	0.40	0.19	4	NO_2	0.35	0.21	4

[a] Values are given in centimeters of water × minutes per liter.

Table A.20. Dynamic Compliance in Resting Dogs Exposed to 5 ppm Nitrogen Dioxide[a]

Data Collection Period	Clean Air Control Day				NO_2 Exposure Day			
	Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Clean air	0.0470	0.0146	4	Clean air	0.0432	0.0069	4
2	Clean air	0.0475	0.0123	4	Clean air	0.0428	0.0083	4
3	Clean air	0.0517	0.0204	4	NO_2	0.0466	0.0103	4
4	Clean air	0.0488	0.0135	4	NO_2	0.0489	0.0107	4
5	Clean air	0.0568	0.0171	4	NO_2	0.0488	0.0092	4
6	Clean air	0.0514	0.0155	4	NO_2	0.0505	0.0105	4
7	Clean air	0.0480	0.0128	4	NO_2	0.0524	0.0183	4
8	Clean air	0.0487	0.0097	4	NO_2	0.0567	0.0186	4

[a] Values are given in liters per centimeter of water under BTPS conditions.

Table A.21. Breath Time[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	2.32	0.78	5	Clean air	2.44	0.75	6
2	Run 5 km/hour 7.5% grade	Clean air	1.66	0.23	6	Clean air	1.57	0.31	6
3	Run 5 km/hour 7.5% grade	Clean air	1.64	0.26	6	HCHO + AMN	1.68	0.27	6
4	Run 5 km/hour 7.5% grade	Clean air	1.65	0.32	6	HCHO + AMN	1.68	0.34	6
5	Run 5 km/hour 7.5% grade	Clean air	1.63	0.32	6	HCHO + AMN	1.66	0.37	6
6	Run 5 km/hour 7.5% grade	Clean air	1.56	0.33	6	HCHO + AMN	1.66	0.45	6
7	Run 5 km/hour 7.5% grade	Clean air	1.66	0.34	6	HCHO + AMN	1.62	0.39	6
8	Run 5 km/hour 7.5% grade	Clean air	1.68	0.45	6	HCHO + AMN	1.58	0.46	6
9	Rest	Clean air	2.78	1.00	6	HCHO + AMN	2.71	1.39	6

[a] Values are given in seconds.

Table A.22. Expired Tidal Volume[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.144	0.030	5	Clean air	0.175	0.028	6
2	Run 5 km/hour 7.5% grade	Clean air	0.258	0.073	6	Clean air	0.286	0.083	6
3	Run 5 km/hour 7.5% grade	Clean air	0.271	0.090	6	HCHO + AMN	0.298	0.083	6
4	Run 5 km/hour 7.5% grade	Clean air	0.274	0.074	6	HCHO + AMN	0.293	0.089	6
5	Run 5 km/hour 7.5% grade	Clean air	0.268	0.076	6	HCHO + AMN	0.299	0.090	6
6	Run 5 km/hour 7.5% grade	Clean air	0.255	0.070	6	HCHO + AMN	0.299	0.104	6
7	Run 5 km/hour 7.5% grade	Clean air	0.274	0.081	6	HCHO + AMN	0.294	0.090	6
8	Run 5 km/hour 7.5% grade	Clean air	0.263	0.083	6	HCHO + AMN	0.274	0.094	6
9	Rest	Clean air	0.203	0.061	6	HCHO + AMN	0.211	0.072	6

[a] Values are given in liters under BTPS conditions.

Table A.23. Minute Ventilation[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	4.03	1.02	5	Clean air	4.63	1.39	6
2	Run 5 km/hour 7.5% grade	Clean air	9.25	1.87	6	Clean air	10.83	2.62	6
3	Run 5 km/hour 7.5% grade	Clean air	9.78	2.25	6	HCHO + AMN	10.54	2.02	6
4	Run 5 km/hour 7.5% grade	Clean air	10.03	2.29	6	HCHO + AMN	10.31	1.82	6
5	Run 5 km/hour 7.5% grade	Clean air	9.79	1.61	6	HCHO + AMN	10.63	1.32	6
6	Run 5 km/hour 7.5% grade	Clean air	9.71	1.42	6	HCHO + AMN	10.59	1.67	6
7	Run 5 km/hour 7.5% grade	Clean air	9.80	1.62	6	HCHO + AMN	10.77	1.20	6
8	Run 5 km/hour 7.5% grade	Clean air	9.35	1.33	6	HCHO + AMN	10.33	0.73	6
9	Rest	Clean air	4.62	1.58	6	HCHO + AMN	5.28	1.47	6

[a] Values are given in liters per minute under BTPS conditions.

Table A.24. Fractional Expiration Time[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.473	0.043	5	Clean air	0.476	0.034	6
2	Run 5 km/hour 7.5% grade	Clean air	0.454	0.027	6	Clean air	0.435	0.029	6
3	Run 5 km/hour 7.5% grade	Clean air	0.431	0.041	6	HCHO + AMN	0.437	0.024	6
4	Run 5 km/hour 7.5% grade	Clean air	0.437	0.042	6	HCHO + AMN	0.449	0.034	6
5	Run 5 km/hour 7.5% grade	Clean air	0.440	0.036	6	HCHO + AMN	0.455	0.037	6
6	Run 5 km/hour 7.5% grade	Clean air	0.435	0.044	6	HCHO + AMN	0.447	0.041	6
7	Run 5 km/hour 7.5% grade	Clean air	0.441	0.040	6	HCHO + AMN	0.440	0.043	6
8	Run 5 km/hour 7.5% grade	Clean air	0.459	0.052	6	HCHO + AMN	0.442	0.041	6
9	Rest	Clean air	0.491	0.023	6	HCHO + AMN	0.493	0.024	6

[a] Fractional expiration time is the ratio of expiration time to total breath time.

Table A.25. Peak Inspiratory Flow Rate[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	15.29	2.20	5	Clean air	16.80	3.58	6
2	Run 5 km/hour 7.5% grade	Clean air	27.94	6.89	6	Clean air	30.04	7.21	6
3	Run 5 km/hour 7.5% grade	Clean air	28.49	7.30	6	HCHO + AMN	29.10	7.41	6
4	Run 5 km/hour 7.5% grade	Clean air	29.11	8.47	6	HCHO + AMN	29.41	6.42	6
5	Run 5 km/hour 7.5% grade	Clean air	29.11	7.53	6	HCHO + AMN	29.80	5.59	6
6	Run 5 km/hour 7.5% grade	Clean air	29.06	8.92	6	HCHO + AMN	29.82	6.34	6
7	Run 5 km/hour 7.5% grade	Clean air	29.42	7.65	6	HCHO + AMN	30.39	7.16	6
8	Run 5 km/hour 7.5% grade	Clean air	29.35	7.49	6	HCHO + AMN	29.79	6.42	6
9	Rest	Clean air	16.99	1.98	6	HCHO + AMN	18.25	3.62	6

[a] Values are given in liters per minute under BTPS conditions.

Table A.26. Peak Expiratory Flow Rates[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	12.28	3.38	5	Clean air	14.00	4.54	6
2	Run 5 km/hour 7.5% grade	Clean air	34.51	10.60	6	Clean air	38.79	14.68	6
3	Run 5 km/hour 7.5% grade	Clean air	37.95	13.42	6	HCHO + AMN	38.60	11.61	6
4	Run 5 km/hour 7.5% grade	Clean air	37.51	13.25	6	HCHO + AMN	36.11	9.55	6
5	Run 5 km/hour 7.5% grade	Clean air	34.99	7.47	6	HCHO + AMN	35.46	6.52	6
6	Run 5 km/hour 7.5% grade	Clean air	36.05	8.04	6	HCHO + AMN	37.90	8.82	6
7	Run 5 km/hour 7.5% grade	Clean air	35.98	9.19	6	HCHO + AMN	37.97	7.79	6
8	Run 5 km/hour 7.5% grade	Clean air	33.29	8.21	6	HCHO + AMN	35.10	4.60	6
9	Rest	Clean air	13.10	3.58	6	HCHO + AMN	14.51	2.98	6

[a] Values are given in liters per minute under BTPS conditions.

Table A.27. Respiratory Exchange Ratio[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.841	0.073	5	Clean air	0.805	0.058	6
2	Run 5 km/hour 7.5% grade	Clean air	0.798	0.030	6	Clean air	0.792	0.069	6
3	Run 5 km/hour 7.5% grade	Clean air	0.791	0.015	6	HCHO + AMN	0.796	0.079	6
4	Run 5 km/hour 7.5% grade	Clean air	0.781	0.022	6	HCHO + AMN	0.773	0.057	6
5	Run 5 km/hour 7.5% grade	Clean air	0.778	0.027	6	HCHO + AMN	0.761	0.048	6
6	Run 5 km/hour 7.5% grade	Clean air	0.774	0.023	6	HCHO + AMN	0.774	0.064	6
7	Run 5 km/hour 7.5% grade	Clean air	0.757	0.023	6	HCHO + AMN	0.768	0.066	6
8	Run 5 km/hour 7.5% grade	Clean air	0.757	0.023	6	HCHO + AMN	0.767	0.081	6
9	Rest	Clean air	0.753	0.025	6	HCHO + AMN	0.757	0.083	5

[a] Values are expressed as the ratio of the volume of carbon dioxide produced per minute to the volume of oxygen consumed per minute ($\dot{V}_{CO_2}/\dot{V}_{O_2}$).

Table A.28. Carbon Dioxide Production[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.080	0.004	5	Clean air	0.101	0.022	6
2	Run 5 km/hour 7.5% grade	Clean air	0.253	0.080	6	Clean air	0.262	0.064	6
3	Run 5 km/hour 7.5% grade	Clean air	0.267	0.090	6	HCHO + AMN	0.260	0.072	6
4	Run 5 km/hour 7.5% grade	Clean air	0.269	0.088	6	HCHO + AMN	0.254	0.065	6
5	Run 5 km/hour 7.5% grade	Clean air	0.256	0.075	6	HCHO + AMN	0.258	0.073	6
6	Run 5 km/hour 7.5% grade	Clean air	0.261	0.064	6	HCHO + AMN	0.250	0.074	6
7	Run 5 km/hour 7.5% grade	Clean air	0.255	0.064	6	HCHO + AMN	0.245	0.073	6
8	Run 5 km/hour 7.5% grade	Clean air	0.242	0.066	6	HCHO + AMN	0.232	0.068	6
9	Rest	Clean air	0.112	0.027	6	HCHO + AMN	0.111	0.010	5

[a] Values are expressed as the volume of carbon dioxide produced in liters per minute uuder STPD conditions.

Table A.29. Oxygen Consumption[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.095	0.006	5	Clean air	0.125	0.027	6
2	Run 5 km/hour 7.5% grade	Clean air	0.320	0.113	6	Clean air	0.337	0.108	6
3	Run 5 km/hour 7.5% grade	Clean air	0.340	0.120	6	HCHO + AMN	0.333	0.119	6
4	Run 5 km/hour 7.5% grade	Clean air	0.346	0.121	6	HCHO + AMN	0.334	0.109	6
5	Run 5 km/hour 7.5% grade	Clean air	0.333	0.110	6	HCHO + AMN	0.344	0.118	6
6	Run 5 km/hour 7.5% grade	Clean air	0.339	0.091	6	HCHO + AMN	0.331	0.124	6
7	Run 5 km/hour 7.5% grade	Clean air	0.338	0.095	6	HCHO + AMN	0.326	0.119	6
8	Run 5 km/hour 7.5% grade	Clean air	0.322	0.095	6	HCHO + AMN	0.313	0.125	6
9	Rest	Clean air	0.149	0.040	6	HCHO + AMN	0.148	0.013	5

[a] Values are expressed as the volume of oxygen consumed in liters per minute under STPD conditions.

Table A.30. Ventilation Equivalent for Oxygen[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	0.977	0.224	5	Clean air	0.848	0.163	6
2	Run 5 km/hour 7.5% grade	Clean air	0.687	0.135	6	Clean air	0.768	0.222	6
3	Run 5 km/hour 7.5% grade	Clean air	0.676	0.111	6	HCHO + AMN	0.751	0.183	6
4	Run 5 km/hour 7.5% grade	Clean air	0.682	0.108	6	HCHO + AMN	0.726	0.146	6
5	Run 5 km/hour 7.5% grade	Clean air	0.696	0.137	6	HCHO + AMN	0.739	0.161	6
6	Run 5 km/hour 7.5% grade	Clean air	0.668	0.113	6	HCHO + AMN	0.794	0.224	6
7	Run 5 km/hour 7.5% grade	Clean air	0.669	0.082	6	HCHO + AMN	0.814	0.247	6
8	Run 5 km/hour 7.5% grade	Clean air	0.676	0.105	6	HCHO + AMN	0.842	0.305	6
9	Rest	Clean air	0.704	0.058	6	HCHO + AMN	0.854	0.398	5

[a] Values are expressed as the ratio of air expired per minute to oxygen consumed per minute in liters per millimole under BTPS conditions.

Table A.31. Ventilation Equivalent for Carbon Dioxide[a] in Exercising Dogs Exposed to 10 ppm Formaldehyde Plus Ammonium Nitrate Particles at 1 mg/m^3

Data Collection Period	Exercise Condition	Clean Air Control Day				HCHO Plus AMN Exposure Day			
		Exposure	Mean	SD	*n*	Exposure	Mean	SD	*n*
1	Rest	Clean air	1.17	0.26	5	Clean air	1.05	0.17	6
2	Run 5 km/hour 7.5% grade	Clean air	0.86	0.15	6	Clean air	0.96	0.21	6
3	Run 5 km/hour 7.5% grade	Clean air	0.85	0.13	6	HCHO + AMN	0.94	0.16	6
4	Run 5 km/hour 7.5% grade	Clean air	0.89	0.13	6	HCHO + AMN	0.93	0.14	6
5	Run 5 km/hour 7.5% grade	Clean air	0.89	0.15	6	HCHO + AMN	0.97	0.16	6
6	Run 5 km/hour 7.5% grade	Clean air	0.86	0.14	6	HCHO + AMN	1.01	0.22	6
7	Run 5 km/hour 7.5% grade	Clean air	0.88	0.10	6	HCHO + AMN	1.04	0.24	6
8	Run 5 km/hour 7.5% grade	Clean air	0.89	0.13	6	HCHO + AMN	1.07	0.28	6
9	Rest	Clean air	0.94	0.09	6	HCHO + AMN	1.09	0.35	5

[a] Values are expressed as the ratio of air expired per minute to carbon dioxide produced per minute in liters per millimole of CO_2 nnder BTPS conditions.

APPENDIX B. Measurements of Respiratory System Uptake and Penetration of Gases and Aerosols in Tracheostomized Beagle Dogs

Table B.1. Uptake of 1 ppm Nitrogen Dioxide in the Lung as a Function of Ventilation Rate[a]

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake
8/12/85	9	51.00	0.11	0.00	5.51	0.90
		60.00	0.11	2.10	6.60	0.87
		51.00	0.17	3.90	8.42	0.92
		51.00	0.23	5.50	11.88	0.94
5/14/86	9	12.70	0.11	0.00	1.40	1.11
		18.90	0.22	3.40	4.16	0.85
		33.00	0.29	6.10	9.57	0.79
3/18/86	10	45.50	0.38	5.80	17.29	0.75
		14.10	0.08	0.00	1.13	1.08
		20.80	0.18	3.80	3.74	0.85
4/10/86	10	18.00	0.20	0.00	3.60	0.87
		17.30	0.23	4.00	3.98	0.85
		27.80	0.29	6.00	8.06	0.84
		19.50	0.26	3.80	5.07	0.82
5/22/86	11	16.50	0.12	0.00	1.98	1.08
		20.00	0.15	2.30	3.00	1.04
		19.60	0.25	4.20	4.90	0.95
		18.70	0.30	6.10	5.61	0.94
		15.30	0.20	2.30	3.06	0.97
		16.80	0.15	0.00	2.52	0.99
5/27/86	11	21.50	0.19	0.00	4.09	0.88
		16.90	0.23	2.00	3.89	0.87
		15.00	0.27	4.00	4.05	0.86
		21.00	0.30	6.00	6.30	0.93
		27.50	0.48	7.90	13.20	0.94
10/1/86	12	23.70	0.14	0.00	3.32	0.92
		22.00	0.16	1.90	3.52	0.94
		23.70	0.24	4.00	5.69	0.85
		37.30	0.36	6.00	13.43	0.93
11/20/86	13	36.50	0.09	0.00	3.28	0.87
		45.40	0.11	2.20	4.99	0.88
		19.20	0.13	3.80	2.50	0.90
		46.30	0.14	3.80	6.48	0.95
		61.60	0.23	5.80	14.17	0.92
		15.20	0.09	0.20	1.37	0.97
4/14/87	14	39.00	0.11	2.20	4.29	0.86
		50.50	0.17	5.70	8.59	0.72

[a] Uptake is corrected for apparatus dead space.

Table B.2. Uptake of 5 ppm Nitrogen Dioxide in the Lung as a Function of Ventilation Rate[a]

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake
5/14/85	9	14.00	0.10	0.00	1.40	1.16
		26.00	0.20	4.00	5.20	0.93
		38.30	0.31	6.50	11.87	0.92
8/12/85	9	39.00	0.16	0.00	6.08	0.93
		39.00	0.17	2.00	6.63	0.84
		48.00	0.21	3.70	9.98	0.94
		52.00	0.27	5.50	13.78	0.97
3/18/86	10	20.30	0.23	5.70	4.67	0.87
		16.30	0.20	4.00	3.26	0.85
		13.40	0.10	2.20	1.34	1.12
		15.10	0.35	1.00	5.28	0.83
4/10/86	10	17.60	0.22	2.10	3.87	1.04
		21.30	0.22	4.00	4.69	1.04
		50.50	0.33	6.00	16.67	1.01
5/22/86	11	12.90	0.14	0.00	1.81	1.00
		16.20	0.18	2.40	2.92	0.95
		15.70	0.25	4.10	3.93	0.94
		23.50	0.35	6.00	8.23	0.93
5/27/85	11	22.00	0.18	0.00	3.96	0.86
		24.50	0.18	2.20	4.41	0.86
		21.50	0.25	4.00	5.38	0.89
		23.70	0.32	6.00	7.58	0.91
		24.00	0.43	8.00	10.32	0.93
10/1/86	12	33.80	0.12	0.00	4.06	0.92
		30.00	0.19	2.30	5.70	0.93
		30.00	0.23	3.80	6.90	0.95
		29.40	0.36	5.90	10.58	0.98
11/20/86	13	26.00	0.10	0.20	2.60	0.98
		18.10	0.13	2.00	2.35	0.95
		67.00	0.18	3.80	12.06	0.94
		62.40	0.19	6.30	11.86	0.95
4/14/87	14	42.00	0.14	2.20	5.88	0.89
		36.50	0.42	4.80	15.33	0.93

[a] Uptake is corrected for apparatus dead space.

Table B.3. Respiratory Tract Uptake of 1 and 5 ppm Nitrogen Dioxide Through Oral Breathing

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake	Fractional Penetration
5 ppm							
4/13/87	9	12.00	0.31	5.80	3.72	0.324	0.676
		6.50	0.29	2.40	1.88	0.538	0.462
5/8/86	10	16.50	0.23	5.30	3.80	0.511	0.489
2/13/87	11	26.50	0.15	2.20	3.97	0.595	0.405
2/11/87	12	6.00	0.27	0.00	1.62	0.616	0.384
		16.50	0.34	3.90	5.61	0.352	0.648
		8.50	0.30	0.00	2.55	0.712	0.288
2/11/87	13	21.50	0.18	0.00	3.87	0.315	0.685
		32.50	0.23	3.90	7.48	0.178	0.822
		25.00	0.26	5.50	6.50	0.178	0.822
4/14/87	14	17.00	0.34	5.50	5.78	0.315	0.685
		14.00	0.25	2.10	3.50	0.308	0.692
1 ppm							
4/13/87	9	21.00	0.22	5.80	4.62	0.561	0.439
		26.00	0.19	2.30	4.94	0.565	0.435
4/29/86	10	11.43	0.20	0.00	2.29	0.821	0.179
		10.87	0.25	2.00	2.72	0.725	0.275
		12.40	0.27	3.90	3.35	0.625	0.375
		11.30	0.25	5.90	2.83	0.577	0.423
5/8/86	10	12.60	0.20	0.00	2.52	0.719	0.281
		11.50	0.20	0.00	2.30	0.732	0.268
		12.50	0.20	2.00	2.50	0.665	0.335
		15.40	0.20	5.20	3.08	0.668	0.332
2/13/87	11	14.50	0.21	2.20	3.05	0.496	0.504
2/11/87	12	14.50	0.25	0.00	3.63	0.592	0.408
		22.50	0.32	3.80	7.20	0.479	0.521
2/11/87	13	16.50	0.20	1.80	3.30	0.469	0.531
		11.00	0.16	0.00	1.76	0.673	0.327
		12.00	0.24	4.00	2.88	0.430	0.57
		20.00	0.30	5.60	6.00	0.312	0.688
4/14/87	14	20.50	0.33	5.80	6.77	0.403	0.597
		15.50	0.28	2.20	4.34	0.646	0.354

Table B.4. Respiratory Tract Uptake of 1 and 5 ppm Nitrogen Dioxide Through Nasal Breathing

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake	Fractional Penetration
5 ppm							
4/12/87	9	18.00	0.26	2.30	4.68	0.319	0.681
		34.50	0.22	5.80	7.59	0.538	0.462
4/29/86	10	14.00	0.26	0.00	3.64	0.570	0.43
		14.00	0.23	2.10	3.22	0.518	0.482
		13.90	0.21	4.00	2.92	0.488	0.512
		14.40	0.25	6.20	3.60	0.459	0.541
5/8/86	10	21.75	0.24	0.00	5.22	0.463	0.537
		20.25	0.12	2.00	2.43	0.535	0.465
		19.25	0.20	3.70	3.85	0.408	0.592
		24.00	0.25	6.00	6.00	0.332	0.668
1/14/87	12	8.80	0.23	0.00	2.02	0.796	0.204
		12.60	0.44	5.80	5.54	0.482	0.518
		11.20	0.40	4.20	4.48	0.548	0.452
		18.90	0.50	8.20	9.45	0.270	0.73
1/15/87	13	24.00	0.18	4.00	4.32	0.987	0.013
		17.70	0.20	6.00	3.54	0.989	0.011
4/14/87	14	21.50	0.25	2.90	5.38	0.404	0.596
		22.00	0.36	5.50	7.92	0.335	0.665
1 ppm							
11/19/85	9	10.50	0.20	0.00	2.10	0.802	0.198
		10.30	0.19	2.20	1.96	0.790	0.21
		12.80	0.30	4.30	3.84	0.716	0.284
		17.80	0.34	6.40	6.05	0.552	0.448
11/27/85	9	9.75	0.20	0.00	1.95	0.598	0.402
		15.90	0.20	0.00	3.18	0.801	0.199
12/3/85	9	9.70	0.28	0.00	2.72	0.745	0.255
		37.50	0.17	6.20	6.38	0.478	0.522
		38.50	0.18	6.10	6.93	0.408	0.592
4/13/87	9	19.00	0.16	2.00	3.04	0.980	0.02
		19.50	0.25	5.80	4.88	0.858	0.142
4/18/86	10	10.70	0.23	0.00	2.46	0.971	0.029
		9.55	0.25	2.00	2.39	0.983	0.017
		12.00	0.30	4.00	3.60	0.968	0.032
		15.50	0.34	6.10	5.27	0.899	0.101
		17.60	0.38	8.40	6.69	0.864	0.136
4/29/86	10	9.71	0.24	0.00	2.33	0.964	0.036
		13.00	0.20	2.20	2.60	0.955	0.045
		17.65	0.30	5.90	5.29	0.679	0.321
		15.75	0.33	3.80	5.20	0.689	0.311
		14.50	0.22	2.10	3.19	0.760	0.24
		13.39	0.22	2.10	2.95	0.805	0.195
		12.80	0.25	5.80	3.20	0.580	0.42
11/28/86	12	7.20	0.22	0.20	1.58	0.742	0.258
		7.00	0.26	2.00	1.82	0.625	0.375
		9.90	0.22	3.60	2.18	0.742	0.258
1/14/87	12	19.80	0.44	6.00	8.71	0.873	0.127
		7.50	0.40	4.20	3.00	0.946	0.054
		16.70	0.54	7.90	9.02	0.734	0.266
		10.10	0.42	5.20	4.24	0.824	0.176
1/15/87	13	12.00	0.16	6.00	1.92	0.938	0.062
		9.50	0.14	4.00	1.33	0.968	0.032
4/14/87	14	13.00	0.27	1.90	3.51	0.545	0.455
		22.00	0.35	5.80	7.70	0.344	0.656

Table B.5. Respiratory Tract Uptake of 2 and 10 ppm Inhaled Formaldehyde

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake	Fractional Penetration
Upper Respiratory Tract 10 ppm via Mouth Breathing							
3/5/86	9	12.00	0.20	1.9	2.40	0.992	0.008
		16.00	0.30	4.2	4.80	0.913	0.087
		24.70	0.34	6.1	8.40	0.816	0.184
		11.40	0.19	0.0	2.17	0.960	0.040
3/4/87	11	16.00	0.30	3.7	4.80	0.917	0.083
		25.40	0.40	5.7	10.16	0.883	0.117
		14.80	0.30	2.1	4.44	0.902	0.098
3/4/87	12	15.20	0.25	4.0	3.80	0.915	0.085
		17.40	0.35	5.9	6.09	0.909	0.091
		14.80	0.30	7.4	4.44	0.864	0.136
4/8/87	12	12.20	0.22	2.1	2.68	0.971	0.029
		14.60	0.30	4.2	4.38	0.871	0.129
4/8/87	14	11.40	0.21	2.1	2.39	0.979	0.021
		19.00	0.30	4.1	5.70	0.988	0.012
Upper Respiratory Tract 10 ppm via Nose Breathing							
2/27/86	9	7.88	0.22	0.0	1.73	0.992	0.008
		9.79	0.26	2.2	2.55	0.992	0.008
		12.60	0.30	4.0	3.78	0.968	0.032
		9.94	0.40	6.0	3.98	0.927	0.073
3/5/86	9	10.70	0.24	0.0	2.57	0.951	0.049
3/5/87	9	24.20	0.20	4.0	4.84	1.000	0.000
		20.60	0.18	2.0	3.71	0.980	0.020
2/20/87	11	15.20	0.24	2.1	3.65	0.986	0.014
		16.30	0.35	4.0	5.71	0.895	0.105
		14.70	0.40	6.0	5.88	0.868	0.132
3/4/87	12	20.40	0.22	3.9	4.49	0.935	0.065
		22.00	0.35	5.8	7.70	0.983	0.017
2/20/87	13	11.30	0.18	0.0	2.03	0.988	0.012
		15.60	0.20	2.0	3.12	0.997	0.003
		37.40	0.08	3.9	2.99	0.934	0.066
		30.00	0.15	6.0	4.50	0.966	0.034
4/8/87	14	43.00	0.25	4.0	10.75	0.962	0.038
		14.20	0.20	1.7	2.84	0.997	0.003

(Table continues next page.)

Table B.5. (*continued*)

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake	Fractional Penetration
Lung 2 ppm							
10/10/85	9	73.00	0.11	0.0	7.81	0.827	
		51.00	0.25	4.0	12.65	0.922	
		22.00	0.29	3.0	6.29	0.910	
7/16/86	9	92.00	0.15	0.0	13.80	0.985	
		124.00	0.12	2.0	14.88	0.795	
7/16/86	11	36.70	0.11	0.0	4.04	0.779	
		32.00	0.17	2.0	5.44	0.908	
		30.30	0.24	4.1	7.27	0.827	
		28.20	0.34	5.9	9.59	0.827	
1/9/87	11	34.50	0.26	6.0	8.97	0.958	
		32.00	0.25	4.1	8.00	0.918	
		30.00	0.15	2.0	4.50	0.908	
		24.50	0.12	0.0	2.94	1.005	
1/7/87	12	21.50	0.44	6.2	9.46	0.964	
		15.00	0.36	4.2	5.40	0.908	
		10.00	0.28	2.0	2.80	0.888	
		40.00	0.44	7.5	17.60	0.912	
1/8/87	13	56.40	0.25	5.0	14.10	1.009	
		47.50	0.24	4.0	11.40	0.907	
		76.40	0.16	2.2	12.22	0.786	
		22.90	0.14	2.9	3.21	1.005	
4/8/87	14	38.00	0.25	4.0	9.50	0.964	
		45.50	0.16	2.0	7.28	0.827	

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PUBLICATIONS RESULTING FROM THIS RESEARCH

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Table B.6. Respiratory Tract Uptake of Inhaled Ammonium Nitrate Particles

Date	Dog	Breathing Frequency (breaths/min)	Tidal Volume (L)	CO_2 Concentration (%)	Ventilation Rate (L/min)	Fractional Uptake	Fractional Penetration
Upper Respiratory Tract via Mouth Breathing							
4/6/87	9	17.2	0.17	1.9	2.92	0.526	0.474
		21.0	0.30	6.0	6.30	0.806	0.194
4/10/87	11	13.7	0.15	3.2	2.05	0.639	0.361
4/16/87	11	33.8	0.30	5.8	10.14	0.660	0.340
3/19/87	12	10.3	0.28	0.0	2.88	0.817	0.183
4/3/87	13	19.7	0.25	3.5	4.93	0.595	0.405
4/6/87	13	17.6	0.33	6.0	5.81	0.556	0.444
		13.3	0.25	1.9	3.33	0.697	0.303
4/1/87	14	16.5	0.26	2.0	4.29	0.251	0.749
		14.7	0.38	4.5	5.59	0.516	0.484
Upper Respiratory Tract via Nose Breathing							
3/19/87	9	14.2	0.15	1.2	2.13	0.171	0.829
		24.1	0.28	6.2	6.75	0.226	0.774
4/6/87	11	19.4	0.18	1.8	3.49	0.307	0.693
		22.7	0.35	5.9	7.94	0.703	0.297
3/19/87	12	9.8	0.24	0.0	2.35	0.673	0.327
		11.1	0.32	3.7	3.55	0.304	0.696
4/3/87	13	22.6	0.22	1.9	4.97	0.553	0.447
		24.2	0.30	3.5	7.26	0.542	0.458
3/19/87	14	24.8	0.34	3.9	8.43	0.215	0.785
		13.4	0.25	1.9	3.35	0.323	0.677
Lung							
3/18/87	9	55.9	0.28	6.0	15.65	0.481	
		38.1	0.16	2.0	6.10	0.729	
3/18/87	11	61.8	0.32	5.4	19.78	0.305	
		39.6	0.13	1.7	5.15	0.820	
3/18/87	12	29.7	0.16	2.0	4.75	0.730	
		27.5	0.38	6.0	10.45	0.493	
4/3/87	13	36.4	0.15	2.0	5.46	0.946	
		47.0	0.28	5.0	13.16	0.339	
3/18/87	14	25.4	0.14	2.0	3.56	0.934	
		27.5	0.35	6.0	9.63	0.543	

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ABBREVIATIONS

AMN	ammonium nitrate
ANOVA	analysis of variance
BTPS	body temperature and pressure, saturated with water vapor

CO_2	carbon dioxide
HCHO	formaldehyde
HEPA	high-efficiency particulate air (filters)
^{85}Kr	krypton-85
MMAD	mass median aerodynamic diameter
NO_2	nitrogen dioxide
O_2	oxygen
O_3	ozone
ppm	parts per million
SD	standard deviation
SE	standard error
SO_2	sulfur dioxide
STPD	standard temperature and pressure, dry
URT	upper respiratory tract
$\dot{V}$	ventilation rate; volume of gas per unit of time
$\dot{V}_{CO_2}$	volume of carbon monoxide released per minute
V_D	dead space volume
$\dot{V}_E$	volume of expired air per minute
$\dot{V}_{O_2}$	volume of oxygen uptake per minute
V_T	tidal volume

INTRODUCTION

In the summer of 1983, the Health Effects Institute issued a Request for Applications (RFA 83-3) that solicited proposals for studies on "Dose of Airborne Pollutants to Target Tissues." In response to this RFA, Drs. Michael T. Kleinman and William J. Mautz, from the University of California at Irvine, submitted a proposal entitled "The Effects of Exercise on Dose and Dose Distribution of Inhaled Automotive Pollutants." Following the submission of a revised proposal, the HEI Research Committee approved the three-year study, which began in June 1984. Total expenditures were $371,104. The Investigators' Report for the study was received at the HEI in May 1989, and a revised report was received in October 1990. The revised report was accepted for publication by the Health Review Committee in October 1990.

During the review of the Investigators' Report, the Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Health Review Committee's Commentary. The Health Review Committee's Commentary is intended to place the Investigators' Report in perspective as an aid to the sponsors of the HEI and to the public.

REGULATORY BACKGROUND

The U.S. Environmental Protection Agency (EPA) sets standards for oxidants (and other pollutants) under Section 202 of the Clean Air Act, as amended in 1990. Section 202(a)(1) directs the Administrator to "prescribe (and from time to time revise) . . . standards applicable to the emission of any air pollutant from any class or classes of new motor vehicles or new motor vehicle engines, which in his judgment cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare." Sections 202(a), (b)(1), (g), and (h) and Sections 207(c)(4), (5), and (6) impose specific requirements for reduction in motor vehicle emissions of certain oxidants (and other pollutants). Section 202(i) calls for the Administrator to study whether or not additional reductions in light-duty vehicle and light-duty truck emissions should be undertaken.

In addition, Section 109 of the Clean Air Act provides for the establishment of National Ambient Air Quality Standards (NAAQS) to protect the public health. The current primary NAAQS for nitrogen dioxide is 0.053 parts per million (ppm) as an annual arithmetic mean concentration. Because the determination of the appropriate standards for emissions of oxidants and their precursors depends, in part, on an assessment of the health risks that they present, research into the health effects of oxides of nitrogen in studies such as this one is essential to the informed regulatory decision-making required by the Clean Air Act.

Formaldehyde has been regulated by the EPA under emission standards for organic material hydrocarbon equivalents produced in connection with the operation of various classes of vehicles using methanol fuel.

Several changes in the Clean Air Act instituted by the 1990 Amendments to the Act deal with formaldehyde. Section 211(k) of the Act, as added by Section 219 of the 1990 Amendments, establishes a program for the use of reformulated gasoline. The program is designed, at least in part, to reduce the "emissions of toxic air pollutants" such as formaldehyde. Similarly, the "clean-fuel vehicle" program emission standards set out in Section 243, as added by the 1990 Amendments, require that certain formaldehyde emission targets be met.

Section 202(l) of the Clean Air Act, as added by Section 206 of the 1990 Amendments, requires the EPA to "complete a study of the need for, and feasibility of, controlling emissions of [certain] toxic air pollutants." Section 202(l) states that the study "shall focus on those categories of emissions that pose the greatest risk to human health or about which significant uncertainties remain." Defined as a "hazardous air pollutant" under Section 112(b) of the Act, as amended by Section 301 of the 1990 Amendments, formaldehyde is one of the three emissions specifically mentioned in Section 202(l)(1).

SCIENTIFIC BACKGROUND

Emissions from motor vehicle engines contain a mixture of diverse particles and gases produced by the fuel combustion process. Because these emission products are released into the atmosphere, inhalation is the primary route by which toxic compounds in these emissions can enter the human body. To understand the human health effects of emission products, it is important to determine the dose delivered to the respiratory tract and the subsequent fate of these inhaled particles and gases. By identifying the target sites for different emission components and the physical and chemical characteristics of the components, researchers can define more clearly their toxicity and possible role in the pathogenesis of lung disease. The present study evaluated the effects produced by inhaling three pollutants

related to motor vehicle emissions: nitrogen dioxide, formaldehyde, and ammonium nitrate particles. The study focused on how these pollutants affected breathing patterns, how deeply the pollutants were able to penetrate into the respiratory tract, and the amount of pollutants retained in the respiratory tract.

Dividing the respiratory tract into three basic anatomical compartments provides a clearer understanding of where and why particles and gases deposit in the lungs (Schlesinger 1988). The nasopharyngeal, or extrathoracic, compartment extends from the nose to the entrance to the trachea (Miller et al. 1988). This compartment, particularly in the head, is irregular and tortuous, and has a high ratio of surface area to volume (Morgan and Frank 1977). It serves to heat and humidify the inspired air and to increase air turbulence. This design enhances gas absorption and deposition of particles, a function termed "scrubbing." A mucous layer secreted by the epithelial cells lining these cavities facilitates the entrapment of particles. This layer also can react with gases in the inspired air before they reach the trachea. The tracheobronchial compartment, which is also covered with mucus, consists of all of the airways leading from the tracheal entrance to the region where the oxygen and carbon dioxide exchange occurs. These airways are also lined with ciliated epithelial cells that perform the important clearance action of sweeping inhaled particles, infectious organisms, and mucus up and out of the lungs. The third compartment is the pulmonary region, where gas exchange occurs. Although some particles and gases are exhaled, this region is the ultimate site for the deposition of particles and gases not removed by the upper airways.

The distribution and fate of inhaled compounds depend not only on the anatomy of the respiratory tract but also on several different processes (Brain and Valberg 1979; Schlesinger 1985). These processes are essential for understanding what amount of dose reaches the target tissues because the dose that an animal or human inspires through the nose or mouth seldom equals the dose that is actually retained in the respiratory tract. Deposition is the first process that occurs. Scientists measure deposition by determining the fraction of inspired gas or particles that fails to exit with the expired air and remains in the lungs. After deposition, clearance, the second process, removes the deposited materials from the respiratory tract. A predominant clearance mechanism is the mucociliary escalator, which links the ciliated cells with the mucous layer that lines the airways. Gases and particles entrapped in the mucous layer can be transported up and out of the lungs through movement of the cilia. Particles engulfed by pulmonary macrophages that are present in both the airways and alveolar spaces can similarly exit by this escalator. Alternatively, depending on their solubility, particles may dissolve in the lung fluids or be dissolved by cells and subsequently be removed via the lymph system. Scientists measure retention, the third process, by determining the amount of inhaled material retained in the lungs at any one time point after an exposure. This measurement is the net difference between the amount deposited and the amount cleared from the respiratory tract.

If an inhaled agent is toxic, its physical and chemical characteristics determine where the compound may reach its maximum dose in the respiratory tract and where it will produce injury. These characteristics include whether or not the agent is a particle or a gas, its size, hygroscopicity, solubility, concentration, pH, reactivity with constituents of the respiratory tract, and interactions with other coexisting pollutants.

For particles, size is a critical determinant for sites of deposition and retention in the respiratory tract (Hounam and Morgan 1977; Brain and Valberg 1979; Brain et al. 1989). Mathematical methods are used to describe the overall aerodynamic shape and size distribution of inhaled particles, and thus predict their deposition patterns. In general, small particles penetrate deeper into the lungs than larger ones. For example, particles with an overall aerodynamic size greater than 1 μm, or 1/1,000th of a millimeter, will deposit predominantly in the nasopharyngeal region, whereas those less than 0.5 μm in size will deposit primarily in the gas exchange region. Because inhaled air is warmed and humidified in the respiratory tract, hygroscopic growth of the particles can occur. Hygroscopicity is a property of some particles to change in size, shape, and density when they absorb and retain water, and depends on temperature and relative humidity (Martonen et al. 1985; Miller et al. 1988). This phenomenon can affect size distribution and subsequent deposition patterns in the respiratory tract.

Solubility can also dramatically affect the total deposition and distribution of gases and particles in the lungs (Brain and Valberg 1979). Solubility affects absorption from the airstream into the mucous layer and the partitioning of particles and gases between the gaseous and liquid phases of the mucous layer. Very soluble gases and particles dissolve quickly in the lung tissues and then can be absorbed into the bloodstream. Particles that can dissolve slowly in the lung tissue or fluid eventually may be removed via the lymph system (Miller et al. 1988). For gases, solubility particularly influences uptake by the upper airways. For example, sulfur dioxide is a more soluble gas than nitrogen dioxide, and it is more readily taken up by the cavities in the nasal compartment (Brain 1970).

The behavior of particles and gases differs in the lungs because of their physical and chemical characteristics. Because particles often do not dissolve as readily as gases,

clearance mechanisms may transport them to different regions in the respiratory tract. As a result, the site where the particles exert their toxicity may differ from the initial deposition site.

The fate of gases in the lungs depends more on concentration, solubility, and reactivity than on size, a critical parameter for particles (Miller et al. 1988). Reactive gases, such as ozone, produce injury primarily at the sites where they are absorbed by lung tissues. Inhaled gases may also produce effects that cause airways to constrict, thereby altering breathing characteristics. In some cases, inhaling noxious fumes or gases may produce changes in the airway lumen at sites distant from the site of initial deposition (Sant'Ambrogio and Sant'Ambrogio 1991). This effect, called reflex bronchoconstriction, may affect a subject's ability to inhale normally, and thus may subsequently alter breathing patterns.

Although the physical and chemical characteristics of a compound are key determinants for deposition sites in the lungs, anatomical and physiological factors, such as mode and rate of breathing, are important as well (Brain and Valberg 1979). More inhaled particles and gases will reach deeper regions of the lungs and potentially be retained during slow, deep breathing than during rapid, shallow breathing. Experiments with human subjects have shown that increased ventilation during exercise increases the total lung burden of an inhaled aerosol (Bennett et al. 1985). Similarly, a greater percentage of the inhaled material will reach the tracheobronchial tree and beyond via oral breathing, thus bypassing the scrubbing action of the tortuous upper airway system of the nose and pharynx. Physiologically, the amount of air one breathes at rest or during exercise is defined as ventilation or minute volume, the volume of air exhaled in a minute. Anatomical factors are also important because the geometry of respiratory tract airways can vary considerably among species. When animals are used for inhalation studies, interpretation and extrapolation of the effects to humans must account for species differences in the respiratory tract, especially in the nasal cavity.

Various experimental approaches have been used to measure and locate the uptake of particles and gases in the respiratory tract (Brain and Valberg 1979). The simplest approach to quantify the dose to the lungs is to measure the difference between the inspired and expired concentrations of an inhaled gas or particle. Brain (1970) used this technique to determine the percentage of sulfur dioxide absorbed by the upper airways of dogs. The animals inhaled a known concentration of sulfur dioxide, and samples of inspired air were drawn through a cannula inserted into the trachea. The author was then able to compare sulfur dioxide uptake in the trachea after nasal and oral breathing. Although this approach is very useful for studies of the upper airways, one drawback is that it provides no information on deposition sites in the lower airways and lung tissue. With the aerosol bolus technique, a bolus of an inert aerosol with known size characteristics is inhaled as part of a measured volume of inspired air, and then a profile of the aerosol's concentration in the exhaled breath is charted. Data from these measurements can be used to determine particle deposition patterns in the lungs of human subjects (Heyder 1988; Stahlhofen et al. 1989).

Radioactive tracers are valuable tools for deposition and clearance studies in animals and humans. Subjects can inhale radiolabeled materials, and the deposition and clearance patterns can be monitored externally with special detectors (Lippmann 1977; Bennett et al. 1985). Dissection is a tedious but productive method that can be used with radioactive tracers in animal studies to quantify the dose to specific areas in the airways and lung tissue. The lungs can be sectioned after inhalation of a radioactive particle or gas, and the radioactivity in each section can be measured to determine the dose to specific areas (Brain and Valberg 1979).

Mathematical models have been used to estimate the dose of inhaled toxicants in the respiratory tract and to extrapolate the results from animal studies to events that may occur in humans (Miller et al. 1982, 1985; Yu and Xu 1987; Ultman 1988; Yu and Yoon 1991). These complex models take into account numerous parameters, including breathing patterns, physical and chemical properties of the inhaled material, airway branching patterns, thickness and rheologic properties of the mucous layer, and clearance mechanisms. These models must be validated, however, with data obtained from laboratory studies of animals and humans to test how well actual data fit with the mathematical estimates. For many pollutants, including those in motor vehicle exhaust, such data are not yet available.

The major focus of the present study was to examine and compare the fate of three different constituents of motor vehicle emissions in the respiratory tract. Because the physical and chemical properties of the three compounds varied greatly, the patterns of uptake could be compared with each other. The authors planned to use the data from these experiments to test the validity of mathematical models that they had devised for describing the fate of inhaled pollutants in the lungs.

The first pollutant evaluated was nitrogen dioxide, a reactive gas that is a secondary product of emissions from motor vehicle engines. During the fuel combustion process, chemical reactions between oxygen in the air and nitrogen in the fuel and in the surrounding heated air predominately produce nitric oxide. Nitric oxide, itself, exerts little biological toxicity at ambient concentrations; it can, however, be

oxidized readily to the more toxic nitrogen dioxide through several chemical pathways. Inhaling nitrogen dioxide can damage various lung cell components, particularly the cilia on ciliated cells in the airways (Mustafa and Tierney 1978). Findings from epidemiological studies on the human health effects of nitrogen dioxide suggest that inhaling nitrogen dioxide is related to alterations in lung function and an increased susceptibility to respiratory infections in children and adults (Speizer et al. 1980; Samet et al. 1987). Other evidence, although somewhat controversial, indicates that certain susceptible populations, such as persons with asthma, may incur a greater increase in resistance to airflow in their airways after inhalation of levels of nitrogen dioxide four to six times the NAAQS than subjects without asthma (Orehek et al. 1976; Bauer et al. 1986; Mohsenin 1987). The effects of chronic inhalation of nitrogen dioxide are not well known.

The second pollutant studied was formaldehyde. It is a product of incomplete fuel combustion in motor vehicle engines, as well as an important chemical industry product used in the manufacture of a wide variety of materials. There have been comprehensive reviews of the toxicity of formaldehyde (Report on the Consensus Workshop on Formaldehyde 1984; U.S. Environmental Protection Agency 1987; Heck et al. 1990). In the range of 1 to 3 ppm, the acute effects of inhaled formaldehyde in humans are chest tightness, shortness of breath, wheezing, nausea, excess phlegm production, and irritation of the eyes, nose, and throat (National Research Council 1981; Feinman 1988).

Formaldehyde is a more soluble gas than nitrogen dioxide, and, therefore, does not penetrate as deeply into the respiratory tract. As a potent upper respiratory irritant, formaldehyde primarily affects the upper airways rather than the gas exchange region of the lungs. From inhalation experiments conducted with dogs, Egle (1972) reported an uptake of formaldehyde by the upper respiratory tract of more than 95 percent. Formaldehyde is a very water soluble compound that can form cross-links with a variety of cell macromolecules, including DNA, RNA, and proteins. As reviewed by Heck and colleagues (1990), mutations produced by formaldehyde may be related to cross-links formed between DNA and protein that lead to subsequent errors during DNA replication. Long-term inhalation studies with rats have indicated an association between chronic formaldehyde inhalation and the development of nasal tumors (Swenberg et al. 1980; Kerns et al. 1983). On the basis of data from animal studies and limited epidemiological studies, formaldehyde has been classified as a probable human carcinogen (International Agency for Research on Cancer 1982; U.S. Environmental Protection Agency 1987).

The third pollutant investigated was an aerosol of ammonium nitrate particles. These particles are formed naturally in the atmosphere when nitric acid vapor reacts with base ammonia to form ammonium nitrate salt (Finlayson-Pitts and Pitts 1986). This is an equilibrium reaction and depends on the temperature and humidity in the atmosphere. Ammonium nitrate particles are a constituent of ambient air and are important contributors to the reduced visibility caused by air pollution (Kleinman et al. 1979; Russell et al. 1983). Ammonium nitrate is regarded as a secondary pollutant because the particles are formed in the atmosphere from reactions between gaseous precursors. Exposures to a combination of formaldehyde and the ammonium nitrate aerosol were also conducted to determine if the distribution and effects of this combination differed from those of either compound alone. Particularly relevant concerns included whether or not the distribution of the more soluble and less penetrating formaldehyde would be altered by the presence of the particles, and what effects would be produced by the exposure.

Determining profiles for the toxic effects produced by each of the thousands of individual components in motor vehicle emissions is an enormous scientific challenge. This problem becomes even more difficult when one considers the additive and synergistic effects that may be produced by the complex mixture that constitutes engine emissions. Additive interactions occur when the effects of combined pollutants equal the simple sum of their individual effects. Synergistic interactions occur when the effects of the combination are either greater than or less than the sum of their individual effects. Because emissions contain both particles and gases, an additional complicating interaction that may occur is the binding of gaseous compounds to particles. As a result, compounds that by themselves might have deposited at certain sites in the lungs may be transported to different sites while bound to particles. This combination may produce a profile of effects different from those of either gases or particles alone.

To understand the biological response of an organ, such as the lungs, to an individual toxic compound or its combined effects with other compounds, information about the dose to that particular tissue is critical. It is, therefore, important to improve the data base regarding the fate of motor vehicle emission components in the respiratory tract. Such information will facilitate more accurate predictions of the sites for deposition and retention of emission constituents and their potential contributions to lung disease.

JUSTIFICATION FOR THE STUDY

The HEI is concerned primarily with the health effects

produced by inhaling the particles and gases in motor vehicle emissions. Considerable attention has been focused on evaluating the biological responses caused by these emission products, but less effort has been devoted to quantifying the dose delivered to the lungs. The lack of accurate dose measurements limits the value of descriptions of the responses to inhaled pollutants. Information about dose to a target tissue, such as the lungs, is essential to understanding dose-response relationships and their impact on diseases caused by inhaled emission toxicants.

Drs. Kleinman and Mautz proposed to evaluate how pollutant concentration and composition, airway anatomy, ventilation rates, and interactions between pollutants and tissue surfaces influence the dose of selected pollutants to the respiratory tract. Using beagle dogs, the authors proposed to measure the respiratory uptake of various pollutants with different chemical properties. The pollutants proposed for the study were nitrogen dioxide, formaldehyde, and aerosols of ammonium nitrate and carbon particles. The investigators proposed to deliver each aerosol alone or in combination with nitrogen dioxide or formaldehyde. They also proposed to evaluate the effects of these inhaled pollutants on a group of intact dogs and a group of dogs that had been fitted with tracheostomy tubes. The investigators wanted to measure pollutant uptake and distribution and a variety of pulmonary function parameters under conditions of rest and exercise.

The HEI Research Committee favorably evaluated the methods proposed by Drs. Kleinman and Mautz to study the distribution of inhaled pollutants in the respiratory tract. The Committee members thought that an examination of the influence of respiratory rates and different physiological states on the deposition of these pollutants represented an important approach for understanding dose assessment. In addition, these investigators documented extensive previous experience with this type of study. After discussing the proposal, the Committee requested that Drs. Kleinman and Mautz submit a revised proposal clarifying the hypotheses for their experiments and proposing methods to interpret the potentially large amount of data from their study. The investigators submitted a revised proposal, and the Committee subsequently recommended the project for funding.

OBJECTIVES AND STUDY DESIGN

The overall objective of this study was to obtain data for developing and validating mathematical models to predict the deposition patterns and fate of pollutants inhaled during exercise. The investigators wanted to determine how changes in ventilation rate and the route of entry of three different pollutants into the respiratory tract affected the uptake of inhaled gaseous and particulate pollutants associated with automobile emissions. The study design included exposures of a group of six intact beagle dogs at rest and during exercise. A separate group of six dogs was fitted with permanent tracheostomy tubes. These tubes permitted independent measurement of pollutant uptake by the upper and lower respiratory tracts.

The authors defined several terms for describing the results of their study. Fractional penetration, P, was defined as the ratio of the amount of pollutant that was not taken up or absorbed by the respiratory tract relative to the amount that was inhaled. Fractional uptake, $1 - P$, was the fraction of the pollutant retained by the respiratory tract.

The authors wanted to test the following hypotheses: (1) pollutant penetration into the lungs is greater during oral breathing than during nasal breathing; (2) penetration is greater during exercise than at rest; (3) penetration of insoluble gases is greater than that of soluble gases; (4) the presence of a droplet aerosol can alter the penetration of a water-soluble gas; and (5) increased pollutant uptake produces increased physiological effects. The investigators' goal was to determine how well their mathematical model predicted pollutant uptake when the type of pollutant or ventilation rate was changed.

The groups of intact and tracheostomized dogs were exposed to individual pollutants or to combinations of pollutants, and measurements were made of pollutant uptake by the respiratory tract. One dog was used in both groups. The authors also measured a variety of pulmonary function variables, including breath time, expired tidal volume, fractional expiration time, minute ventilation, oxygen consumption, carbon dioxide production, ventilation equivalents for oxygen and carbon dioxide, pulmonary airway resistance, and dynamic compliance. These variables were measured to determine the effects of the inhaled pollutant on breathing characteristics.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

Overall, the authors achieved the specific goals outlined in their proposal. A broad range of functional endpoints relating to breathing characteristics was obtained after exposures to the three pollutants separately and exposures to combinations of the pollutants. The investigators also developed and successfully used an experimental apparatus to evaluate separately the uptake of inhaled pollutants by the upper and lower respiratory tracts.

The authors originally proposed to evaluate whether or not an increase in the penetration of pollutants into the respiratory tract would increase their impact on pulmonary function. However, the pulmonary function changes at the pollutant concentrations chosen for this study were too small to test this hypothesis. In addition, a shift in experimental priorities during the course of the study prohibited any experiments with aerosols of carbon particles.

METHODS AND STUDY DESIGN

The authors' rationale for selecting the three pollutants was sound. They chose two gases with markedly different solubilities, nitrogen dioxide and formaldehyde, so that they could compare values for fractional uptake and penetration in the respiratory tract. They predicted that the relatively insoluble nitrogen dioxide would penetrate more deeply into the respiratory tract than the more soluble formaldehyde. Ammonium nitrate was selected as the aerosol particle because it constitutes a major fraction of the fine particles in ambient air and is a secondary pollutant of motor vehicle emissions (Kleinman et al. 1979; Russell et al. 1983).

The intact dogs were exposed to 5 ppm nitrogen dioxide at rest and during exercise and to a mixture of 10 ppm formaldehyde and ammonium nitrate particles during exercise. The exercise exposures were performed in a refrigerated treadmill exposure system so that the dogs would not have to pant to thermoregulate their temperature. A broad range of pulmonary function measurements was obtained during these exposures, allowing the investigators to compare the changes in breathing characteristics produced by the different pollutants.

The authors developed an ingenious device to measure the penetration of inhaled agents into the respiratory tract of tracheostomized dogs. This device was an artificial external loop, complete with valves and sampling ports, that was attached at the level of the trachea. For pollutant exposures with the tracheostomized dogs, a head-only exposure chamber was used. It was designed as a glove box so that the trainer could observe a dog during the experiment. Using this entire exposure system, the authors were able to measure the amount of an inhaled pollutant that reached the trachea when an exposure occurred through either oral or nasal breathing. The investigators also were able to bypass the upper airways and deliver inhaled pollutants through the trachea into the lungs. They then could measure the amount of the selected pollutant or pollutant mixture retained by the lower respiratory tract. Because this exposure system did not accommodate exercise by this group of dogs, the investigators added 0 to 8 percent carbon dioxide to the air in the head-only chamber to increase the rate of breathing and to simulate the effects of exercise.

With the tracheostomized dogs, the authors evaluated how changes in ventilation rate and route of entry altered pollutant penetration into the lungs. The dogs were exposed to 1 and 5 ppm nitrogen dioxide, to 2 and 10 ppm formaldehyde, and to an aerosol of ammonium nitrate particles. The amounts of pollutants that reached the trachea after oral or nasal breathing and amounts of pollutant uptake by the lower respiratory tract after breathing through the tracheostomy tube were measured at various ventilation rates. The authors also estimated the extent to which individual differences in penetration between dogs could be explained by anatomical differences. Because the animals were always exposed to clean air before exposure to the pollutant, there is a possibility that bias was introduced into the results because the order of the exposures was not randomized.

Although the artificial external loop was a clever approach, it presented several technical problems. One problem was the need to consider the dead space volume added to the tidal volume by the loop. This was an important factor for calculating the fractional uptake of the pollutant and interpreting the response to the pollutant. The authors did measure and correct for the dead space volume added by the valve and tracheostomy apparatus, but it is not clear if the assumptions used in deriving the correction equation were validated.

Another problem with the artificial loop was the potential loss of pollutants in the tubing and valves. This problem was particularly applicable to the soluble gas, formaldehyde. Formaldehyde loss to the tubing and valves could have had an impact on the assumptions concerning gas concentrations. This loss could introduce uncertainty about the actual concentrations delivered to and expired from the respiratory tract. In addition, there were some problems with the sensitivity of the instrumentation used to analyze the levels of the different pollutants. In some cases, the reported fractional uptake of the pollutants exceeded a value of 1.

One drawback of the head-only chamber exposures for the tracheostomized dogs was that the experimental protocol required technician interaction during the exposures, including encouragement of the dogs. Because the technician knew when the pollutant exposures were occurring, this interaction may have affected measurement outcome.

RESULTS AND INTERPRETATION

The findings of this study confirm the general conceptual understanding of the uptake of inhaled gases and particles by the respiratory tract. As anticipated, oral breathing led to greater penetration into the respiratory tract than nasal breathing because the scrubbing effects contributed by the nasal passages were absent. Increasing the nitrogen dioxide

concentration from 1 to 5 ppm tended to decrease fractional uptake by the upper respiratory tract and, in turn, increase the fractional penetration in the tracheal sampling port. The total respiratory uptake of 5 ppm nitrogen dioxide was also greater during exercise than at rest.

The authors reported that the water-soluble formaldehyde tended to be scrubbed out in the upper airways; therefore, the amount of this reactive compound reaching the lower respiratory tract was decreased. These findings are consistent with measurements of formaldehyde uptake by the upper respiratory tracts of dogs reported by Egle (1972).

In nontracheostomized dogs, some rapid breathing was observed after exposure to 5 ppm nitrogen dioxide at rest, but not during the exercise exposure. Exposure to 10 ppm formaldehyde plus the ammonium nitrate aerosol during exercise resulted in slow, deep breathing. In addition, total respiratory system uptake of formaldehyde during the combined formaldehyde and ammonium nitrate exposure was greater at rest than during exercise. This result suggests that the ammonium nitrate aerosol altered the regional pattern of formaldehyde deposition toward the lower respiratory tract.

To measure fractional uptake accurately, a sufficient amount of expired pollutant had to be collected for the analyses. This measurement required a relatively long collection period for both the ammonium nitrate aerosol studies and the formaldehyde studies. For example, the authors stated that the ammonium nitrate aerosol studies required 15 minutes of stable breathing by the dogs. Because the dogs had to maintain relatively constant breathing patterns during the entire collection period, this factor added a level of complexity to several of the experiments. Although the authors do refer to the length of the collection period, they do not discuss whether or not this time factor accounted for the unexpected result that uptake of the ammonium nitrate was greater for nasal breathing than for oral breathing.

In retrospect, although ammonium nitrate particles are relevant to motor vehicle emissions, this may have been an unfortunate choice of aerosol, because, as discussed by the authors, the particles are hygroscopic. As a result, size distribution and deposition patterns of the ammonium nitrate aerosol particles presumably were altered because of water absorption in the humidified airways of the respiratory tract. This factor confounded interpretation of the results and suggests that further research is needed to substantiate the investigators' findings with ammonium nitrate particles.

The finding that fractional uptake of formaldehyde increased when formaldehyde was combined with an aerosol of ammonium nitrate particles was an important observation. This confirmed previous studies showing that highly water-soluble compounds normally scrubbed out by the upper respiratory tract can be delivered to the lower airway when simultaneously inhaled with particles (Speizer and Frank 1966). The investigators' interpretation that this effect was due most likely to absorption of the water-soluble formaldehyde into the hygroscopic ammonium nitrite particles is probably correct. It would have been very interesting if the authors had confirmed this finding by direct measurement. However, the general concept that the delivery of reactive agents, such as sulfur dioxide or formaldehyde, to the lower respiratory tract is increased when these agents are inhaled simultaneously with particles is important for understanding pollutant interactions.

Carbon dioxide was added to the air inspired by the tracheostomized dogs to increase their breathing frequency, thereby simulating the breathing patterns of exercising, nontracheostomized dogs. Although exercise and inhalation of carbon dioxide produced comparable minute volume values in the two groups of dogs, there are important physiological differences between these two approaches. As noted by the authors, inhaled carbon dioxide does not mimic the exercise-induced cardiovascular changes, airway caliber changes, or alveolar recruitment. It was also noted that the four- to five-fold increases in ventilation produced in the dogs by exercise or carbon dioxide inhalation were modest. A healthy human will often have minute volumes 10 to 15 times greater during exercise than at rest and will naturally shift from nasal to oral breathing at these ventilation rates.

The investigators also were interested in the relationship between breathing patterns and respiratory uptake. Previous studies showed that ozone inhalation produced a switch from normal breathing rates to rapid, shallow breathing (Hazucha 1987). This presumably is a protective reaction against ozone uptake. However, in the present study, changes in pollutant uptake relative to altered breathing patterns in response to an inhaled agent were not large enough to be distinguished from one another.

Finally, the authors provided some estimates comparing pollutant uptake in dogs and humans based on ratios of airway surface area to ventilation rates. Because they estimated that this ratio is more than five times greater for dogs than humans, the authors suggested that pollutant fractional uptake by the upper respiratory tract would be much higher for dogs than humans.

REMAINING UNCERTAINTIES AND IMPLICATIONS FOR FUTURE RESEARCH

It is important to understand the dose and distribution of

inhaled agents to different respiratory tract compartments because they may relate to the pathogenesis of lung disease caused by inhaled pollutants. Investigations on the penetration of pollutants into the lungs should be augmented with studies using new techniques to determine the intrapulmonary distribution of dose from the trachea to the alveoli and the effects of clearance mechanisms on this distribution. The work of these and other investigators in recent years has provided insight into the mechanisms governing these processes, much of which has had predictive value. These findings should be used to validate mathematical models for predicting regional and local dose of pollutants to lung cells and tissues.

In the future, studies should be directed toward obtaining similar types of data in humans. Data from experiments with radioactive tracers, the aerosol bolus technique, and other noninvasive methods can expand our data base and lead to more accurate estimates of human lung burdens of inhaled particles and gases. Such information will facilitate prediction of the deposition and retention sites of emission constituents and their potential contribution to lung disease.

CONCLUSIONS

The investigators' major contribution was to provide empirical data that can be used in mathematical models for predicting the deposition and fate of inhaled agents under conditions of rest and exercise. These data will provide a baseline for further development and validation of models to study the effects of exercise on pulmonary deposition and the fate of inhaled toxicants.

Many of the authors' findings were predictable. They reported that fractional penetration of pollutants was greater when the dogs breathed orally rather than nasally because of the absence of the scrubbing action of the nasal cavities. Similarly, fractional penetration increased with increased concentrations of nitrogen dioxide. The authors also reported that fractional uptake of formaldehyde exceeded that of the less water-soluble gas, nitrogen dioxide. Interestingly, they also found that formaldehyde uptake by the lower respiratory tract was higher when the formaldehyde was mixed with the ammonium nitrate aerosol. These results have important implications for other mixtures of environmental pollutants composed of gases and particles; they also provide interesting insights into gas and particle uptake that are worthy of future exploration.

An important result from these experiments is the extrapolation of the authors' findings from laboratory animals to humans. The investigators provided a useful discussion of the scaling factors needed to extrapolate findings from airway studies in dogs to humans.

The results of these studies contribute to the authors' long-term goal to develop a mathematical model capable of predicting the deposition and fate of pollutants inhaled during work-related exercise. This is an ambitious goal, and although some progress was made in this study, the results suggest that additional basic studies will be needed before this goal is attained.

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