Toxicologic Methods: Controlled Human Exposures

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The assessment of risk from exposure to environmental air pollutants is complex, and involves the disciplines of epidemiology, animal toxicology, and human inhalation studies. Controlled, quantitative studies of exposed humans help determine health-related effects that result from breathing the atmosphere. The major unique feature of the clinical study is the ability to select, control, and quantify pollutant exposures of subjects of known clinical status, and determine their effects under ideal experimental conditions. The choice of outcomes to be assessed in human clinical studies can be guided by both scientific and practical considerations, but the diversity of human responses and responsiveness must be considered. Subjects considered to be among the most susceptible include those with asthma, chronic obstructive lung disease, and cardiovascular disease. New experimental approaches include exposures to concentrated ambient air particles, diesel engine exhaust, combustion products from smoking machines, and experimental model particles. Future investigations of the health effects of air pollution will benefit from collaborative efforts among the disciplines of epidemiology, animal toxicology, and human clinical studies. *Key words*: air pollution, asthma, cardiac, chronic obstructive pulmonary disease, experimental design, exposure, gases, human, inflammation, methods, particles, pulmonary function, respiratory. — *Environ Health Perspect* 108(suppl 4):605–613 (2000).
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The assessment of risk for acute and/or chronic inhalation of low-level environmental air pollutants is complex. Typically, the database for risk assessment arises from three separate investigational approaches: epidemiology, animal toxicology, and human inhalation studies. Carefully controlled quantitative studies of exposed humans utilize laboratory atmospheric conditions, considered relevant to outdoor pollutant levels, or concentrated particles from the ambient air, and document health-related effects that result from breathing the atmosphere. Advantage is taken of the highly controlled environment to identify responses to individual pollutants and characterize exposure–response relationships. In addition, the controlled environment provides an opportunity to study interaction among pollutants per se or with other variables such as exercise, humidity, or temperature. Insofar as individuals with acute and chronic cardiopulmonary diseases can participate in exposure protocols, potentially susceptible populations can be studied. However, controlled human exposures have important limitations: for practical and ethical reasons, studies are limited to small groups, presumably representative of larger populations, to short durations of exposure, and to pollutant concentrations expected to produce only mild and transient responses. Furthermore, efforts to predict chronic health effects from acute transient responses in clinical studies lack validation.

In this article we examine the exposure methodologies and study design issues essential for performance of controlled clinical studies. Subsequently, we focus on the investigative tools available to evaluate respiratory, cardiovascular, and pharmacokinetic outcomes. Finally, our current understanding of responses of susceptible populations as determined by the clinical study is reviewed.

Experimental Design and Methods

Controlled human exposure studies of inhaled gases and particles, especially those designed to evaluate short-term effects of airborne pollutants, have been performed in multiple laboratories over the past decade. The major unique feature of the clinical study is the ability to select, control, and quantify pollutant exposures of subjects of known clinical status and determine their effects on cardiopulmonary performance under ideal conditions for conducting physiologic evaluations.

Typically, modern clinical studies use exposures to single pollutants or simple pollutant mixtures, exposure durations of 0.5–8 hr, and a double-blind, crossover design. Exposures are often conducted in environmentally controlled chambers (25–75 m^3 volume) with a single passage of the pollutant(s). Physiologic responses may include lung function, or cardiac rate, rhythm, and variability. Techniques used to detect potential biomarkers include bronchoscopy, nasal lavage, or sputum induction. Techniques are also available to measure airway size and mucociliary clearance rates using aerosol probes and radiolabeled aerosol techniques. An additional approach is the use of nasal masks or mouthpiece exposures of individual subjects in place of an exposure chamber. These design features affect and often determine the technologic and methodologic approaches used for pollutant-generated monitoring and quantitative sampling (*1*).

There is a major difference with regard to pollutant generation requirements between studies utilizing facemask or mouthpiece exposures and those conducted in relatively large environmental chambers. With the mouthpiece, a pollutant–air mixture must be produced that only slightly exceeds the individual subject's respiratory intake requirements, e.g., from 5 L min^{-1} to 50 L min^{-1} with exercise. In contrast, for chambers operating with a single pass (no recirculation), 5 to 25 m^3 min^{-1} is the likely flow rate requirement, i.e., as much as 1,000 times greater. Usually the exposure duration required for this greater generation capacity is also longer with chamber studies than with mouthpiece or facemask studies.

Monitoring devices are used to determine whether exposure levels in the breathing zone are stable or changing. Ideally, monitors are real-time devices calibrated to indicate continuously the absolute pollutant concentrations. This level of performance can be achieved with certain pollutant analyzers such as for ozone (O_3), but often the investigator is provided only qualitative information by monitors. In this situation, if quantitative measurements of pollutant levels are required, complementary analytical devices must be used that often are not real-time and only serially sample the exposure conditions.

The controlled clinical study provides the opportunity to examine both healthy volunteers and individuals with underlying cardiopulmonary diseases. Subjects typically are classified by age, gender, race, lung function, and cardiovascular status. Normal volunteers are characterized by the absence of allergies, often documented by skin testing, the lack of

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hyperresponsive airways assessed by inhalation challenge tests, and absence of hypertension or cardiac arrhythmias. Subgroups of healthy volunteers may include adolescents, elderly subjects, and smokers. Healthy volunteers typically are able to perform vigorous exercise for extended periods.

Exercise performed either on a treadmill or bicycle is an important component of the exposure study. Exercise enhances the pollutant dose both by increasing ventilation and by causing a switch from nasal to oral breathing, effectively bypassing the nose. The nose may remove some particles and gases, variably reducing their delivery to the lower respiratory tract (*2*). In addition, the effect of exercise on airway drying may enhance the response to pollutants.

Finally, because clinical studies often use small numbers of volunteers, it is essential that sample size calculations be performed to make certain the study has adequate power. This is often an important limitation in controlled human exposures. Statistical issues in controlled clinical study designs have been considered by Van Ryzin (*3*).

Assessing Responses in Human Studies

The choice of outcomes to be assessed in human clinical studies can be guided by both scientific and practical considerations. Clearly, the outcomes of interest depend on what is known and expected about the effects of the pollutant being studied. Results from epidemiologic investigations, occupational or accidental exposures, or animal exposure studies may suggest the range of effects of a given agent. The chemical behavior of the agent may predict its effects. For example, SO_2 is a reactive and soluble gas and therefore exerts its effects predominantly in the upper airways and major bronchi where it is completely absorbed. O_3, although very reactive, is relatively insoluble. Because it is not entirely dissolved in the epithelial lining fluid of the upper airway, it persists in the inhaled air, even to the alveolar space. Thus, effects are expected and found throughout the respiratory tract. In contrast to these irritant gases, carbon monoxide passes into the blood with virtually no pulmonary toxicity; its health effects result from binding to hemoglobin and resulting tissue hypoxia.

Practical considerations obviously guide and limit outcome measures in human clinical studies. For safety and comfort reasons, human studies utilize exposure protocols and pollutants designed to elicit transient, clinically insignificant effects in relatively healthy subjects, using minimally invasive measurement techniques. The usefulness of human studies has been extended with the development of techniques for safely sampling cells of the lower airway and by the ongoing search for markers of pollutant effects. This section reviews traditional and more recent outcome measures used in human clinical studies of air pollution.

Respiratory Responses

Virtually every clinical test designed to assess the presence or severity of respiratory disease has been used to measure the effects of inhaled pollutants in either clinical or epidemiologic studies. The use of traditional tests of lung mechanics in human clinical studies has been reviewed (*4*), and is discussed only briefly; emphasis instead is placed on more recently developed methods for assessing respiratory responses.

Pulmonary function tests. Despite the development of newer and more direct measures of pollutant effects on the airways, simple spirometry remains a mainstay in human clinical studies. The measurement techniques, equipment, and interpretation have been reviewed extensively (*5*). The most commonly evaluated parameters obtained from spirometry are the forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), and the maximal flow-volume curve. During the past 10 years, the vast majority of human pollutant exposure studies have evaluated responses with at least one of these parameters. The reasons for selecting these tests are obvious. They are simple to perform, and the results are reproducible within subjects. Standards for spirometry have been established by the American Thoracic Society (*6*), and ranges of normal values have been established (*7*). Results of testing, especially FEV_1, have been found to correlate well with functional status. For example, in one study in healthy subjects and in patients with chronic bronchitis, a reduction in FEV_1 of more than 5% in the healthy subjects and of more than 15% in patients with airflow limitation was required to be considered significant (*8*).

One potentially confounding problem with spirometry is that the test itself may alter the parameters being tested. For example, the deep inspiration that precedes measurements of forced expiratory flow causes transient bronchodilation and reduces induced bronchoconstriction in the healthy individual but may cause bronchoconstriction in the asthmatic. Performance of spirometry also appears to increase the airway production of nitric oxide (*9*), a measurement used in some studies to assess airway inflammation noninvasively. These changes should be considered in designing and interpreting the results of clinical studies.

Other tests of pulmonary mechanics include the measurement of airway resistance or its reciprocal, conductance, analysis of partial flow-volume loops and flow rates at specific lung volumes, and closing volumes. Airway resistance testing is influenced by changes in the major bronchi and upper airway, including the larynx. Partial volume flow rates and closing volumes were designed to provide more sensitive measures with greater sensitivity for small changes, particularly in the more distal airways (*10*). Weinmann et al. (*11*) have used partial volume flow rates to indicate possible delayed small airway effects following O_3 exposure. Although these and other techniques may prove more sensitive than FEV_1 for detecting mild obstruction or peripheral airway changes, the methods often lack reproducibility and there is uncertainty regarding their interpretation.

Airway responsiveness. The testing of nonspecific airway hyperresponsiveness has proven useful for assessing airway responses to low concentrations of environmental airway pollutants. These tests measure responses to inhalation of pharmacologic bronchoconstricting agents such as methacholine, carbachol, or histamine. Other stimuli of bronchoconstriction used in clinical studies include isocapnic cold air hyperventilation and inhalation of increasing concentrations of SO_2. Responses are measured with the usual pulmonary function measures, typically FEV_1 or airways resistance. Increasing concentrations of a bronchoconstricting agent are inhaled to construct a dose–response curve, and the results are expressed as the provocative concentration (PC) necessary to produce a given change in function, such as a decrease in FEV_1 of 20% (PC_{20}). The PC_{20} is obtained from the log-dose–response curve by linear interpolation of the last two points; the lower the PC_{20}, the greater the responsiveness.

Asthma is characterized by a significant increase in nonspecific airways hyperresponsiveness compared with that in healthy subjects. Studies of subjects with asthma therefore need to use considerably lower initial concentrations of the bronchoconstricting agent. Furthermore, airway diameter influences the results of challenge testing. For example, an airway already constricted to 50% of its baseline diameter needs a much smaller concentration of methacholine to achieve a further 20% reduction. Thus, the results of challenge testing are difficult to interpret if bronchoconstriction is already present at the time of testing.

Airway challenge studies have been used to assess the effects of pollutant exposure on airway responsiveness and to determine whether baseline levels of responsiveness predict lung function decrements in response to pollutant exposure. For example, subjects with mild asthma demonstrated increased airway responsiveness following exposure to NO_2 (*12*) or sulfuric acid aerosol (*13*).

Healthy subjects experienced increased responsiveness to carbachol following 6-hr exposures to 2.0 ppm NO_2, in the absence of changes in lung function (*14*). Utell et al. (*15*) found a relationship between baseline airways responsiveness assessed by carbachol in asthmatic subjects and responsiveness to an inhaled sulfuric acid aerosol. In contrast, airways responsiveness to methacholine was not predictive of the FEV_1 decrement in response to O_3 exposure in either smokers or nonsmokers (*16*).

Fiberoptic bronchoscopy. Development of fiberoptic bronchoscopy in the 1970s revolutionized the diagnostic evaluation of patients with pulmonary disease, and this technique is now widely used to sample the respiratory tract for research purposes. Its use in studies of asthma has been reviewed recently (*17*). Bronchoscopy was first used by Seltzer and colleagues (*18*) to detect airway inflammation in response to O_3 exposure. Since then, bronchoscopy has allowed investigators to characterize the nature of the airway inflammatory response to single and repeated exposures to O_3 in healthy, allergic, and asthmatic subjects (*19–22*). It has been used to study the effects of exposure to many pollutants in addition to O_3, including NO_2 (*23–25*), SO_2, (*26*), acid aerosols (*27–29*), and diesel exhaust (*30*).

A growing number of techniques have been developed using the access provided by the fiberoptic bronchoscope to sample fluids and cells of the lower airway (Table 1). Bronchoalveolar lavage (BAL), the serial installation and removal of fluid through a bronchoscope wedged in a distal airway, has provided an opportunity to sample the epithelial lining fluid and cells of the distal airways. Evidence suggests that cells recovered by BAL reflect those present in the pulmonary parenchyma in disease states such as sarcoidosis and idiopathic pulmonary fibrosis (*31*). However, cells from the pulmonary interstitium, some of which may have a key role in the pulmonary immune response (*32*), are not sampled using this technique. A modification of the original technique of serial instillation of aliquots of saline involves separate analysis of the first returned portion of fluid, which preferentially samples the distal conducting airways in which the bronchoscope is wedged. Fluid obtained from subsequent aliquots more closely reflects alveolar sampling (*33*). This concept of regional lavage was extended with the use of proximal airway lavage (*34*), in which a catheter with two inflatable balloons and a port between them is inserted through the bronchoscope into a mainstem bronchus. The balloons are inflated to isolate the bronchus, and repeated small-volume washes are performed using the port between the balloons. Care must be taken during the procedure to avoid prolonged balloon inflation, as some subjects may experience significantly decreased arterial oxygen tension during occlusion of the airway. The technique has been used successfully in both healthy volunteers and subjects with mild asthma (*35*). A modification of this technique has been used to study the effects of O_3 exposure in healthy and asthmatic subjects (*36*). However, this technique is not often used because of its increased complexity and variability as well as difficulty in interpreting the findings.

The fiberoptic bronchoscope has been used to instill particles into a single subsegmental airway in human subjects to examine localized epithelial responses in an isolated lung region (*36*). The exposed segment is then sampled subsequently using BAL and/or biopsy. This approach is similar to segmental allergen challenge in studies of the pathophysiology of asthma. It permits the delivery of relatively high concentrations of particles directly into the airway without the potential risks involved with inhalation exposures.

Epithelial cells and tissue can also be obtained through the bronchoscope using brushes or biopsy forceps, respectively. Endobronchial biopsies have been shown to be safe in healthy volunteers and subjects with mild or moderate asthma. Although bleeding can occur at the site of a biopsy, it is almost never clinically significant. The tissue samples are small, but sufficient material can be obtained to examine cellular patterns of gene expression using immunologic staining and *in situ* hybridization. In studies utilizing pollutant and control exposures, with each subject serving as his/her own control, it is important to avoid carryover effects, in which mucosal repair processes may influence the results of the second study. The combination of pollutant exposure and endobronchial biopsies may induce subtle changes in airway cytokine expression that persist as much as three weeks after exposure (*37*).

Endobronchial biopsy should not be confused with transbronchial biopsy, a procedure designed to obtain alveolar tissue from the human lung for clinical diagnostic purposes. The biopsy forceps are advanced into

Table 1. Bronchoscopic procedures used in clinical studies.

Procedure	Description	Purpose and advantage	Disadvantages
BAL	Bronchoscope wedged in a distal airway; sequential instillation and recovery of sterile saline	Samples distal airway and alveolar space, with recovery of epithelial lining fluid, surfactant components, and cells; relatively reproducible findings	Protocols differ among labs; serial measurements not possible; findings may not reflect tissue effects
Bronchial lavage	Separate analysis of first recovered aliquot of BAL (see above)	Preferentially samples distal conducting airways in comparison with subsequently recovered aliquots	Sample is of mixed bronchial and alveolar origin; protocols differ among labs
Proximal airway lavage	Double-balloon occlusion catheter placed in a main bronchus; lavage performed via port between occluding balloons	Samples large conducting airway separate from alveolar space and distal airways	Relatively invasive; difficult to perform; may cause transient hypoxemia
Bronchial brush biopsy	Passage of small brush through suction port, brush airway surface	Samples of airway epithelial cells; cells can be cultured and passaged	Low viability and limited number of recovered cells
Endobronchial biopsy	Passage of tiny alligator forceps through suction port; obtain 1–2-mm tissue sample from bronchial epithelium	Samples epithelium *in situ*; provides tissue for histology, immunocytochemistry, *in situ* hybridization	Limited to major conducting airways; small tissue samples; relatively invasive; bleeding can occur (rarely significant)
Transbronchial biopsy	Passage of tiny alligator forceps through the bronchial wall; obtain small samples of alveolar tissue	Samples alveolar tissue and distal bronchial epithelium	Small tissue samples; invasive; Potential complications include pneumothorax, hemorrhage
Bronchial instillation	Agent (allergen, particles) instilled via suction channel of bronchoscope; sampling with BAL, biopsy	Most useful for studies of airway cellular responses *in vivo*; allows safe study of high tissue doses	Effects of instillation may differ from inhalation; difficult to control dose

the most distal airways and samples obtained either blindly or under fluoroscopic guidance. This procedure may be complicated by pneumothorax and rarely, lung hemorrhage, and has not been used in clinical studies of air pollution. Recent studies have utilized transbronchial biopsy to demonstrate the presence of inflammation at the alveolar level in subjects with nocturnal asthma (*38*).

Sputum induction. In recent years, sputum induction has emerged as a research tool for sampling the cells and epithelial lining fluid of the lower respiratory tract in humans (*39,40*). Sputum can be reliably induced in asthmatic subjects as well as in most healthy subjects using nebulized 3–5% saline. The cells recovered are representative of the lower airways and provide a measure of airway inflammation. Asthmatic subjects have more eosinophils and polymorphonuclear leukocytes (PMNs) in sputum than healthy subjects. Sputum inflammatory cells increased with asthma exacerbations (*41,42*) and allergen challenge (*43*), and decreased with prednisone therapy (*44*). The results appear to be qualitatively similar to findings in the first (bronchial) aliquot of BAL fluid (*45*).

The process of sputum induction itself appears to induce a mild airway inflammatory response. For example, when two inductions are performed 24 hr apart, the second induction has a higher recovery of PMNs (*46,47*). Thus, caution must be used in designing studies with serial measurements. Sputum induction is well tolerated, even in those with severe asthma, but some asthmatic subjects experience bronchoconstriction in response to the nebulized saline. Pretreatment with bronchodilators is effective in preventing the aerosol-induced bronchoconstriction.

Some subjects are unable to produce sputum following induction. Approximately two-thirds of healthy subjects, and more than 90% of asthmatic subjects, are able to be induced. Individuals able to produce sputum do so on subsequent challenges and the findings on repeat sputum inductions are quite consistent ($r = 0.80$) among individuals (*48*). Fahy et al. (*49*) demonstrated that sputum induction detects airway inflammation following exposure to 0.4 ppm O_3 for 2 hr in healthy subjects. In contrast, exposure to 0.3 ppm NO_2 for 1 hr, with moderate intermittent exercise, did not alter cell recovery in induced sputum in subjects with mild asthma or chronic obstructive pulmonary disease (COPD) (*50*). These findings are consistent with findings in BAL fluid following exposures to O_3 and to NO_2. Sputum induction therefore holds promise as a noninvasive method for assessing airway inflammation in clinical and field studies.

Markers in exhaled air. Biochemical processes at the epithelial level may release gaseous products, some of which can be detected in the exhaled breath. Investigators have sampled exhaled air for a variety of substances as markers of either airway inflammation or injury, including hydrogen peroxide (*51*), CO (*52*), isoprene (*53*), ethane (*54*), and pentane (*55*). The technology has been developed to measure concentrations of over 100 volatile chemicals in the exhaled breath, with potential applications in field studies of environmental exposures (*56*). Foster et al. (*53*) found small but significant increases in exhaled isoprene levels in healthy subjects following exposure to O_3 at 0.15–0.35 ppm for 130 min.

Measurements of exhaled nitric oxide have attracted wide interest as a means of detecting lung inflammation (*57*). NO is produced from the action of nitric oxide synthase (NOS) on L-arginine. A variety of cells express one or more of the three isoforms of NOS, including airway epithelial cells and endothelial cells. Concentrations of NO are increased in the exhaled air of people with asthma compared with healthy subjects (*58*). Mild asthmatics not requiring inhaled or oral corticosteroids have exhaled NO levels 7-fold greater than normal subjects (*59*). NO levels increase further with clinical exacerbations, correlate with the degree of airway hyperresponsiveness in steroid-naïve asthmatics (*60*), and decrease following therapy with corticosteroids (*61,62*). A preliminary report (*63*) indicates that exhaled NO is increased in healthy subjects following exposure to 0.25 ppm O_3 2 hr daily for 3 days, suggesting NO may prove to be a useful marker of pollutant-induced inflammation. However, the high concentrations of NO found in the pharynx and nasal passages can confound the measurement of NO production by the lower airways. Furthermore, the lungs have a very high diffusing capacity for NO, and most studies have not taken into account the removal of NO from the airways by capillary blood (*64*).

The measurements of gases and volatile organic molecules in exhaled air show promise as noninvasive markers of pollutant effects, but considerable work remains to understand the significance of the observed changes.

Cardiovascular Responses

Until recently, concern about possible cardiovascular effects of exposure to air pollution was limited almost exclusively to CO because of the known detrimental effects of CO on oxygen delivery. Few studies have assessed cardiovascular effects of other pollutants. In 1985 Linn and co-workers (*65*) found small reductions in systemic blood pressure in healthy subjects exposed to NO_2, but the finding has not been confirmed in subsequent studies. Drechlser-Parks (*66*) used a noninvasive impedance cardiographic method to estimate changes in cardiac output following 2-hr exposures of healthy subjects to 0.60 ppm NO_2, 0.4 ppm O_3, and a combination of NO_2 and O_3. Compared with air exposure, cardiac output was reduced following exposure to the combination of NO_2 and O_3, but not for the individual pollutants. Changes in blood pressure were not reported. Gong and co-workers (*67*) performed perhaps the most definitive study of cardiac effects of O_3 exposure. Men with and without stable hypertension underwent arterial and right heart catheterization, and then performed intermittent exercise in 0.3 ppm O_3 for 3 hr. No differences from air exposure were seen for a variety of indices of cardiac function. However, heart rate, the rate–pressure product (an index of left-ventricle work), and the alveolar–arterial gradient in oxygen tension all showed greater increases following O_3 but not air exposure. This combination of effects could be clinically important in individuals with critical coronary lesions.

Epidemiologic studies of ambient particles have shown a surprisingly robust association with cardiovascular events, but no mechanism has been found to explain the relationship. Proposed hypotheses explaining cardiac effects of inhaled particles have included hypoxemia (*68*), direct effects of particles or their reaction products on myocardial cells (*69*), and cardiac consequences of airway inflammation or injury (*70,71*). Recent studies in healthy and compromised animals from the laboratories of the U.S. Environmental Protection Agency (*69*) and from Harvard University (Boston, MA) (*72*) have suggested that inhalation of particulate pollutants may induce changes in cardiac rhythm or repolarization. Pope and co-workers (*68*) used pulse oximetry to study panels of elderly subjects residing at elevation. They found no changes in oxygen saturation, but there were small but significant increases in heart rate associated with outdoor particle concentrations. A 100-μg/m^3 increase in the previous-day level of particulate matter < 10 μm in diameter (PM_{10}) was associated with a 95% increase in the odds of a 10-beat increase in the heart rate. These observations have intensified efforts to understand the relationship between pollutant exposure and changes in cardiac function.

One tool being used in this effort is continuous cardiac monitoring, or Holter recording. Originally used to identify silent arrhythmias in cardiac patients, cardiac monitoring has revealed that both the pattern of variation in heart rate, or heart rate variability, and the pattern of electrical repolarization of the heart are markers of cardiac health or disease. Decreased heart rate variability as well as abnormalities in the duration, dynamics, and heterogeneity of repolarization are established noninvasive predictors of arrhythmic events in

patients with cardiovascular diseases (*73–77*) and in healthy subjects (*78*). Dispersion of repolarization, a noninvasive electrocardiographic (EKG) measure of spatial heterogeneity of repolarization, was increased in patients with COPD (*79*), but also in healthy subjects in response to hypoxemia (*80*).

These observations suggest that detailed analyses of the continuous ambulatory EKG for specific parameters, including heart rate variability and repolarization, might provide insights into the nature of the cardiac effects of pollutant exposure.

Pharmacokinetics of Inhaled Vapors

Although inhaled chemicals such as NO_2, O_3, and SO_2 may cause direct damage to airway and lung tissue, other vapors diffuse very rapidly from the alveolar spaces, through the lung tissue, and into blood, causing no direct lung injury. CO diffuses into the blood and binds avidly to hemoglobin. Other volatile chemicals are absorbed by inhalation and subsequently cause toxicity at sites distal to the respiratory tract. In addition, with some of these chemicals, it is not the parent compound that is most toxic but the metabolites, which may be more reactive and more damaging to the tissue.

The ultimate objective of many toxicologic studies is to take results from animal studies and attempt to predict or describe expected human responses. Physiologically based pharmacokinetic (PBPK) models are used in an attempt to describe the overall disposition of an inhaled chemical by simulating the uptake, distribution, metabolism, and elimination of the inhaled material (*81*). The PBPK model is then used in extrapolation between species based on animal scaling techniques. The adequacy of interspecies models should be tested by extrapolating the results of experiments with animal models to humans and comparing them with the relatively limited data available from controlled human exposures.

Although the strengths and limitations of the PBPK approach are beyond the scope of this article, Gargas and Andersen (*81*) previously reviewed the necessary parameters required to develop such models as well as the application to several chemicals. Such models have been developed for styrene (*82*) comparing data for rats and humans. The extrapolated model performed very well, predicting the concentration profile in humans exposed to 80 ppm styrene. Utell and co-workers (*83*) examined the pharmacokinetics of cyclic siloxanes in quantitative controlled exposure studies. Silicones are commonly found in personal care products including antiperspirants and hair sprays, with potential for exposure by the respiratory route. Octamethlycyclotetrasiloxane (D4), a volatile siloxane, is a major silicone component of these consumer products and as many as 200 million Americans are exposed to D4 in personal care products on a daily basis.

In these studies, 12 healthy subjects inhaled 10 ppm D4 (122 µg/L) or air (control) during a 1-hr exposure via a mouthpiece. Inspiratory and expiratory D4 levels were continuously measured as well as exhaled air and plasma levels before, during, and after exposure. Mean D4 intake was 137 mg, with a mean uptake of 13 mg (intake × deposition fraction). The mean deposition fraction at rest was 13% and with exercise it fell to 7%. Plasma measurements of D4 gave a mean peak value of 79 ng/g and indicated a rapid nonlinear blood clearance. These types of data regarding the mean D4 deposition fraction and uptake are applicable to estimates of exposure from both consumer product use and occupational exposure to D4 vapor. Subsequent studies using ^{14}C-labeled D4 should allow even better quantitative assessments and detection of metabolites in blood and urine. Comparison with data from ongoing animal studies (*84,85*) will allow development of PBPK models for extrapolation to humans. The data from controlled human studies should contribute in a meaningful way to the risk assessment process.

Studying Susceptible Subjects

The concept of susceptibility is highly relevant to public health protection and to the delivery of health care. The United States Clean Air Act (*86*) mandated that national ambient air quality standards be established to protect the health of all susceptible groups within the population.

There is a high degree of variability in human responses to environmental agents. For example, decrements in FEV_1 following 4-hr exposures to 0.22 ppm O_3 varied from 0 to more than 50% (Figure 1) (*16*), and yet the determinants of this variability are largely unknown. Some of the known and postulated factors influencing variability in human responses to pollutants are as follows: age, gender, body size, exercise, pre-existing disease, atopy, infection, airway geometry, smoking, pregnancy, exposure history, airways responsiveness, nutritional status, antioxidant vitamin intake, and antioxidant enzyme expression. Variability is generally increased in populations with respiratory disease, especially asthma. Some of the observed variability may be related to the actual dose delivered to the lung epithelium or to the distribution of the inhaled pollutant. For example, retention of insoluble 2 µm particles was increased 50% in subjects with COPD compared with healthy subjects, and the increased deposition correlated with the severity of obstruction (*87*). The diversity of human responses and responsiveness must be considered in designing and conducting human clinical studies of pollutant effects, and careful attention must be given to subject selection and characterization.

The concept of susceptibility is often oversimplified by both researchers and regulators. Rather than a single characteristic of a given population, susceptibility varies by pollutant and by the health effect being considered. There may be multiple health effects of pollutant exposure (i.e., symptoms, lung function decrements, airway inflammation, infection, cardiac effects, etc.), with a number of susceptible populations for which mechanisms and susceptibility factors differ. Susceptibility for a given health effect may not confer susceptibility to a different health effect. A striking example is exposure to O_3: individuals who experience the greatest reductions in lung function are not necessarily more likely to experience airway inflammatory effects (*19,35*). Furthermore, the mechanisms by which pollutant exposure contributes to respiratory mortality may differ from those responsible for excess cardiovascular mortality.

An understanding of the mechanisms by which ambient concentrations of pollutants increase mortality will require human clinical studies of susceptible subjects. However, such studies are limited by the special needs and changing health status of the subjects. Increased variability in measured parameters often requires that studies of susceptible subjects have larger numbers of subjects per study group compared with studies of healthy subjects. Medication use may alter responses in some subjects. Measures must be taken to protect the safety of susceptible subjects, who may be more vulnerable to adverse effects or complications from testing procedures or pollutant exposures.

In this section we focus on the increased susceptibility conferred by three chronic conditions: asthma, COPD, and cardiovascular disease.

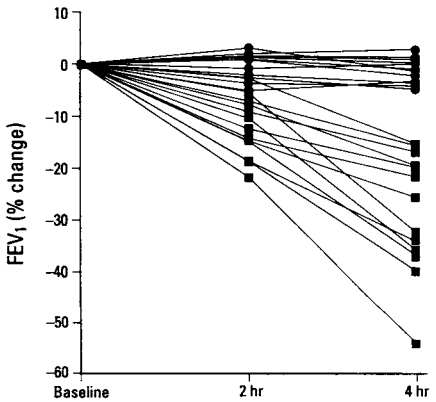

Figure 1. Percent change in FEV_1 during and after exposure to 0.22 ppm ozone, with intermittent exercise, in healthy nonsmoking subjects who were either nonresponsive (change in FEV_1 < 5%; ●) or responsive (change in FEV_1 > 15%; ■) to ozone. Data from Torres et al. (*19*).

Asthma

In clinical studies, asthmatics exhibit exaggerated lung function responses to SO_2, acidic aerosols, and in some studies, NO_2 and O_3. Asthmatics may also experience an enhanced airway inflammatory response to O_3.

In the laboratory, the most striking effect of acute exposure to SO_2 at concentrations of 1.0 ppm or below is the induction of bronchoconstriction in asthmatics after exposures lasting only 5 min (*88*). In contrast, inhalation of concentrations in excess of 5.0 ppm causes only small decrements in airway function in normal subjects. Similarly, clinical studies have identified exercising adolescent asthmatics (*89*) and adult asthmatics (*90*) as susceptible to sulfuric acid aerosols at high ambient concentrations, levels that do not affect healthy volunteers. Although several controlled human studies have found asthmatics responsive to low levels of NO_2, the findings have not been consistent (*91*). The conflicting results among these studies are probably related to differences in subject selection and exposure protocols.

A number of epidemiologic studies have suggested that emergency room and hospital visits for asthma are increased on high-O_3 days. It is therefore surprising that most controlled clinical studies generally have not found striking differences in lung function responses to O_3 in asthmatic compared with healthy subjects. Several possible explanations exist. In contrast to studies with healthy volunteers, few studies of asthmatic subjects have been performed using prolonged exposures or repeated daily exposures. Furthermore, few studies with asthmatic subjects have incorporated multiple periods of moderate-to-intense exercise, a factor that contributes to changes in airway function with low-level O_3 exposure in healthy volunteers.

One study has addressed some of these issues. Horstman and colleagues (*92*) exposed 17 subjects with clinically active asthma and 13 healthy subjects to 0.16 ppm O_3 for 7.6 hr, with multiple prolonged periods of mild exercise. As shown in Table 2, asthmatic subjects had significantly larger decrements in FEV_1 and in FEV_1/FVC, despite occasional use of bronchodilators before and during the exposure. This study suggests that people with clinically active asthma may be at increased risk for bronchoconstriction following prolonged exposures to environmentally relevant concentrations of O_3.

Two recent studies suggested that mild asthmatics may experience a neutrophilic airway inflammatory response to O_3 exposure that is more intense than in healthy subjects (*22,93*). Recent studies have also shown that, in asthmatics, O_3 exposures at concentrations sufficient to induce an airway inflammatory response increase responsiveness to a subsequent allergen challenge (*94*). Clinical investigations therefore suggest that the mechanisms for exacerbation of asthma following O_3 exposure include bronchoconstriction, worsening of airway inflammation, and increased responsiveness to allergen challenge.

Asthmatics may also be more sensitive to combinations of pollutants. Frampton et al. (*95*) examined the effects of prior exposure to low-level sulfuric acid aerosol on the airway response to O_3 in healthy and asthmatic subjects. Exposure–response relationships were examined using three levels of O_3, 0.08, 0.12, and 0.18 ppm. The exposures were preceded 24 hr earlier by exposure to 100 $\mu g/m^3$ H_2SO_4 or NaCl aerosol. The acidic aerosol and oxidant exposures were 3 hr in duration. Thirty healthy and 30 allergic asthmatics were studied. The findings revealed an interactive relationship between the O_3 exposure concentration and H_2SO_4 or NaCl aerosol preexposure in asthmatics but not in healthy subjects. For the asthmatic subjects, O_3 concentration-related differences in lung function were observed with H_2SO_4 preexposure but not with NaCl preexposure. These effects were observed for both FVC and FEV_1 immediately and 4 hr after O_3 exposure. These data suggest that preexposure to H_2SO_4 aerosols may alter responses to O_3 in exercising asthmatics.

Chronic Obstructive Pulmonary Disease

Effects of inhaled pollutants in COPD have not been extensively examined. Patients with COPD demonstrated similar or decreased lung function responsiveness to O_3 at levels up to 0.30 ppm compared with healthy nonsmokers of comparable ages (*96*). To determine if low-level NO_2 induces changes in pulmonary function, Morrow et al. (*97*) investigated responses to inhalation of 0.3 ppm NO_2 for 4 hr in 20 COPD subjects with a mean age of 60 years, and all with a history of cigarette smoking. These subjects were compared with 20 elderly healthy subjects. Criteria for inclusion included dyspnea on exertion, airways obstruction [FEV_1/FVC = 0.58 ± 0.09 (SD)] and a lack of response to inhaled bronchodilators. During intermittent light exercise breathing NO_2, COPD subjects demonstrated progressive decrements from baseline in FVC and FEV_1 and no change with air. Subgroup analyses suggested that responsiveness to NO_2 decreased with increased severity of the COPD.

Cardiovascular Disease

Studies of low-level exposure to CO have focused on subpopulations with ischemic heart disease and peripheral vascular disease. In patients with exertional angina, early onset of angina pectoris and depression of the ST-segment on the electrocardiogram (a marker of myocardial ischemia) have been consistently observed at carboxyhemoglobin (COHb) levels of 2–4% by several investigative teams. In the largest of these studies, the Health Effects Institute Multicenter CO Study (*98*), 5 and 12% decreases in the time to onset of ST-segment depression were observed at COHb levels of 2 and 4%, respectively. Significant decreases in time to onset of angina were also demonstrated at these COHb levels. These end points are remarkably consistent and are compatible with the hypothesis that an elevated COHb level impairs the response of the myocardium to increased metabolic demands.

New Experimental Approaches

Concentrated Ambient Air Particles

Ambient air contains a complex mixture of particles and gases containing a variety of chemicals at trace levels that may interact in causing health effects. A technology recently developed allows fine particles in the air to be concentrated in real time. Air is drawn through a series of virtual impactors designed so that particles less than 2.5 μm in aerodynamic diameter are progressively concentrated up to 25- to 30-fold (*99*). Ambient gases, and particles in the ultrafine size range (< 100 nm diameter) are not concentrated using this methodology.

This instrument has been used to demonstrate effects of ambient particulate matter in animals. For example, Gordon and colleagues (*100*) exposed rats to concentrated ambient air particles (CAPs) or filtered air for 3 hr, and found a significant but transient increase in circulating PMNs along with small alterations in heart rate associated with CAPs exposure. Godleski and colleagues (*72*) exposed 12 dogs, 6 of which had surgically placed devices to induce transient coronary occlusions, to CAPs or filtered air on multiple occasions. In the 6 surgically treated dogs, exposure to CAPs was associated with a shortened time to ST elevation and an increased magnitude of ST elevation during

Table 2. Lung function changes following exposure to 0.16 ppm ozone for 7.6 hr.[a]

	Asthmatic		Nonasthmatic		
	Air	Ozone	Air	Ozone	p^b
FVC (% change)	−0.3 ± 1.5	−12.2 ± 3.1	0.5 ± 0.9	−8.3 ± 1.8	0.33
FEV_1 (% change)	2.7 ± 3.0	−16.7 ± 4.2	1.2 ± 0.8	−8.6 ± 1.9	0.04
FEV_1/FVC (%)	2 ± 1	−4 ± 2	0 ± 1	−1 ± 1	0.02

[a]All values represent postexposure minus preexposure, means ± SE. [b]*p*-value: asthmatic vs nonasthmatic. Data from Horstman et al. (*92*).

coronary occlusion. The implications of these studies for human health remain unclear, and human studies using CAPs are now in progress (*101,102*).

The key strength of this technology is the ability to separate particle effects from gaseous pollutant effects in the atmospheric mix. However, there are several potential problems. The actual exposure concentration and particle composition may vary day to day and even hour to hour, depending on changes in outdoor particle levels and sources during the experiment. The limited ability to define the chemistry of the CAPs, and the day-to-day changes in particle composition, introduces an additional element of variability into the experimental design. In addition, the failure to concentrate ambient ultrafine particles may have significance. Animal exposure studies suggest that ultrafine particles have increased potential to induce an airway inflammatory response when compared on an equal mass basis with larger particles, perhaps because of their greater number and surface area (*103*). The relative absence of ultrafine particles and gaseous pollutants must be considered when interpreting the results of CAPs studies.

Diesel Engine Exhaust

Another approach to the problem of mixtures is illustrated by recent studies of exposure to diesel exhaust. Diesel engines have gained favor in may parts of the world because of reduced operating costs and reduced emissions of CO, CO_2, and hydrocarbons, compared with gasoline engines. However, they appear to release greater quantities of fine and ultrafine particles and nitrogen oxides (*104*).

Salvi et al. (*30*) exposed healthy subjects for 1 hr to dilute exhaust from an operating Volvo diesel engine in a specially designed exposure facility. Bronchoscopy with bronchoalveolar lavage and bronchial biopsy were performed 6 hr after exposure. In comparison with control air exposures, a significant increase was observed in epithelial leukocytes and mast cells, with increased expression of the adhesion molecules ICAM-1 and VCAM-1 in submucosal endothelium. Thus, diesel exhaust induces airway inflammation in healthy subjects in the absence of effects on lung function.

Nasal challenge with diesel exhaust particles has been shown to enhance the local ragweed-specific IgE response in subjects with allergic rhinitis and to drive the nasal cytokine response toward the Th2, or allergic phenotype (*105*). These findings raise concerns about diesel exhaust as a contributor to the increased prevalence of allergic rhinitis and asthma.

"Smoking" Machines

Using smoking machines, controlled exposure studies have examined the role of environmental tobacco smoke (ETS) in the exacerbation of asthma (*106*). Of course, by design, participants cannot be blinded to the ETS exposure. Typically, brief exposure to ETS produces symptoms such as eye and nasopharyngeal irritation. Although there are a few positive studies with ETS, the majority document no significant effect on FEV_1 or measures of airway responsiveness. However, study design has not been optimal; limited sample size, brief duration of exposure, and unrealistic exposure designs all emphasize the need for careful protocol development if this important clinical and social issue is to be better understood.

Model Particle Exposures

Studies of CAPs, diesel exhaust, and other real-world particles are unable to determine which particle characteristics, or combinations of characteristics, are responsible for the observed effects. One approach to this problem is to use particles with specific size characteristics or chemical composition to test specific hypotheses. This approach permits careful control of the particle to be tested, and permits precise exposure–response assessments. However, the particle design must be guided by detailed chemical and size characterization of ambient particles and by preliminary animal exposure studies to assure safety.

Our laboratory is using this approach to test the hypothesis that particles in the ultrafine size range have greater potential than larger particles to induce airway inflammation, systemic acute phase responses, and changes in cardiac repolarization. We have developed and tested a facility that permits exposures of humans to ultrafine particles of varying composition and that also permits the quantitative determination of exposure levels, respiratory intakes, and depositions of the aerosol. Using this facility, we have initiated clinical studies of exposure to ultrafine particles in healthy human subjects at rest and exercising (*107*).

Conclusions and Future Directions for Human Studies

Controlled clinical studies provide a means for examining responses to air pollutants and chemical vapors, especially those identified from epidemiologic studies. Well-characterized exposures have been performed either in environmental chambers or by mouthpiece and the responses assessed primarily by respiratory mechanics or from direct sampling of respiratory tract fluids, cells, and tissues. BAL studies have demonstrated pollutant-induced inflammation, lung injury, and decreased host defense capacity. Less invasive techniques such as nasal lavage and induced sputum also have been used to show pollutant-induced inflammation. Clinical studies have used a wide range of potentially sensitive subpopulations including asthmatics, children, the elderly, and people with COPD and coronary artery disease. To date, clinical studies with air pollutants have identified susceptible populations, characterized exposure–response relationships, and examined lowest-effect levels. With recent epidemiologic observations linking elevated particle concentrations with adverse cardiovascular events, clinical studies will focus on markers of systemic effects and changes in cardiac function linked with acute cardiac events. The introduction of the particle concentrator should strengthen the ability of clinical studies to study real ambient mixtures.

A variety of opportunities exist for extension of clinical studies into new arenas. Zelikoff et al. (*29*) examined effects of H_2SO_4 aerosol exposures, comparing responses in a laboratory animal model commonly used for toxicologic assessment with those observed in human volunteers. The purpose of this collaborative study was to provide a basis for extrapolation of findings in animals to humans. Substantial concordance between humans and rabbits was found with regard to the cells recovered in BAL fluid, and alveolar macrophage immuno-responsiveness, in relation to H_2SO_4 exposure. For example, in both species, a single 2-hr inhalation exposure to H_2SO_4 aerosol failed to evoke an inflammatory response, alter lavageable protein levels, or produce changes in cell viability. The results of directly comparative studies can provide data necessary for risk assessment and for predicting the accuracy of extrapolation modeling.

Finally, there are unique opportunities to combine the strengths of clinical and epidemiologic studies in the effort to understand mechanisms of susceptibility to pollutant effects. Hackney et al. (*108*) recognized the usefulness of this approach, and in a very limited study, found that four healthy subjects residing in Canada showed greater decrements in FEV_1 following laboratory exposures to O_3 than did four residents of southern California. This was considered to be evidence that chronic exposure to O_3 in California blunted responsiveness in those subjects, although the number of subjects was too small to derive any definite conclusions.

A parallel approach may prove useful in determining the mechanisms involved in responses to particle exposure. For example, groups of subjects identified from an epidemiology or panel study as susceptible (symptoms, lung function, medication use, heart rate variability, etc.) or nonsusceptible to particle effects could be studied in the laboratory under controlled conditions, either to concentrated ambient particles or to a model particle. Conversely, groups of individuals determined to be responders in a clinical study could be followed in a panel study to determine whether laboratory responses were

reproduced with actual ambient exposures. These approaches will require a new level of collaboration between investigators with expertise in epidemiology and human clinical studies but may provide key clues in our understanding of the mechanisms for susceptibility to pollutant effects.

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