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Authors

Rom, William N
Boushey, Homer
Caplan, Arthur

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Perspective

Experimental Human Exposure to Air Pollutants Is Essential to Understand Adverse Health Effects

William N. Rom[1], Homer Boushey[2], and Arthur Caplan[3]

[1]Division of Pulmonary, Critical Care, and Sleep Medicine, and [3]Division of Medical Ethics, Department of Population Health, New York University School of Medicine, New York, New York; and [2]Division of Pulmonary and Critical Care Medicine, University of California, San Francisco, San Francisco, California

Air pollution has been found to cause significant global mortality, with 6.8 million excess deaths attributed to air pollution each year, and similarly large numbers of exacerbations of asthma, chronic obstructive pulmonary disease, and cardiovascular diseases. Epidemiological research has identified associations, and experimental human exposure has provided critical information on dose–response relationships of adverse effects caused by controlled human exposure to individual pollutants. Human exposures further enable examination of the relationship of adverse effects such as symptoms and pulmonary function changes to presumed mechanisms of disease revealed through analysis of bronchoalveolar lavage fluid obtained from the lower respiratory tract. In this Perspective, we analyze the ethics of human exposure, the importance of the information gained, and the risks of such exposure. We find that these studies appear to have been done with proper approval of institutional review boards, were done with informed consent from the participants, and have rarely been associated with serious adverse events.

Keywords: chamber studies; ozone; particulate matter

A 2012 World Health Organization (WHO) study found that 3.5 million people die prematurely every year from indoor air pollution. Another 3.3 million die from outdoor air pollution (1). That air pollution can increase mortality was brought home forcefully by the air pollution disasters in Donora, Pennsylvania, in 1948 and London's great smog in 1952. Subsequent studies chronicled associations between air pollution and excess hospitalizations, particularly in the elderly, for chronic obstructive pulmonary disease (COPD), congestive heart failure, acute myocardial infarction, and stroke. With the advent of air pollution measurements, at first for black smoke and later for fine particulate and gaseous pollutants, epidemiological studies showed associations in metropolitan areas between varying levels of pollution exposure and negative health effects. Animal and *in vitro* studies substantiated these findings, prompting passage of the Clean Air Act and its amendments, requiring the U.S. government to set National Ambient Air Quality Standards (NAAQS). These guidelines were written to protect not just healthy individuals but also sensitive subgroups within the population. The Environmental Protection Agency (EPA) prepares a Criteria Document on scheduled pollutants (CO, NO_2, SO_2, $PM_{2.5}$, ozone, and lead) that is an integrated assessment of scientific studies followed by a staff paper on policy, risk assessments, and recommendations on ranges of standards for consideration. The reviews are supposed to be done every 5 years. These recommendations are reviewed by the Clean Air Scientific Advisory Committee with public input before promulgation of a new standard. Linkage of attaining the standards to the receipt of federal funds encouraged state and local health and air quality divisions to meet them; however, sanctions have rarely been applied. Estimates of the costs and benefits of achieving the standards have generally shown highly favorable ratios, on the order of 1:30.

While the initial studies calling attention to the associations between increases in morbidity and mortality and air pollution were epidemiological, elucidation of associations with specific air pollutants, including the "criteria" pollutants specifically named in the Clean Air Act, required approaches permitting greater control and precision to overcome the limitations of epidemiologic studies. These include correcting for imprecision in the measurement of actual pollutant exposures, the presumption of attributing observed effects to a dominant pollutant among the mix of different pollutants always present in urban atmospheres, and the effects of confounding personal behaviors (e.g., smoking, occupational, socio-economic, and local environmental factors).

Absent the ability to demonstrate mechanisms of effect, epidemiologic studies can only provide circumstantial evidence of associations between exposures and outcomes. While information on the mechanisms of air pollutant effects can be inferred from studies of laboratory animals, the generalizability of the findings to humans is uncertain, especially to a possibly particularly sensitive subgroup of the population, such as those with asthma or COPD. Studies of carefully characterized human volunteers have thus proved necessary to establish the relationship between exposure to a known concentration of a defined pollutant or pollutant mixture and an adverse health effect, to establish the relevance of mechanisms of action observed in animals to the effects of pollutant exposure in humans, and, especially, to examine the responsiveness of possibly unusually sensitive subpopulations. These scientific considerations underlie the use of precisely defined exposures of informed human volunteers to particular pollutants in carefully regulated environments. We carefully explore the medical ethics of chamber or controlled exposure studies to particulate matter (PM) and ozone with consideration of adverse health effects as end-points.

THE ETHICAL RATIONALE FOR "CHALLENGE" STUDIES

Studies that involve exposing human subjects to varying levels of pollutants are known as "challenge" studies. Since there is no possibility or intent to benefit subjects, and since some are recruited precisely because of the suspicion that they are especially reactive to various pollutants and toxic substances, challenge studies are

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Correspondence and requests for reprints should be addressed to William N. Rom, M.D., M.P.H., Division of Pulmonary, Critical Care, and Sleep Medicine, New York University School of Medicine, NYU Langone Medical Center, 550 1st Ave, Bellevue Chest Service Room 7N24, New York, NY 10016. E-mail: william.rom@med.nyu.edu

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seen by many as controversial and by some as simply unethical (2). Given the importance of challenge studies in establishing precise parameters for exposures and the need to demonstrate mechanisms of effect to provide evidence sufficient to secure regulation and enforcement of air pollution standards, what must be done to ensure the ethics of research where no direct benefit to subjects is possible?

First, studies involving human subjects being exposed to air pollutants need review by the local ethics committee; in the United States this is an Institutional Review Board (IRB). An IRB must have at least five members with sufficient diversity with respect to gender, race, cultural background, and professional expertise. Each IRB must include members whose primary concerns are in scientific areas and at least one member not affiliated with the institution. Most IRBs are composed of translational scientists covering the broad spectrum of organ systems being evaluated, plus experts and knowledgeable individuals on ethics including religious authorities, trained ethicists, nurses, and patient advocates as well as one or more community members. The primary duties of the IRB are to ensure that risk to subjects are minimized; that the risks to subjects are reasonable in relation to anticipated benefits; that the selection of subjects is equitable; that the purposes, procedures, and risks of the study are fully described in obtaining informed consent; that such consent is obtained from every subject or their legal surrogate; and that this informed consent is documented. The IRB additionally scrutinizes the form used for obtaining consent for clarity of language and proper grade level for understanding.

In assessing challenge studies the IRB must ensure that there is no other way to obtain the requisite information other than deliberate human exposure; that the risks involved are minimized and are no greater than a rational, autonomous individual might choose to accept to help advance knowledge; and that the information sought is worth the risks to subject welfare. While some might argue that no challenge study can meet the test of reasonable risk, a risk-free environment is not required to conduct human subjects research. IRBs can and do approve studies in which there is risk and no prospect of benefit to subjects, or in which benefit is highly unlikely (i.e., Phase One studies). Since many of those involved in challenge studies are compensated for their time and effort participating in a study, one of the challenges for an IRB is ensuring that payment is reasonable and not so overwhelming as to blind prospective subjects to the reality of the risk that many challenge studies entail.

The other ethical norm that is crucial to the conduct of challenge study research is informed consent. The subject must fully understand the risks involved and must be willing to face the possibility of risk to their health, even if minimal in terms of severity or likelihood. Subjects are allowed to choose to participate in research with more than minimal risk, so long as the description of the nature, severity, and likelihood of the risks entailed are described in a consent form that has been approved by an IRB and so long as the subject appears to fully understand them and is well aware of their right to withdraw from a research study at any time. This means that children and other vulnerable populations who cannot give credible voluntary consent are very difficult to enroll in challenge studies regardless of the benefit of the knowledge that might be gained. These subjects could be enrolled in such studies only in extraordinary circumstances regardless of the benefit of the knowledge that might be gained.

The U.S. EPA has both supported and conducted human exposure to PM and ozone. The EPA has been sued by entities opposed to environmental regulations with allegations that the informed consent process was unethical and that susceptible individuals were exposed to dangerous and life-threatening levels of PM or ozone. Criticisms have focused on health impairments of study subjects including asthma, age, metabolic syndrome including obesity, and even modest payments that were challenged as coercive to subjects of severely limited means. Furthermore, the EPA has been challenged that the exposures could cause death, and that the informed consent process fails to adequately warn of this dire possibility.

In addition to review by committees and informed participant consent, environmental justice may play a role in conducting challenge studies. There is a heterogeneous, inequitable distribution of environmental and occupational exposure in the United States. Disadvantaged groups include various racial and ethnic minority populations, particularly those living within urban areas, and the poor living in both developed and in developing nations. These individuals may encompass a new form of susceptibility in that their baseline level of pollutant exposure is raised and then they are intentionally exposed to an air pollutant in a research study (3). Subject selection in challenge studies must take into account how the burden of involvement in such studies can be fairly borne in societies with economic disparities. Unfortunately, too often the poor bear both the burden of excess pollution and participation in clinical research.

NATURE OF HUMAN EXPOSURE EVIDENCE

The nature of the evidence provided by human exposure studies necessarily differs for studies of acute versus chronic effects of exposure. For acute effects, such as discomfort (e.g., conjunctival irritation, cough, chest pain on inspiration), or transient impairment (e.g., reduced maximal exercise capacity), or changes in direct tests of organ function (e.g., decline in vital capacity or oxygen saturation, increase in carboxyhemoglobin), the findings of human exposure studies are direct and absolute. They provide evidence that exposure to a particular concentration of a named pollutant under defined conditions of exposure results in symptoms or impairments of measurable severity in a particular proportion of the population from which the volunteers were drawn (e.g., nonsmoking healthy young adults, adults with mild intermittent asthma, adult cigarette smokers with mild airflow obstruction, etc.). Studies of acute adverse health effects have thus included such outcomes as responses to respiratory symptom questionnaires, pulmonary function studies, tests of exercise capacity, and, in volunteers with asthma, their response to inhalational challenges. If a study of the effects of controlled exposure shows a change in an acute effects outcome that qualifies on its own as clinically important, that in itself is directly relevant to the setting of an ambient air quality standard for the pollutant in question. Thus, for example, establishing that 2 hours of exposure to levels of ozone that had been occasionally reached or exceeded in U.S. cities in the 1960s (150 ppb) caused chest pain on inspiration and a fall in FEV_1 greater than 5% or greater than 15% in SGaw in 6/20 healthy young nonsmoking adults (4, 5) could be considered sufficient evidence of an adverse health effect. This evidence justified setting a NAAQS at a lower level, on the grounds that a margin of safety was needed to protect members of the population in whom similar or even less severe chest pain or falls in FEV_1 would be considered important adverse health effects on their own merits—for example, in people of advanced age or with chronic lung disease. The case would be similar for a study showing that controlled exposure to a comparable level of ozone increased the response of subjects with asthma to inhalation of levels of, say, grass pollen achieved in outdoor air. Once it has been established that some level of ozone causes an acute adverse effect, additional exposure studies are no longer necessary to justify setting a NAAQS. They are necessary to determine whether similar effects might be caused by exposure to lower levels by people with different characteristics

or under different conditions of exposure (e.g., after repeated exposure, with intermittent exercise, under conditions of different temperature or humidity, etc.).

The health endpoints are different for studies of the effects of repeated exposure on chronic effects, such as accelerated loss of pulmonary function, increase in risk for development of asthma or COPD, or development of some disease outside the respiratory system, like hypertension or coronary artery disease. For these, the evidence from controlled human exposures is often indirect and less certain. The endpoints selected are most often biomarkers of injury and repair or surrogate markers of respiratory and cardiovascular health. The outcomes analyzed thus might include cells and mediators in blood, induced sputum, exhaled breath condensates, and the products of bronchial biopsy or bronchoalveolar lavage (BAL). The limitation of these endpoints has to do with uncertainty over the relationship between the changes observed after a few, or, most often, a single exposure and the development of disease associated with long-term chronic exposure. The relevance of the finding of a change in one of these endpoints to setting a NAAQS is a function of degree of confidence in the rationale linking a change in the endpoint after acute exposure to the development of disease with chronic exposure.

Views on this type of inference can certainly differ. What significance should be attributed, for example, to the finding of an influx of neutrophils in bronchial lavage fluid after brief exposure to a level of ozone causing no symptoms or impairments in pulmonary function (6, 7)? There can be no argument as to whether the ozone exposure has caused inflammation, but concluding that this constitutes an adverse health effect reflects the belief that the induction of inflammation after a single exposure suggests a heightened risk after repeated exposures for development of disease, or aggravation of a preexisting disease. The certainty of this belief often depends on inferences about the mechanisms of disease drawn from animal or tissue studies of the same or similar pollutants, or from epidemiological studies of associations between cumulative exposure and incidence of disease in populations. Seen in this light, studies of the effects of controlled exposures of human volunteers constitute only one leg of a tripod of evidence (epidemiological and laboratory studies being the other legs). It should be mentioned that the tests to evaluate the response themselves have risk; for example, BAL is done with local anesthesia including lidocaine which, if used in excess, can cause neurological symptoms and even death (8).

These general considerations about the relevance of findings of studies of controlled human exposures to pollutants can be illustrated specifically by review of the findings of controlled human exposure to ozone and to PM. Ozone is selected because it is the dominant member of the complex collection of oxidant pollutants found commonly in urban atmospheres, especially in areas with heavy contributions from mobile source combustion of petrochemical products, principally automotive combustion of gasoline. PM is selected because of its association with another product of petrochemical product combustion, especially from diesel-powered mobile sources.

EXPOSURE TO PM AND RESPONSES THOUGHT TO INDICATE HEIGHTENED RISK OF DEVELOPING ADVERSE HEALTH EFFECTS

Epidemiologic studies have suggested an association between exposure to PM and increases in cardiovascular mortality. To examine possible mechanisms of such an association, the EPA National Health and Environmental Effects Research Laboratory studied healthy volunteers ($n = 19$) to ultrafine concentrated ambient particulates (CAPS) (~ 50 $\mu g/m^3$) with intermittent exercise with BAL 18 hours after exposure (9). Surprisingly, there were no changes in pulmonary function or in the BAL cell counts. Interleukin-8 (IL-8) was, however, significantly increased in BAL, consistent with mild inflammation. Moreover, D-dimer was increased in blood, suggesting fibrin formation with attendant fibrinolysis. Changes in indices of variability in cardiac repolarization, increased QT variability index, and an increase in frequency domain markers of heart rate variability indicative of elevated vagal input to the heart were consistent with a cardiac response to PM. The most dramatic response to CAPS exposure may be the one described in a case report from the EPA Health Effects Laboratory. This report described a 58-year-old female who developed atrial flutter during CAPS exposure for 23 min (112 $\mu g/m^3$) without symptoms or changes on physical examination (10). She had had premature atrial contractions before the exposure, and had had similar findings when examined 2 years earlier. She was a never-smoker with mild hypertension treated with lisinopril and hydrochlorothiazide. Two hours after the onset of the arrhythmia, she spontaneously reverted to a normal sinus rhythm. Approximately 6 weeks later, atrial ectopy could not be provoked on an electrophysiologic study, but a reentrant circuit was ablated to prevent potential future episodes of atrial flutter. This was the only case report of a severe adverse cardiac event from controlled air pollutant exposure, but since cardiovascular effects were described from $PM_{2.5}$ exposure (11), its occurrence highlights the greater risk of exposure in subgroups with preexisting cardiovascular diseases and the importance of disclosing this risk to them in obtaining informed consent.

The approach used in this case report—analyzing cells and tissues obtained from the lungs after exposure to determine whether acute exposure caused changes thought from animal and epidemiological studies to be related to increased risk of development of disease with repeated exposure—was pioneered at the Pulmonary Branch of the National Heart, Lung, and Blood Institute (12). Research on the mechanisms of pulmonary fibrosis in individuals exposed to asbestos, coal, or silica showed that bronchoscopy with BAL was well tolerated with low risk of severe adverse events (13).

Increased levels of particulate pollution are associated with asthma exacerbations, increased respiratory symptoms, decreased lung function, increased medication use, and increased hospital admissions. PM exposure has also elucidated adverse health effects in humans. Ghio and Devlin extracted PM from air pollution filters collected near a steel mill in Utah Valley prior to a strike, during closure, and after reopening (14). Aqueous extracts containing 500 to 1,500 μg PM were instilled through the bronchoscope into the lungs of nonsmoking volunteers, and 24 hours later the same subsegment was lavaged. Exposure to aqueous extracts of PM collected before closure and after reopening of the steel mill provoked a greater inflammatory response with increased BAL neutrophils, protein, fibronectin, and IL-1β, IL-8, and TNF-α relative to PM extract acquired during the plant shutdown. This was reproduced in a similar study from Germany in which increased neutrophils and oxidant radicals were measured in the BAL cells (15). Concentrated ambient air particle exposure to 38 healthy human volunteers for 2 hours with intermittent exercise to concentrations ranging from 23 to 311 $\mu g/m^3$ (16) was performed with no respiratory symptoms noted and no pulmonary function test abnormalities detected after the exposures. BAL was performed 18 hours after exposure. There was an increase in the total number and percentage of neutrophils after exposure (2.5% CAPS vs. 0.8% air, $P = 0.016$). A lower range of exposures to CAPS (21–80 $\mu g/m^3$ for 2 h sitting to 10 persons aged 60–80 yr) found mild decreases in heart rate variability, with two subjects having increases in premature atrial contractions and three subjects

having greater than 5-fold increase in bradycardia of uncertain significance (17).

Fine particulate was noted to have effects on biomarkers in the blood, suggesting either of two hypotheses: that fine particulate caused changes in the lung that had systemic effects, or the particulate translocated across the epithelial–endothelial barrier and migrated directly into the blood. Mills and colleagues at the University of Edinburgh studied 20 men with stable coronary artery disease who had a history of myocardial infarction more than 6 months before enrollment (18). Men with angina pectoris, a history of arrhythmia, diabetes mellitus, uncontrolled hypertension, renal or hepatic failure, or unstable coronary disease were excluded. Exercise stress testing was performed, and those with left bundle branch block or early electrocardiographic ST-segment depression greater than 2 mm were ineligible. Exposures were for 1 hour with two 15-minute periods of exercise to 300 $\mu g/m^3$ diesel particulate with median particle diameter of 54 nm; these levels can be reached in heavy traffic or in the world's largest cities. The study subjects reported no symptoms of angina and had no major arrhythmias during exposure or in the subsequent 24 hours. Myocardial ischemia was detected during exercise in all study subjects, with greater maximum ST-segment depression during exposure to diesel exhaust than during exposure to filtered air. Acute plasma concentrations of tissue plasminogen activator (t-PA), PA-I, or blood counts or C-reactive protein were not affected by diesel exposure; however, endogenous fibrinolytic capacity measured by bradykinin-induced plasma t-PA was suppressed by 35% ($P = 0.009$). This effect on endogenous fibrinolysis was delayed until 6 hours, and is consistent with the observations of Peters and colleagues, who reported a second peak in the incidence of myocardial infarction 5 to 6 hours after exposure to traffic (19). Together, ST segment depression and reduced t-PA indicate an important thrombotic effect of diesel exhaust inhalation that may promote coronary thrombosis. Importantly, this study demonstrated the safety of studying susceptible subgroups.

EXPOSURE TO OZONE IN CHAMBERS WITH EXERCISE AND BAL MIMIC AMBIENT CONDITIONS

EPA's National Health and Environmental Effects Research Laboratory further developed the use of BAL in air pollution exposure studies (20–22), and investigators at the University of California, San Francisco undertook human exposure studies to evaluate relationships between changes in BAL to changes in pulmonary function and in bronchial reactivity to methacholine. Their findings showed that ozone exposure induced an influx of neutrophils into the bronchial mucosa and BAL in humans as it had previously been shown to do in dogs (23). Healthy volunteers were exposed to air or to ozone at 0.4 or 0.6 ppm for short periods of time (2 h) with intermittent exercise (24). Specific airway resistance increased in all 10 subjects immediately after both doses of ozone exposure with a dose response; BAL showed a significant difference in percent neutrophils: 2.3 ± 2.6% after sham exposure versus 18.5 ± 11.7% after exposure to O_3 (24). The 0.6 ppm O_3 exposure increased airway responsiveness for 24 hours (25). Five healthy humans exposed to O_3 at 0.3 ppm for 1 hour with exercise on four separate days induced cough, shortness of breath, chest discomfort on deep inspiration, throat irritation, chest congestion, and a fall in FEV_1 (26); analysis of BAL fluid showed a rise in the percent neutrophils from 3.7% at 1 hour, to 16.5% at 6 hours, and to 9.2% at 24 hours. Another study of 14 healthy athletes exposed to O_3 at 0.2 ppm for 4 hours with moderate exercise, followed by BAL 18 hours later, showed an increase in neutrophils from 2.1 ± 3.0 after filtered air exposure to 7.6 ± 3.9% after ozone exposure ($P < 0.002$) (27). The investigators also obtained bronchial biopsies and noted a significant increase in tissue $PMNs/cm^2$ compared with air ($P < 0.0025$). In both of these studies O_3 produced an inflammatory effect that occurred later and was uncorrelated with early reductions in FEV_1 and FVC.

EPA's Health Effects Research Laboratory collaborated with the University of North Carolina's Center for Environmental Medicine and Lung Biology: in their first study, they exposed nonsmoking, healthy volunteers ($n = 11$) to O_3 0.4 ppm or air for 2 hours with intermittent exercise with BAL performed 18 hours later (20). FEV_1 declined by 960 ± 180 ml after O_3 exposure compared with air exposure. The percentage of neutrophils in BAL increased 8.2-fold after exposure to O_3, and immunoreactive neutrophil elastase increased by 3.8-fold in BAL fluid and 20.6-fold in the BAL cells. The levels of fibronectin and prostaglandin E increased as well. These studies further compared nasal lavage (NL) to BAL using the same exposure protocol and again noted a significant increase in the percentage of neutrophils by 7.7-fold immediately after O_3 and 6.1-fold 18 hours after O_3 exposure (21). The NL percentage of neutrophils was an order of magnitude higher than BAL after O_3 exposure.

A nonchamber, "field exposure" study of joggers on Governors Island in New York in the summer ozone season compared with winter found the BAL fluid obtained in the summer to contain increased concentrations of lactic dehydrogenase, IL-8, and PGE_2 (28).

The consistency of evidence showing ozone exposure to cause inflammatory effects in the lungs of healthy volunteers, and the coincidentally growing body of evidence of chronic bronchial inflammation as important to the pathogenesis of asthma, prompted studies of whether asthma is associated with heightened sensitivity to ozone. Beginning in 1991, a series of chamber studies exposing subjects with atopic asthma to 0.12 to 0.27 ppm of ozone for 1 to 7.6 hours with or without exercise showed the ozone exposure reduced the amount of inhaled allergen needed to provoke a fall in FEV_1 (29) or an increase in airway eosinophilia in induced sputum (29–33). A UCSF study examined 14 subjects with asthma exposed to 0.2 ppm O_3 or air for 1 hour with intermittent exercise on separate days, followed a half hour later with challenge to *Dermatophagoides farinae*, and evaluated with spirometry and BAL (33). Ozone and air exposures did not differ in their effects on FEV_1 or on the provocative dose of allergen causing a 15% fall in FEV_1 (PC15), although the authors commented that allergen responsiveness appeared to have increased in a subgroup of the subjects with asthma. Another study of five subjects with atopic asthma and five normal volunteer subjects showed that exposure to 0.2 ppm O_3 for 6 hours with moderate exercise increased neutrophil numbers and, IL-8, IL-6, and albumin concentrations in BAL fluid obtained 18 hours later (6). There were no differences between subjects with asthma and healthy control subjects in baseline or post-exposure FEV_1, FVC, FEV_1/FVC, and sRAW. Linn and colleagues (34) found no significant changes in either pulmonary function or respiratory symptoms in a study of 22 subjects with asthma exposed to 0.2 to 0.25 ppm ozone for 2 hours with intermittent light exercise. McDonnell and coworkers (35) studied 28 atopic volunteer subjects exposed to 0.18 ppm ozone, finding no differences in response compared with nonatopic subjects.

Taken together, the evidence that brief exposure to levels of ozone that occur in urban areas induces changes in the cells and mediators of inflammation in the lungs and airways of healthy subjects and of subjects with asthma is remarkably consistent from study to study and from investigator team to investigator team. While, strictly speaking, the development of inflammation in the absence of development of distressing symptoms or of declines in pulmonary function suggesting important limitations

in respiratory function is not in itself an adverse health effect, the known importance of inflammation in the pathogenesis of asthma, chronic bronchitis, and pulmonary fibrosis certainly make it reasonable to presume that the induction of inflammation from a single exposure is a marker of risk for development of disease, or for aggravation of preexisting disease. Accordingly, the 1979 NAAQS standard of an hourly average not exceeding 0.12 ppm more than once a year was revised in 1997 to 0.08 ppm, as the annual fourth-highest daily maximum concentration averaged over 3 years. This was lowered further to 0.075 ppm in 2008.

Chamber studies at EPA's Health Effects Research Laboratory increased their exposure time to O_3 to 6.6 hours to resemble the waking work-day. In addition, exposures in the real world were lower than 0.12 ppm, requiring human experimental research at lower levels of O_3. Devlin and colleagues exposed nonsmoking males to either 0.10 ppm ($n = 10$) or 0.08 ppm ($n = 18$) ozone for 6.6 h with moderate exercise followed by BAL 18 h after exposure (22). There was a significant ($P < 0.05$) increase in the percentage of neutrophils after each O_3 exposure in the BAL (O_3 0.10 ppm from 1.6 ± 0.3 to 3.8 ± 0.7 and O_3 0.08 ppm from 1.8 ± 0.4 to 2.9 ± 0.6). In addition, the BAL fluid showed increases in prostaglandin E_2, fibronectin, protein, and IL-6 at both doses. An important exposure study was performed at UC Davis by Schelegle and coworkers using 31 healthy nonsmokers aged 18–25 years who completed five 6.6-hour chamber exposures: filtered air and four O_3 concentrations of 60, 70, 80, and 87 ppb (parts per billion) (36). Compared with filtered air, statistically significant decrements in FEV_1 and increases in total subjective symptom scores were measured after exposure to mean concentrations of 70, 80, and 87 ppb. There was a dose–response ranging from mean FEV_1% predicted at the end of the protocol. They used both a two-way ANOVA with repeated measures as well as nonparametric and parametric approaches for analysis of post-exposure FEV_1 data, finding similar results regardless of statistical method. To respond to these biostatistical considerations, Kim and colleagues at the National Health and Environmental Effects Research Laboratory studied a larger sample of 59 subjects exposed to a single dose of O_3 at 60 ppb for 6.6 hours compared with filtered air with moderate exercise during the winter, when ambient O_3 levels would be at their lowest (37). They found a significant ($P = 0.008$) decrease in FEV_1 of 1.7 ± 0.5% compared with filtered air, and a significant decrease in FVC of 2.3 ± 0.4%. They also found a 16% increase in the percentage of neutrophils in induced sputum after O_3 compared with filtered air ($P < 0.001$). Mudway and Kelly reviewed almost two dozen publications, performing a metaanalysis of O_3 studies with early decrements on lung function and late increases in parameters of inflammation (7). They characterized the neutrophil influx as a linear exposure relationship with onset at 3 hours and a mean of 16% neutrophils at the 18- to 24-hour postexposure BAL. Interestingly, they calculated that the threshold response for neutrophilia (645 mg/m^3) would be exceeded at the 0.08 ppm standard over 8 hours, at moderate V_E values of 10–11 L/minute/m^3. With this in mind, they suggested that further emphasis should be placed on limiting physical activity during pollution episodes. There may be chronic impacts on various health indices from cumulative exposures below the threshold values.

SUMMARY AND CONCLUSIONS

In summary, there has been a remarkable progression of translational research science over several decades using precise human exposures to model the real-world air pollution experience. The value of these exposure studies is at least 2-fold. They provide precise, irrefutable evidence of acute effects that can occur in human subjects on exposure to a particular pollutant or pollutant mixture under the conditions of exposure. In addition, they have elucidated mechanisms through measurements of lung function and inflammation using the powerful research tool of BAL. Since health standards must provide a margin of error and protect susceptible subgroups, individuals with asthma or coronary artery disease have recently been studied to ascertain their susceptibility. These studies have not only been illustrative, but have been remarkably safe; even exposure of members of sensitive subgroups, including individuals with asthma and individuals with atherosclerosis, appears so far to have a most minimal risk of severe adverse effects requiring medical intervention (38, 39). Since fine particulate matter can cross the alveolar–capillary barrier and enter the blood stream, acute effects can be identified on the cardiovascular system. In this regard, quasi-experimental studies on nonsmoking medical residents and healthy adults before, during, and after the Beijing Olympics demonstrated lower air pollutants during the Olympics that correlated with reduced pulmonary inflammation and oxidative stress (40, 41). An intervention using a highly efficient facemask used by 100 patients with coronary heart disease on Beijing streets showed reduced personal exposure to fine particulate that correlated with reduced symptoms and electrocardiographic ST segment depression (42). The remarkable record of safety of studies of controlled chamber and "real world" exposures is at least partially attributable to the intimate involvement of IRBs in reviewing protocols in detail, checking the wording of the informed consent, the mechanisms for recruitment, conducting audits, and mandating data safety monitoring plans for continuous ongoing survey of expected and unexpected adverse events. Subgroups of children and pregnant women are more difficult for research study, but children have been studied for induced sputum in novel exposure situations, and regression equations show inverse relationships between amount of carbon in alveolar macrophages and decline in lung function (43). Children also have reduced growth in lung function related to increased exposure to $PM_{2.5}$ (44). Limitations of human chamber studies have been low exposures for safety reasons, short exposure times, single or limited exposures that cannot model the multipollutant exposure of the real world, and the difficulty of separating the physiological effects of exercise from the effects of air pollutants. Studies in humans in real time are nonetheless essential to the understanding the adverse health effects of air pollution, and importantly, chamber studies have ethically modeled exposures at levels of pollutants above, at, or below promulgated air quality standards, measured adverse health effects, and performed this with a remarkable safety record.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, *et al*. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012;380: 2224–2260.
2. Hope T, McMillan J. Challenge studies of human volunteers: ethical issues. *J Med Ethics* 2004;30:110–116.
3. American Thoracic Society. What constitutes an adverse health effect of air pollution? Official statement of the American Thoracic Society. *Am J Respir Crit Care Med* 2000;161:665–673.
4. Kulle TJ, Sauder LR, Hebel JR, Chatham MD. Ozone response relationships in healthy nonsmokers. *Am Rev Respir Dis* 1985;132:36–41.
5. Balmes JR. The role of ozone exposure in the epidemiology of asthma. *Environ Health Perspect* 1993;101:219–224.
6. Basha MA, Gross KB, Gwizdala CJ, Haidar AH, Popovich J Jr. Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers

after controlled exposure to ozone and filtered purified air. *Chest* 1994;106: 1757–1765.
7. Mudway IS, Kelly FJ. An investigation of inhaled ozone dose and the magnitude of airway inflammation in healthy adults. *Am J Respir Crit Care Med* 2004;169:1089–1095.
8. Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B, Kraft M, Levine RJ, Peters SP, Sullivan EJ. Investigative bronchoprovocation and bronchoscopy in airway diseases. *Am J Respir Crit Care Med* 2005; 172:807–816.
9. Samet JM, Rappold A, Graff D, Cascio WE, Berntsen JH, Huang YC, Herbst M, Bassett M, Montilla T, Hazucha MJ, *et al*. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. *Am J Respir Crit Care Med* 2009;179:1034–1042.
10. Ghio AJ, Bassett M, Montilla T, Chung EH, Smith CB, Cascio WE, Carraway MS. Case report: supraventricular arrhythmia after exposure to concentrated ambient air pollution particles. *Environ Health Perspect* 2012;120:275–277.
11. Pope CA III, Muhlestein JB, May HT, Renlund DG, Anderson JL, Horne BD. Ischemic heart disease events triggered by short-term exposure to fine particulate air pollution. *Circulation* 2006;114:2443–2448.
12. Hunninghake GW, Kawanami O, Ferrans VJ, Young RC Jr, Roberts WC, Crystal RG. Characterization of the inflammatory and immune effector cells in the lung parenchyma of patients with interstitial lung disease. *Am Rev Respir Dis* 1981;123:407–412.
13. Rom WN, Bitterman PB, Rennard SI, Cantin AC, Crystal RG. Characterization of the lower respiratory tract inflammation of nonsmoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts. *Am Rev Respir Dis* 1987;136: 1429–1434.
14. Ghio AJ, Devlin RB. Inflammatory lung injury after bronchial instillation of air pollution particles. *Am J Respir Crit Care Med* 2001;164:704–708.
15. Schaumann F, Borm PJA, Herbrich A, Knoch J, Pitz M, Schins RPF, Luettig B, Hohlfeld JM, Heinrich J, Krug N. Metal-rich ambient particles (particulate matter $_{2.5}$) cause airway inflammation in healthy subjects. *Am J Respir Crit Care Med* 2004;170:898–903.
16. Ghio AJ, Kim C, Devlin RB. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. *Am J Respir Crit Care Med* 2000;162:981–988.
17. Devlin RB, Ghio AJ, Kehrl H, Sanders G, Cascio W. Elderly humans exposed to concentrated air pollution particles have decreased heart rate variability. *Eur Respir J Suppl* 2003;40:76s–80s.
18. Mills NL, Törnqvist H, Gonzalez MC, Vink E, Robinson SD, Söderberg S, Boon NA, Donaldson K, Sandström T, Blomberg A, *et al*. Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. *N Engl J Med* 2007;357:1075–1082.
19. Peters A, von Klot S, Heier M, Trentinaglia I, Hörmann A, Wichmann HE, Löwel H; Cooperative Health Research in the Region of Augsburg Study Group. Exposure to traffic and the onset of myocardial infarction. *N Engl J Med* 2004;351:1721–1730.
20. Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Horstman DH, Kozumbo WJ, Becker S, House DE, McDonnell WF, *et al*. Ozone-induced inflammation in the lower airways of human subjects. *Am Rev Respir Dis* 1989;139:407–415.
21. Graham DE, Koren HS. Biomarkers of inflammation in ozone-exposed humans: comparison of the nasal and bronchoalveolar lavage. *Am Rev Respir Dis* 1990;142:152–156.
22. Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schreinemachers D, Koren HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am J Respir Cell Mol Biol* 1991;4:72–81.
23. Holtzman MJ, Fabbri LM, O'Byrne PM, Gold BD, Aizawa H, Walters EH, Alpert SE, Nadel JA. Importance of airway inflammation for hyperresponsiveness induced by ozone. *Am Rev Respir Dis* 1983; 127:686–690.
24. Seltzer J, Bigby BG, Stulbarg M, Holtzman MJ, Nadel JA, Ueki IF, Leikauf GD, Goetzl EJ, Boushey HA. O_3-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J Appl Physiol* 1986;60:1321–1326.
25. Holtzman MJ, Cunningham JH, Sheller JR, Irsigler GB, Nadel JA, Boushey HA. Effect of ozone on bronchial reactivity in atopic and nonatopic subjects. *Am Rev Respir Dis* 1979;120:1059–1067.
26. Schelegle ES, Siefkin AD, McDonald RJ. Time course of ozone-induced neutrophilia in normal humans. *Am Rev Respir Dis* 1991;143:1353–1358.
27. Aris RM, Christian D, Hearne PQ, Kerr K, Finkbeiner WE, Balmes JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *Am Rev Respir Dis* 1993;148:1363–1372.
28. Kinney PL, Nilsen DM, Lippmann M, Brescia M, Gordon T, McGovern T, El-Fawal H, Devlin RB, Rom WN. Biomarkers of lung inflammation in recreational joggers exposed to ozone. *Am J Respir Crit Care Med* 1996;154:1430–1435.
29. Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, Szalai JP, Raizenne M, Slutsky AS, Zamel N. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. *Lancet* 1991; 338:199–203.
30. Ball BA, Folinsbee LJ, Peden DB, Kehrl HR. Allergen bronchoprovocation of patients with mild allergic asthma after ozone exposure. *J Allergy Clin Immunol* 1996;98:563–572.
31. Kehrl HR, Peden DB, Ball B, Folinsbee LJ, Horstman D. Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone. *J Allergy Clin Immunol* 1999;104:1198–1204.
32. Vagaggini B, Taccola M, Cianchetti S, Carnevali S, Bartoli ML, Bacci E, Dente FL, Di Franco A, Giannini D, Paggiaro PL. Ozone exposure increases eosinophilic airway response induced by previous allergen challenge. *Am J Respir Crit Care Med* 2002;166:1073–1077.
33. Chen LL, Tager IB, Peden DB, Christian DL, Ferrando RE, Welch BS, Balmes JR. Effect of ozone exposure on airway responses to inhaled allergen in asthmatic subjects. *Chest* 2004;125:2328–2335.
34. Linn WS, Buckley RD, Spier CE, Blessey RL, Jones MP, Fischer DA, Hackney JD. Health effects of ozone exposure in asthmatics. *Am Rev Respir Dis* 1978;117:835–843.
35. McDonnell WF, Horstman DH, Abdul-Salaam S, Raggio LJ, Green JA. The respiratory responses of subjects with allergic rhinitis to ozone exposure and their relationship to nonspecific airway reactivity. *Toxicol Ind Health* 1987;3:507–517.
36. Schelegle ES, Morales CA, Walby WF, Marion S, Allen RP. 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. *Am J Respir Crit Care Med* 2009;180:265–272.
37. Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC, Schmitt MT, Case M, Devlin RB, Peden DB, *et al*. Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. *Am J Respir Crit Care Med* 2011;183:1215–1221.
38. Törnqvist H, Mills NL, Gonzalez M, Miller MR, Robinson SD, Megson IL, Macnee W, Donaldson K, Söderberg S, Newby DE, *et al*. Persistent endothelial dysfunction in humans after diesel exhaust inhalation. *Am J Respir Crit Care Med* 2007;176:395–400.
39. Frampton MW, Stewart JC, Oberdörster G, Morrow PE, Chalupa D, Pietropaoli AP, Frasier LM, Speers DM, Cox C, Huang L-S, *et al*. Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. *Environ Health Perspect* 2006;114: 51–58.
40. Huang W, Wang G, Lu SE, Kipen H, Wang Y, Hu M, Lin W, Rich D, Ohman-Strickland P, Diehl SR, *et al*. Inflammatory and oxidative stress responses of healthy young adults to changes in air quality during the Beijing Olympics. *Am J Respir Crit Care Med* 2012;186: 1150–1159.
41. Rich DQ, Kipen HM, Huang W, Wang G, Wang Y, Zhu P, Ohman-Strickland P, Hu M, Philipp C, Diehl SR, *et al*. Association between changes in air pollution levels during the Beijing Olympics and biomarkers of inflammation and thrombosis in healthy young adults. *JAMA* 2012;307:2068–2078.
42. Langrish JP, Li X, Wang S, Lee MM, Barnes GD, Miller MR, Cassee FR, Boon NA, Donaldson K, Li J, *et al*. Reducing personal exposure to particulate air pollution improves cardiovascular health in patients with coronary heart disease. *Environ Health Perspect* 2011;119:1507–1512.
43. Kulkarni N, Pierse N, Rushton L, Grigg J. Carbon in airway macrophages and lung function in children. *N Engl J Med* 2006;355:21–30.
44. Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, *et al*. The effect of air pollution on lung development from 10 to 18 years of age. *N Engl J Med* 2004;351:1057–1067.