
Utility of Controlled Human Exposure Studies for Assessing the Health Effects of Complex Mixtures and Indoor Air Pollutants

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The study of health effects induced by exposure to mixtures of pollutants is a complex task. The purpose of this paper is to identify areas of research in which the conduct of human controlled exposure (clinical) studies may contribute to better understanding health effects of exposure to indoor air and other mixtures. The strengths and weaknesses of clinical studies in general are reviewed, as well as examples from the literature of approaches that have been used. Human chamber studies play an important role alongside epidemiologic and animal toxicologic studies in such research. Human chamber studies are limited with regard to assessing chronic effects, rare effects, or effects from long-duration exposures but are powerful in assessing acute, reversible effects from short-duration exposures in humans. The areas in which human chamber studies are most likely to contribute include identification of effects or markers of effects for exposure to a given pollutant or mix of pollutants; direct dose-response assessment of effects for individual compounds and mixtures of set composition; identification of individual compounds responsible for the effects of a mixture; study of the joint effects of a binary mixture; development of markers of acute exposure for particular compounds; development of outcome measurements to be used in the field; and identification, characterization, and testing of sensitive subpopulations. — *Environ Health Perspect* 101(Suppl 4):199–203 (1993)

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Introduction

Inferences about the health effects of exposure to mixtures of air pollutants are based generally upon data from some combination of clinical studies, epidemiologic studies, and animal toxicologic studies. The relative contribution of information from each of these study types is dependent on the exposure of interest, the nature of the health outcome, the relationship between exposure and outcome, the existence of natural experiments, and the availability of suitable animal models, among others. In many circumstances, the data generated are complementary, and simultaneous assessment of information from those sources allows gaps in knowledge to be filled and allows the consistency of findings among the different disciplines to be examined. In other circumstances, hypotheses may be generated in one type of study with subsequent testing in another type.

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The purpose of this paper is to identify ways in which controlled human-exposure studies can be used for direct measurement of exposures and effects, and can be integrated into a program of epidemiologic research to enhance our understanding of the health consequences of exposure to indoor air pollution and other complex mixtures.

Clinical studies are most useful in situations in which the mixtures of interest are well defined and easily produced and measured, and the outcomes of interest occur acutely, are reversible, and are measured easily with little error. At the other extreme are mixtures that are difficult to characterize and generate, and outcomes that occur only after a long period of exposure, are chronic in nature, and are rare and difficult to assess at an early stage. In the former case, effects of exposure can be assessed directly in human chamber studies. In the latter case, clinical studies can provide at best some information that may result in a more efficient epidemiologic study design or may provide information that lends plausibility to observed results.

Strengths and Weaknesses of Controlled Human Exposure Studies

A consideration of the utility of clinical studies must begin with a discussion of the strengths and weakness of such studies. A major advantage includes the control that

one exerts over the conditions of exposure. For example, the effects of either the individual components of a complex mixture or the mixture itself can be studied without having to identify locations with appropriate ambient conditions. This is important particularly when one is interested in studying the individual and joint effects of pollutants, such as ozone and acidic aerosols, which commonly occur together. Control of the exposure also allows one to concentrate on the conditions of most interest. Studies primarily interested in worst-case scenarios may include exposures with high pollutant concentrations and levels of ventilation and long duration, while other studies may focus on lower level exposures similar to those that occur for large segments of the population. Dose-response information can be generated for individual compounds or a specific mixture, and interaction between two compounds theoretically can be studied by varying the relative concentrations of each component. Because controlled human exposure studies are conducted generally in a permanent facility with resident staff, the availability of sophisticated equipment and expertise allows measurement of a greater variety of end points than may be feasible in field studies.

Another strong point of clinical studies is reduction of bias, leading to greater internal validity. Foremost among the strengths of clinical studies is the random

assignment of subjects to treatment groups, which reduces both confounding and selection bias. Elements of exposure, such as concentration, ventilation, and duration, typically can be measured more precisely than under conditions of ambient exposure. Similarly, depending on the end point, health outcome usually can be measured more precisely. Such reduction in measurement error of both exposure and effect reduces misclassification bias experienced in epidemiologic studies. A further advantage of clinical studies is the obvious temporal order in which exposure and effects occur. The ability to manipulate effects by varying exposure greatly increases the confidence that a given exposure causes a particular effect.

While clinical studies are powerful in the assessment of many effects of interest, there are some relevant exposures and outcomes that cannot be studied experimentally in humans for ethical or practical reasons. Obviously, one cannot conduct ethically a study in which permanent effects are induced in subjects. This appropriate ethical concern excludes direct study of the induction of all chronic diseases that generally have major impact on the affected individuals. Furthermore, the study of reversible effects that require prolonged exposure of subjects is not practical. For example, the effect of a season of exposure to ozone and acid aerosols on bronchoalveolar inflammation cannot be studied in an experimental setting.

Another potential limitation of clinical studies is the relatively small number of individuals that can be studied. Exposure chambers usually can accommodate only one to four individuals at a time. Given the duration of exposure, the need for multiple exposures, the amount of time required for measurement of outcomes and for maintenance and auditing of chamber equipment, one realizes quickly the constraints on the clinical study of large numbers of individuals. The statistical limitations imposed by restricted numbers of subjects makes it difficult to study exposures that produce small or imprecisely measured effects. In these cases, it is hard to detect changes that are small due either to the nature of the effect or to bias toward the null. Small numbers also make selection among competing dose-response models for a single compound and assessment of interactions between mixtures of pollutants difficult. Human chamber studies also are inefficient for the direct study of rare events. This deficiency can be overcome somewhat by measuring markers of the outcome of interest that may occur more frequently and that

can be measured with more precision. An example of this would be the production of asthma attacks by mixtures of ozone and acid aerosols. Actual attacks are the outcome of direct interest, but they occur rarely after a single chamber exposure. An increase in airway hyperreactivity, however, which may be associated with increased asthma attacks, can be measured easily in each exposed subject. Other potential approaches to the limitation of small numbers of subjects is aggregation of data from several studies and identification and study of particularly sensitive individuals for whom frequency and magnitude of effect is larger than for the population as a whole.

Experimental study of some complex mixtures is not realistic, either because mixtures are characterized poorly or are heterogeneous or because artificially generated mixtures are not comparable to those experienced in ambient air. An example of the former is the wide variety of volatile organic compounds (VOC) that are found in indoor air and that are suspected of causing sick-building syndrome. Neither one substance, nor a small number of compounds, nor a characteristic mix of compounds has been identified as most likely responsible for the syndrome. Rather different mixtures of compounds have been identified with a variety of sources (1). One approach to the study of poorly defined, variable mixtures has been the exposure of volunteers to a mixture containing 22 volatile organic compounds that are produced from a variety of sources (2,3). An example in which it is difficult to produce the complex mixture found in ambient air involves the study of the effects of acidic aerosols combined with photochemical oxidants. Differences in chemical composition and deposition characteristics may exist between naturally occurring aerosols and those generated artificially, and the mix of photochemical oxidants that occurs in ambient air is difficult to replicate because of the aging of the mixture that normally occurs. As a result, ozone, a chemically active and representative oxidant, has been used in such studies without inclusion of other oxidant species, while the aerosols usually have been limited to one-chemical species. While considerable information can be gained about some mixtures or about representative compounds in this manner, the true impact of ambient exposure to many complex mixtures can only be approximated in chamber studies.

A further concern about human experimental exposure studies as they have been traditionally carried out is that the population represented by the samples studied has

not been consistently well defined. While the subjects often are well characterized, they are generally volunteers recruited through advertising and, in many cases, from university campuses. Furthermore, in the interest of decreasing heterogeneity of responses and increasing internal validity, very homogeneous groups are usually studied, such as very healthy, never-smoking, white, male individuals, or mild asthmatics not on medication. This process for subject selection raises questions about the ability to generalize findings to other segments of the population not represented by these samples. In many instances, this may not be a major concern, and in cases where it is, different methods of recruitment and subject selection can be used to improve the external validity of a given study.

Historical Use of Human Exposure Chambers for Study of Mixtures

Historically, chamber studies of air pollutants have been conducted once an exposure of interest has been identified. For a number of individual pollutants (e.g., ozone and sulfur dioxide), the purposes accomplished include identification and description of health effects, exposure-response characterization, assessment of individual variability in response, identification of sensitive or susceptible populations, quantification of retained dose, and some insights into mechanisms of action. Study of more than one pollutant generally has been limited to comparisons between clean air and complex mixtures or to assessment of the individual and joint effects of a single concentration of each of two substances during simultaneous or sequential exposure. As has been pointed out by Greenland (4), unless the dose-response characteristics of each of the individual pollutants are known, this latter study design is inadequate for completely assessing the nature of the interaction between effects of more than one compound.

Mixtures that have been studied in chambers include, among others, ozone and a variety of acidic aerosols, ozone and sulfur dioxide, ozone and nitrogen dioxide, sulfur dioxide and acidic aerosols, ozone and peroxyacetyl nitrate (PAN), a complex mixture of 22 volatile organic compounds, and environmental tobacco smoke. Stacy et al. (5) exposed individuals to a mixture consisting of one gaseous pollutant (air, ozone, nitrogen dioxide, or sulfur dioxide) and one aerosol pollutant (air, sulfuric acid, ammonium sulfate, ammonium bisulfate, or ammonium nitrate). They observed no

joint effects that were different statistically from those produced by ozone alone, although the mixture of ozone and sulfuric acid produced effects that suggested some additional effect. A number of other investigators have also studied the effects of ozone combined with a variety of aerosolized acidic substances. While one recent abstract suggested that responses to mixtures of ozone and either sulfuric or nitric acid are somewhat larger than the sum of effects of the individual compounds (6), most studies have found no such evidence (7–12). For simultaneous exposures to a mixture of ozone and sulfur dioxide that also may result in production of sulfuric acid particles, some investigators have observed evidence of a joint effect slightly larger than that due to the sum of the effects of the individual pollutants (13,14), while the majority of investigators have not observed such an effect (15–17). For exposure to mixtures of ozone and nitrogen dioxide (18–22) and ozone and carbon monoxide (23), there is little convincing evidence that exposure to any of the mixtures has much effect beyond that attributable to ozone.

Dreschler-Parks et al. (24) reported that a mixture of ozone and peroxyacetyl nitrate causes lung function decrements larger than those due to exposure to individual pollutants. Avol et al. (25) used a slightly different approach in assessing the joint effects of exposure to the mixture of pollutants common to the Los Angeles basin. They exposed volunteers to purified air that contained 0.16-ppm ozone on one occasion, and on another occasion, they exposed the same individuals to ambient air that contained a similar concentration of ozone in addition to the other pollutants commonly found in the Los Angeles basin. Avol et al. observed no differences in the magnitude of acute respiratory responses of the ambient air compared to the purified air with ozone alone. They concluded that the acute respiratory effects of exposure to the complex mixture making up ambient air in Los Angeles could be attributed to ozone.

No single chemical or mixture of chemicals has been observed to be responsible for the variety of complaints that are associated with the sick-building syndrome. Rather, a variety of mixtures of diverse chemicals has been identified in buildings in which the number of complaints seem to be elevated. Molhave et al. (2) and Otto et al. (3) measured responses to a mixture of 22 volatile organic compounds that seem to occur often in buildings in which complaints are recorded. Sensory irritation was observed in both studies, and

memory deficits were observed in one study but not the other. Evidence indicates that this mixture also may result in an influx of inflammatory cells in the nose (26). Using a similar approach of measuring response to an entire mixture, Willes et al. (27) observed that exposure to environmental tobacco smoke (ETS) results in upper-respiratory symptoms and increased nasal resistance.

Use of Human Experimental Exposure Studies in Future Investigation

Having considered the strengths and weaknesses of clinical studies and the type of information that has been collected in the past both for exposure to individual pollutants and to mixtures of pollutants, one can better evaluate the possible contributions that clinical studies can make to direct assessment of effects of indoor air and other complex mixtures and to providing ancillary information that may enhance the design and interpretation of epidemiologic studies. Two approaches described by the U. S. Environmental Protection Agency (EPA) (28) include the “top down” and “bottom up” research strategies. The top down approach involves study of the mixture as a whole, with further study of fractions of the mixture to identify the causative agents and interactions among them. The bottom up approach involves study of the individual compounds as a first step followed by examination of the joint effects of mixtures of these individual compounds. Mauderly (29), in this supplement, refers to similar approaches used in toxicological assessment of mixtures: an integrative approach and a dissective approach (both top down) and a synthetic approach (bottom up). These paradigms also are useful for identifying areas of clinical research that may prove fruitful.

The integrative approach, as part of a top down strategy, concerns itself with assessment of the mixture as it exists in the ambient environment. This generally requires that the mixture, or a reasonable approximation, can be generated in a chamber setting. Two situations that appear worthy of study include the effects of environmental tobacco smoke and the effects of the mixture of 22 volatile organic compounds used to simulate an indoor environment in new buildings. Areas of research that seem most promising include generation of empirical evidence that either of these particular mixtures causes a given effect or a marker of a given effect; direct dose–response assessment of acute, reversible outcomes for

the mixtures; development of markers of acute exposure for particular compounds representative of the mixture; development of outcome measurements that also could be used in the field; and identification or characterization of sensitive subpopulations. Environmental tobacco smoke has been documented to produce symptoms and physiological effects in the nose. The VOC mixture produces nasal inflammation and symptoms and may produce neurobehavioral effects. Both of these mixtures can be produced and controlled during chamber studies: ETS by “smoking machines” that generate sidestream smoke and VOC by evaporation of the mixture of interest. Further elucidation of the spectrum of effects for each of these mixtures and dose–response characterization of these effects seem to be worthwhile pursuits. This may include assessment of nasal and ocular inflammation, stimulation of neural elements in the nasal cavity, alterations in breathing pattern or airway reactivity, and behavioral effects. Because many of the complaints about ETS or sick-building syndrome are subjective, identification of physiological outcomes may help elucidate the mechanisms underlying the symptoms. Many of the outcome measurements developed for chamber studies also could be modified for use in the field to assess effects of exposure in epidemiologic studies. Promising techniques include nasal washes, sampling of tears, and neurobehavioral testing. Questionnaires could be developed and standardized for use in both clinical and epidemiologic studies to facilitate comparison between studies.

Cotinine often is used as a marker of exposure to ETS. Because metabolism of nicotine may vary among individuals and among groups of different age or gender, and because many different exposure scenarios can result in a given cotinine level at one point in time, further work in developing cotinine as a marker of exposure can be carried out during periods of exposure or non-exposure to ETS in chamber studies. Similar pharmacokinetic studies could be carried out for individual VOCs contained in indoor mixtures. Relationships could be established between inhaled dose and concentrations in blood, urine, or exhaled air. Such information potentially could be useful for assessing exposure in free-living individuals participating in epidemiologic studies.

Another promising use of chamber exposures to ETS or VOC is as part of a hybrid epidemiologic–clinical study. In a questionnaire survey of an exposed population, one might identify individuals who

are unusually sensitive and others who are nonsensitive. These groups could be exposed under controlled conditions and examined both for concordance and reported symptoms during ambient exposure and for physiological differences in response that could account for symptom differences. Furthermore, depending upon the question to be addressed, the a priori ability to identify responsive individuals can increase the study efficiency through proper selection of subjects. Alternately, one could document in the chamber the responses of a group of individuals who were to move into a new building. Concordance between responses measured in the chamber and those in the new ambient environment may provide insight into the host factors responsible for differences in response and into the underlying basis for reported symptoms. Such information could be useful for study design and control of confounding in future epidemiologic studies.

Another area that should be explored for feasibility is the use of environments other than existing chambers for quasi-controlled human exposures. For example, many model houses used for air monitoring information exist (30). The pollutants in these structures represent exposures of interest and are well measured. The feasibility of exposing individuals to these mixtures in these facilities and measuring responses should be explored. Similarly, facilities in which the atmospheric chemistry of photochemical oxidants is studied could provide an opportunity to assess the effects of exposure of individuals to a number of representative mixtures, including ozone and acid aerosols. A third approach involves the use of mobile chambers, which would allow the hybrid epidemiologic-clinical studies discussed above to be conducted at many more locations. Atmospheres could be generated for study of individual responses to single compounds or to specific mixtures of pollutants at the site of an epidemiologic investigation. Alternately, ambient air from various locations at an epidemiologic study site could be drawn into the mobile chamber for measurement of individual responses and inhaled doses. Such an approach would allow the random assignment of individuals to environmental conditions.

The dissective component of the top down strategy begins with understanding the effects of exposure to the mixture and then involves further work to identify the individual pollutants responsible for the observed effects. Willes et al. (27) have done some preliminary work in this area by measuring the responses of sensitive individuals to different components of ETS. Such

an approach also could be undertaken with VOC, and the approach of Avol et al. (25) with the mix of photochemical chemicals could be refined and expanded. The use in knowing the compound of greatest interest is that exposure assessment in epidemiologic studies could be directed to that individual compound, and reduction of exposure to a single noxious agent may be a more efficient method of reducing effects than reduction of exposure to the entire mixture.

The bottom up or synthetic approach involves understanding the effects of exposure to individual pollutants (e.g., ozone and one acid-aerosol species) and then assessment of the joint effects of exposure to mixtures of these individual pollutants. This has been the method used most often in human chamber studies. This approach can also be extended to study the joint effects of two complex mixtures, such as VOC and ETS, or one complex mixture with one pure compound, such as ETS and nitrogen dioxide or ozone, and a mix of acid-aerosol species. As mentioned, the chamber study is a powerful tool in establishing causality between a given exposure and effect. From a theoretical perspective, it is very attractive for quantifying the individual and joint effects of two or more substances. Because of practical limitations on the amount of resources that can be devoted to a particular question, however, the actual utility is restricted. This is reflected in the number of subjects that can be studied.

Studies in which maximal utility can be made of this method include the effects of ozone and sulfuric acid aerosol upon respiratory symptoms and changes in lung function, or the effects of ETS and VOC exposure on symptoms and number of leukocytes in nasal lavage. Other studies in which some contribution could be made might include the effects of nitrogen dioxide and ETS on incidence of respiratory infection or the effects of ozone and sulfuric acid aerosol on frequency of asthma attacks. In the former case, some of the outcomes of interest can be measured directly; in the latter case, the incidence of respiratory infection and asthma attacks following a single exposure is likely to be too low to study efficiently. One might use an attenuated virus to study directly the effects of pollutant exposure on infection rates. Alternately, one may choose a surrogate measure for likelihood of infection, such as a decrease in phagocytosis of virus by alveolar macrophage harvested by bronchoalveolar lavage, or a surrogate measure for asthma attacks, such as an increase in responses to methacholine, cold air, exercise, or, more

invasively, antigen. Similarly, identification of outcomes that occur following acute exposure and are in the pathogenetic pathway for a given chronic disease might allow inferences to be drawn from acute responses about the effects of chronic exposure to a given mixture.

In order to make maximal use of this method for assessing risk from exposure to varying levels of two compounds, one must be able to define the response surface for all possible combinations of the two substances. Assuming that response to each substance is nonlinear, one needs at least four concentrations for each pollutant for a total of 16 cells. Depending upon the precision of the measurements of interest and the variability in responsiveness to each pollutant, one needs a minimum of 10 to 20 individuals per cell. Such a study allows description of the entire response surface for the given exposure protocol and might allow one to distinguish between competing statistical models of interaction. Achievement of this latter goal requires substantial a priori knowledge of individual dose-response characteristics so that the optimal concentrations and conditions for study can be chosen and the number of models tested can be kept to a minimum. Definition of the response surface for exposure to two substances is further complicated by adding the multiple dimensions of time. Issues such as duration of exposure and latency period for effect development for each pollutant are critical for definition of response and add tremendously to the complexity and expense of this approach.

A simpler approach, which gives limited information but requires far fewer resources, involves study of fewer combinations of exposure and a selected duration of exposure and times of measurement. Because one often has some information about dose-response characteristics for each component of the mixture, one can usually choose a concentration for each substance that gives reproducible responses and that is either near the threshold of response or on a linear portion of a dose-response curve. Using clean air and a single concentration of each pollutant, one can measure the effect of each pollutant compared to a clean-air exposure and the joint effect of exposure to both compared to the effects of exposure to each and to clean air. While one cannot choose between different models of statistical interaction with this study design, depending upon how exposure concentrations are chosen, one can decide whether the joint effect compared to air exposure is large enough to justify concern, whether addition of a noneffect level of one pollutant to another pollutant produces

increases in response, or whether the combination of two pollutants with small, individual effects results in a much larger effect or in a reduction of effect.

Another use of the synthetic, or bottom up, approach is in identification of sensitive individuals for epidemiologic study. As mentioned for the top down approach, identification and study of individuals with optimal rates of the outcome of interest due to either exposure alone or to the joint exposure can result in more efficient epidemiologic studies. Furthermore, if other risk factors for the response of interest can be identified in

chamber studies, control of potential confounding by these factors can be controlled in subsequent epidemiologic studies.

One can conclude that human experimental exposure studies play an important role alongside epidemiologic and animal toxicologic studies in the investigation of health effects that are the result of exposure to complex mixtures. The human chamber studies are limited with regard to assessing chronic effects, rare effects, or effects from long duration exposure but are powerful in assessing acute, reversible effects from short-duration exposures in the species of interest. The

areas in which chamber studies are most likely to contribute include identification of effects or markers of effects for exposure to a given pollutant or mix of pollutants; direct dose-response assessment of effects for individual compounds and mixtures of set composition; identification of individual compounds responsible for the effects of a mixture; study of the joint effects of a binary mixture; development of markers of acute exposure for particular compounds; development of outcome measurements that can be used in the field; and identification, characterization, and testing of sensitive subpopulations. ♪

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