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Commentary

Can Animal Pulmonary Function Testing Provide Data for Regulatory Decision Making?

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The process of setting health standards requires rigorous, scientifically sound data that relate to man's interaction with his environment. Tests of pulmonary function are especially useful, since they may permit some direct comparisons between animals and man. The development of tests to measure pulmonary function in small animals has been important, and research into the health effects of air pollution may be greatly strengthened with the use of data from such measurements.

Information derived from research by using laboratory animals has traditionally been used in support of regulatory decision making and is an important part of the information which needs to be gathered to define the potential health risks associated with exposure to environmental pollutants or other potentially toxic compounds. More direct approaches including the study of the acute responses in man during exposure to inhaled pollutants in environmental chambers and epidemiologic studies which attempt to define associations between substances in the environment and human health have provided the most important information to the regulatory decision makers. However, these clinical and epidemiological approaches often leave major areas of uncertainty which are difficult to address, but which are important to standards setting. For example, the nature of research that can be undertaken with human subjects is limited by ethical considerations such that only those responses which result from short exposures to low levels of inhaled pollutants can be measured in man. Indeed, before undertaking clinical studies we have to convince ourselves and the Human Rights Committees, which are established to protect the health of our volunteer subjects, that no harmful or deleterious consequences are likely to persist as a result of our research. However, questions relating to the potential development of chronic pulmonary disease can and should be asked with animal research. For this reason, the most useful aspect of animal research is that studies can be undertaken with animals which would not be possible with man. These studies include exposure to well-defined and controlled pollutant environments, either at transiently high concentrations or at low concentrations for extended periods of time. Studies with compounds or exposure conditions which are expected to produce chronic changes are of special interest. Research results derived from such work with laboratory animals can and should be used to fill data gaps for regulatory purposes.

Because the respiratory tree is considered the "portal of entry" for inhaled air pollutants, studies on the changes in pulmonary function, biochemistry, morphology, and immunology are of special interest in environmental toxicology. Tests of pulmonary function have been developed for use with intact small laboratory animals. Since they are based on the principles that are used in human pulmonary function testing it may be possible to compare experimental data from laboratory animals with similar data in humans. However, measurements of pulmonary function in small animals are difficult because signals are smaller, and, therefore, the measuring equipment must have adequate sensitivity, and equipment dead space must be minimized because of the small volumes being measured. In addition, since respiratory events occur more rapidly than they do in humans or large animals, measuring equip-

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ment must also be shown to have adequate frequency response. These requirements have occupied the interest and activity of many small animal physiologists in recent years, and technical and scientific advancements have occurred which satisfy most needs for measuring equipment to record very rapid events in the smallest mammals (*1, 2*). It is now possible to make meaningful measurements of pulmonary function in common laboratory animals (*3*). Such measurements can be accomplished in a humane fashion and, although commonly done only once, it is theoretically possible to make measurements in the same individuals on several different occasions.

A wide selection of pulmonary function tests can be utilized to follow the progression of disease processes during the course of animal exposure and to evaluate the recovery or progression of disease after the exposure has been terminated. For example, functional residual capacity and total lung capacity can be measured by gas dilution techniques (*4*), while the Boyle's Law functional residual capacity, vital capacity, and pressure-volume relationships of the lungs and chest wall can be measured plethysmographically (*5, 6*). Flow-volume relationships can be measured using rapidly responding flow plethysmographs (*2, 7, 8*), and frequency dependence of compliance and resistance and the resonate frequency of the respiratory system can be measured using oscillatory mechanics (*9, 10*). Changes in dynamic compliance and airways resistance can be measured in unanesthetized guinea pigs during exposure to environmental pollutants using methods originally described by Amdur and Mead (*11*). Techniques have been developed to measure the single breath diffusing capacity for carbon monoxide in small animals and this is a useful test of gas exchange (*4*). The distribution of ventilation can be measured using the multiple breath nitrogen washout (*12, 13*), or the slope of Phase III and the onset of phase IV (closing volume) of the single breath oxygen test (*14, 15*).

Interpretation of the changes in pulmonary function of small animals is influenced by our understanding of changes in pulmonary function of humans. Some aspects of structure are different between animals and man; however, the underlying physiology and biochemistry are similar. That is, while quantitative differences may exist (e.g., numbers of certain airway cell types) there are qualitative similarities. Changes in lung volume, diffusing capacity and distribution of ventilation are determined by structural constraints in the lungs such as the architecture of the pulmonary alveolus or capillary bed, while changes in dynamic tests such as measurement of flow-volume relationships or airways resistance are influenced by airway mechanics. Since structure and function appear to be tightly coupled, the functional consequences of lung disease or pulmonary damage can be readily evaluated with such pulmonary function tests.

A useful comparison that can be made when conducting animal research is the direct correlation between the measurement of functional changes and documentation of such changes anatomically by using rigorous quantitative morphologic techniques on lung tissue. Unlike human research, individual organ systems can be studied in great detail or tissues can be prepared for histologic and morphologic evaluations. Therefore, in some studies, one can factor out specific responses and, consequently, can expect greater sensitivity and definition in animal research than might be possible with man. Such comparisons between physiology and morphology are especially useful because of the information on structure-function relationships. For example, in a clinically related study, Hayatdavoudi et al. (*16*) exposed adult rats to 60% oxygen continuously for 7 days. They observed that the total lung capacity measured physiologically was reduced by about 15% following the oxygen exposure and that the deflation limb of compliance curves generated on excised lungs was shifted downwards and to the left. Subsequent, rigorous morphologic evaluations of the fixed lungs from a separate group of exposed animals revealed interstitial thickening as well as changes to the endothelial cells. Although oxygen is known to produce pulmonary damage at high concentrations, supplementary oxygen is often used at such intermediate concentrations to improve blood oxygenation in patients with various forms of respiratory insufficiency. The information derived from this study would probably not have been available from humans because the variability in lung volumes between individuals would have masked any evidence of a difference in the measurement of pulmonary function and because no control measurements would be available under clinical circumstances. The morphometric study could not have been undertaken in humans because of the limitations in collecting fixed lung samples. Even if such samples were available, the investigators would likely have been unable to factor out effects related to the exposure to 60% oxygen, since the disease process which necessitated the oxygen therapy would probably obscure any changes related to the oxygen. However, because this study

was done in experimental animals, it was possible to measure changes in pulmonary function and then to subsequently document with rigorous, quantitative morphometric techniques, the structural changes which had occurred.

Another potential research area is the use of animal models of pulmonary disease which may represent sensitive populations and the interaction of environmental pollutants with these disease models. Several reports have been published on the development of an emphysema model in hamsters which is produced by the intratracheal instillation of elastase (*17*). Raub and his coworkers have described the progressive changes in pulmonary function which occur in hamsters as the severity of the emphysema is increased with increasing doses of elastase (*13*). Previous workers had done range-finding studies and had established the elastase-induced emphysema model using concentrations close to the lethal level. At this level, lung function is almost completely dominated by the pathology of the lesion; therefore, Raub and his colleagues treated hamsters with elastase at concentrations up to those known to produce massive lesions and observed that measurements of pulmonary function were affected in a dose-dependent manner. Using a dose which was approximately one-fourth of that used by previous workers, an emphysemic lesion was produced which was detectable but which did not dominate pulmonary function measurements. They then used this model to study the potential interactions that might occur in hamsters which have mild experimental emphysema and which are also exposed to environmental pollutants (*18*). Groups of treated animals, as well as normal animals, were exposed to a complex mixture of pollutant gases. The diffusing capacity and the nitrogen washout slope in both control animals and those with emphysema were affected by exposure to this complex mix. However, in the animals with emphysema, the diffusing capacity did not change as much as it did in the normals. They postulated that because of the pre-existing disease, the lungs of the hamsters with experimental emphysema could not compensate for changes induced by the environmental insult. In other words, these hamsters, which had chronic emphysema, had a limited capacity to respond to the challenge of an environmental exposure when compared to their normal (or sham-treated) mates. The use of such an animal model may have interesting implications for man. Will individuals who have chronic obstructive lung disease also be less able to respond to the stress of an insult induced by exposure to an inhaled pollutant? Such an hypothesis will be difficult to test but would be very important to the standards setting process.

Animal research offers several logistic advantages for studies of the potential health effects of exposure to air pollutants: greater statistical power can be achieved because relatively large numbers of animals can be exposed in inhalation chambers and subsequently studied in the research laboratory; environments can be carefully controlled and monitored; research protocols can be developed which permit direct comparisons in different exposure groups of the effects produced by individual pollutants; accurate dose-response data can be developed; and total body burdens of pollutants can be measured. Indeed, it is even possible to devise mathematical models and to verify them experimentally, in order to predict deposition and dose of inhaled compounds for many different species over a wide range of animal sizes (*19, 20*).

There is an increasing need for animal research which determines the pulmonary response to a large number of compounds, including criteria air pollutants. For example, newborn rat pups raised in 0.25 ppm ozone for up to 6 weeks had increased total lung distensibility when measured at high lung volumes (*21*). Using matched groups of rat pups, Barry and her co-workers were able to describe quantitative changes in the morphology of the small airways in these developing rats raised in ozone (22). When a group of beagle dogs was exposed to various components of automobile exhaust for 4 years, Gillespie demonstrated that not only did this exposure cause deleterious changes in the pulmonary function, but the pulmonary function continued to deterioriate for at least 2 years following the termination of the exposure (*23*). Chronic animal studies which address the pulmonary response to the inhalation of such air pollutants are time consuming, difficult and expensive. Information about the effect of such exposures is obviously impossible to derive from human experimentation. The need for such data in standards setting is acute, and there is evidence that such research would be fruitful.

Additional models of human pulmonary disease should be developed for use in environmental research. Long-term exposures need to be done. Studies which might lead to development of chronic lung disease should be undertaken. The effects of pollutant exposure on the newborn and young should be studied. Are such effects reversible? That is, would developmental changes be produced, and would such changes be reversed given a period of time for recovery and growth?

Does infection affect the sensitivity of animals to air pollutants? Information already exists which demonstrates that childhood pulmonary infections are associated with a reduction of lung function in later years (*24*). Is there any association to exposure to air pollutants?

The development of models to extrapolate from animal research to man is necessary if we want to use animal data for standard setting. Does pulmonary function change in a consistent manner as body mass progresses from small to large animals? Lung volumes, diffusing capacity and other physiologic and morphologic measurements seem to change in predictable ways (*25-28*). Ideally, changes in pulmonary function which might occur in small animals as a consequence of exposure to environmental pollutants should be interpreted with respect to parallel changes which might occur in man following similar exposures. Information on the scaling of pulmonary function from small animals to man needs to be expanded to develop the data base for extrapolation modeling.

Probably the most important aspect of animal research is to describe the mechanisms of change in pulmonary function, morphology, biochemistry, or immunology following exposure to air pollutants. For example, one effect of ozone that has been measured in humans is the phenomenon termed "adaptation." Following sequential daily exposures to ozone, the human response is changed each day until little response is detected. Is the need for strict controls on ozone exposure minimized since humans apparently adapt, or is this diminished response to ozone actually the result of damage to the airway surface which diminishes the ability of individuals to respond to subsequent ozone exposure? Measurement of effects (i.e., dose response) provides useful data, is relatively easy to derive, and has been useful to the standards setters in terms of regulating exposure. However, a regulatory decision should be based on a thorough understanding of the mechanism of such a response. In this context, the most important use of animal research is to provide information that will help the scientific community and the regulators to understand why pollutants cause responses, not merely the magnitude of the responses.

The process of setting health standards requires rigorous, scientifically sound data that relates to man's interaction with his environment. Tests of pulmonary function are especially useful, since they may permit some direct comparison between animals and man. Research on scaling of pulmonary function, extrapolation modeling, and tissue sensitivity is an urgent need if comparisons are to be made between animal research and man. Work on making structure-function comparisons with, for example, pulmonary function tests and morphometric measurements is needed to document the functional consequences of anatomical changes. The development of tests to measure pulmonary function in small animals has been important and research into the health effects of air pollution may be greatly strengthened with the use of data from such measurements.

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