Chapter 3

Pulmonary Toxicology and Epidemiology

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INTRODUCTION

As individuals move from home to work to various outdoor environments, they breathe air of divergent composition and quality. In addition to the oxygen needed for survival, people inhale a "soup" of gases and particles. The ingredients of that soup range from benign to lethal in their potential and actual effects on the human lung.

Obvious health concerns in the outdoor air include particulate matter, sulfur oxides, nitrogen oxides, ozone, and carbon monoxide—those pollutants associated with a heavily industrialized, fossil-fuel based economy. Workplace concerns differ greatly among occupations, but typically include organic and inorganic dusts and the vapors from various chemicals. Concerns also focus on "indoor" (e.g., home, school, office) air, where substances as ubiquitous as formaldehyde, tobacco smoke, asbestos, woodsmoke, and molds stand accused as potential contributors to respiratory disease.

Exposure to airborne toxicants varies considerably among individuals and among populations. A taxi driver who cooks over a gas stove receives a regulated (vehicle emissions) and unregulated (stove emissions) dose of nitrogen dioxide, while a homemaker in an all-electric home may receive a wholly unregulated dose of formaldehyde (offgassing from furnishings or insulation). A school-age child of smoking parents may be exposed to air pollutants subject to legislative controls (e.g., asbestos in schools) and uncontrolled pollutants (e.g., tobacco smoke in the home) with known synergistic effects. Residents downwind of a coal-burning power plant may worry about sulfur dioxide and particulate matter, while the miners who supply the fuel may worry more about coal dust. Policymakers and regulators must determine how best to protect human health from the potential ill effects of these multiple exposures to toxicants. They wrestle with technical questions (e.g., is there solid evidence that Substance X causes human health problems? If so, at what concentrations?) and socio-economic questions (e.g., does the benefit of avoiding a particular health problem outweigh the cost of reducing the toxic exposure that causes it?).

This chapter examines how toxicology and epidemiology contribute to decisions on whether or how to regulate substances because of pulmonary toxicity (box 3-A). The chapter first describes the framework for most regulatory decision making—risk assessment—and then describes the types of technologies available to complete each step of that process with regard to inhaled pulmonary toxicants. The technologies covered include those that enable assessment of exposure and dose and assessment of health effects. Finally, this chapter examines whether remaining questions about the noncancer health risks of pulmonary toxicants merely await application of existing technologies or whether answers will require development of new tools.

FRAMEWORK FOR STUDYING TOXICANTS

Early efforts to identify and control pulmonary toxicants in the United States were directed at substances that induced obvious disease in highly exposed individuals. In recent years efforts have focused more on attempts to protect the general population from the more nebulous "unacceptable risk of disease" at much lower exposure levels (34). But the objective of reducing mortality and morbidity remains. The statutes that authorize control of pollutants to protect human health explicitly or implicitly require a substantial amount of proof that a substance causes disease or injury before the substance can be subjected to regulatory controls. The framework in which such proof is sought is generally referred to as risk assessment.

Risk assessment is the process of characterizing and quantifying potential adverse health effects that

Box 3-A-General Principles of Toxicology

To evaluate the toxic nature of a substance, including its pulmonary toxicity, scientists have developed several general criteria for consideration, including:

Nature of the Toxic Substance. Toxicologists try to determine the characteristics that render a chemical toxic. Individual molecules may not be toxic in their native states but become toxic after being metabolized. The size and shape of particles may affect their toxicity.

Dose and Length of Exposure. These parameters, together with rates of metabolism and excretion, determine what quantity of a substance is actually affecting the body. A given substance maybe toxic in high doses but nontoxic under conditions of chronic low-dose exposure.

Route of Exposure. The pathway by which a toxicant enters the body (e.g., skin, eye, lungs, or gastrointestinal tract) affects its toxicity. The amount of absorption, ability of the toxicant to combine with native molecules at the entry point (e.g., heavy metals with skin collagen), vulnerability of sensitive areas (e.g., lining of the lung), and condition of the organ at time of contact (e.g., pH a nd content of the stomach) all play a role in subsequent toxicity. This study examines inhalation exposures.

Species Affected. Toxicants exhibit different levels and effects of toxicity depending on the species on which it is tested.

Age. Susceptibility to a toxicant varies with age—the young and the old generally being the most susceptible.

State of Health. The health status of an individual, including the presence of disease, can greatly affect toxicity response. For example, people with asthma may suffer adverse effects from substances that do no harm to most individuals.

Individual Susceptibility. Host factors such as genetic predisposition affect the response of an individual to a toxicant.

Presence of Other Agents. Toxicology often involves evaluating one substance in isolation, yet the body is seldom exposed to agents in this manner. Knowledge about toxic effects of multiple substances is not well-developed because of the practical limitations of testing the infinite number of combinations.

Adaptation/Tolerance. Biological adaptation to a toxicant often occurs when chronically low doses are presented. Adaptation/tolerance must be factored into evaluating the range of individual responsiveness to a toxicant.

SOURCE: Office of Technology Assessment, 1992, based on M.A. Ottoboni, *The Dose Makes the Poison* (Berkeley, CA: Vincente Books, 19$4).

may result from exposures to harmful physical or chemical agents in the environment. As practiced in U.S. Federal agencies, it generally involves four essential elements:

- hazard identification;
- dose-response assessment;
- exposure assessment; and
- risk characterization (9,27).

The process of ***hazard identification*** attempts to determine whether a particular substance or mixture of substances can create a measurable health effect. Dose-***response assessment*** identifies the health effects caused by a given dose of the substance understudy. ***Exposure assessment*** applies measurement and extrapolation technologies to determine what level of human exposure can be anticipated. ***Risk characterization*** integrates the results of the first three steps to estimate the incidence of a health effect for a given population under various conditions of human exposure (24,27).

Bringing a risk assessment of a suspected pulmonary toxicant to a satisfactory conclusion poses tricky problems for an investigator. Hazard identification may involve laboratory and field studies. In vitro tests at the cellular level may indicate that a substance causes a biological response but fail to address whether the effect would be adverse in the whole animal, where defense mechanisms may prevent the toxicant from

reaching the cells being tested. Dose-response assessments often are performed on animals rather than humans (particularly when new substances are being studied), which requires knowledge of whether animals and humans would respond similarly to the substance under study. Animal tests of acute exposures can be conducted with relative ease, but tests of low-level, chronic exposures are time-consuming and costly. Lack of emissions data, lack of knowledge about how substances are transported in the air, and lack of adequate monitoring devices typically complicate the exposure assessment. The following sections on Exposure Assessment and Dosimetry and on Health Effects Assessment describe technologies available for risk assessment of pulmonary toxicants and limits of those technologies.

EXPOSURE ASSESSMENT AND DOSIMETRY

Exposure to a contaminant has been defined as contact between a person and a physical or chemical agent. Exposure differs from ***biologically effective dose,*** which is the amount of a contaminant that interacts with cells and results in altered physiologic function. Regulators direct their efforts toward controlling exposures to populations that can reasonably be expected to result in harmful, biologically effective doses to individuals within those populations. The technologies of exposure assessment and dosimetry contribute to these efforts.

Exposure assessment is the estimation of the magnitude, frequency, duration, and route of exposure to a substance with the potential to cause adverse health effects. ***Dosimetry*** is the estimation of the amount of a toxicant that reaches the target site, in this case the lung, following exposure (30). The following subsections first describe devices that can be used to estimate exposure and determine the amount of a toxicant actually retained by the lung, and then describe technologies available to help scientists predict the biologically effective dose that will be produced by a given human exposure (figure 3-l).

Estimating Exposure and Biologically Effective Dose

A series or combination of physical and biological events may affect whether a toxicant that becomes airborne will create a health effect. Toxicants may be transported and transformed in the environment before human contact. Defense mechanisms in the respiratory system may remove or transform a toxicant before it causes damage. This section describes technologies to measure actual and potential exposures to toxicants and technologies to measure retention by the lung.

Exposure

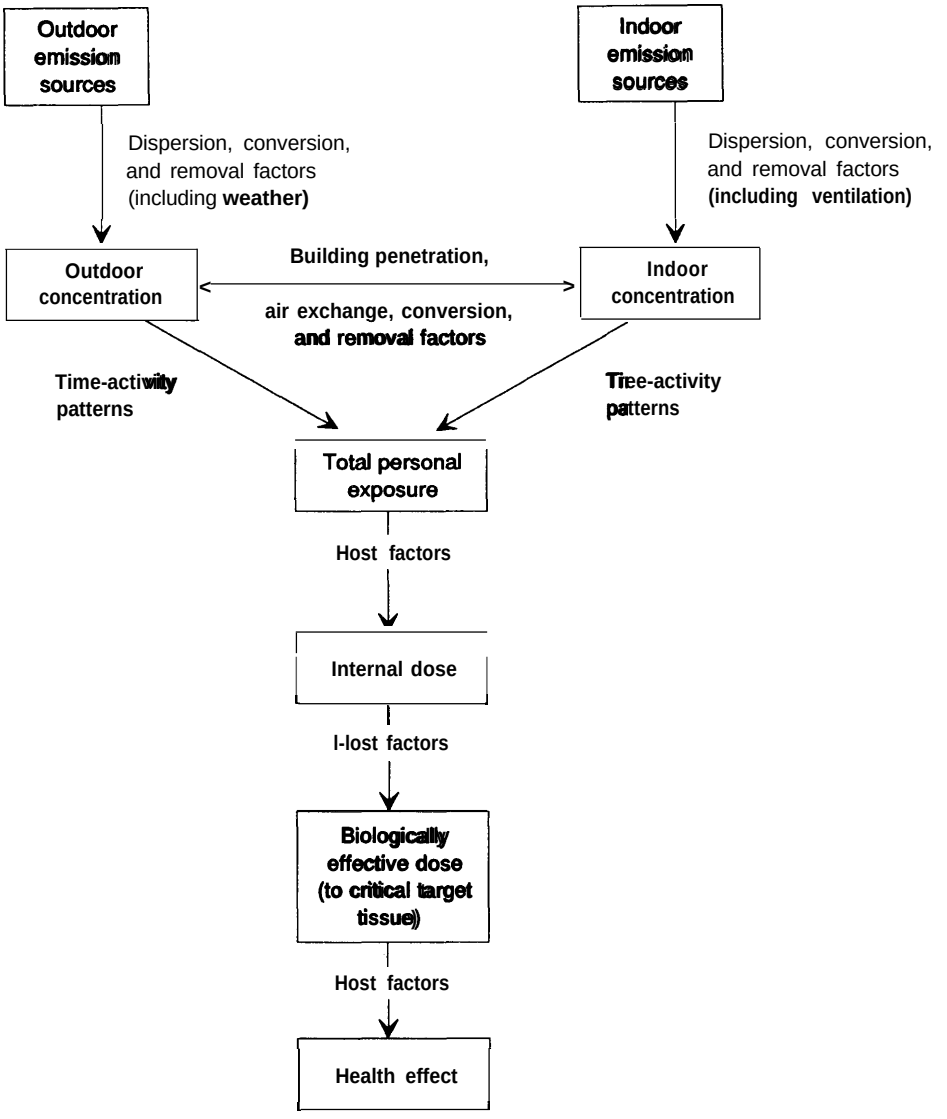
Exposure to airborne toxic substances traditionally has been estimated by sampling community and workplace air. Continuous samplers, used to measure gases, and integrating samplers, used to measure particles, are placed atone or more fixed sites in urban and nonurban areas (22). Contaminant concentrations derived from such measurements of the outdoor air have often been used to estimate an individual's average acute or lifetime exposure. Such estimates assume that all contact with pollutants occurs in the outdoors and that the breathing zone concentration is identical to that at fixed-site monitors.

In reality, people come into contact with polluted air in many different environments. Sophisticated monitoring devices now permit consideration of the multiple opportunities for people to come into contact with polluted air. Indirect (microenvironmental) and direct (personal) monitoring, combined with outdoor measurements, help scientists arrive at more accurate estimates of individuals' total exposure to airborne pollutants. Indirect monitoring uses traditional air sampling techniques but applies them to microenvironments—various indoor (e.g., homes, commercial buildings, worksites, vehicles) and outdoor (e.g., highways, industrial sites, backyards) sites. Personal monitoring requires individuals to wear or carry a sampling device throughout the study period and, generally, to log their daily activities to help associate measured exposures with their sources. Studies have demonstrated the effectiveness of personal monitoring devices (45), but scientists generally agree that detection limits and reliability need improvement. Techniques to enhance or supplement personal activity logs, such as personal location monitors, would also increase the precision achievable with personal monitoring devices (22).

Biologically Effective Dose

Several factors influence the amount of an inhaled contaminant that actually reaches lung tissues and cells following exposure. An individual at rest inhales less air than an exercising individual because exercise increases the ventilation rate. The ambient exposure can

Figure 3-l—Framework for Exposure Assessment



SOURCE: National Research Council, Committee on Epidemiology of Air Pollutants, *Epidemiology and Air Pollution* (Washington, DC: National Academy Press, 1985).

be the same for each, but exercise increases the biologically effective dose. Inhaled toxicants maybe removed from the inspired air before they reach the tracheobronchial and pulmonary regions (the predominant sites of injury that may lead to changes in pulmonary performance; see ch. 2). But individuals who breathe through their mouths rather than noses (e.g., asthmatics) may lose the benefit of that respiratory defense mechanism. Techniques ranging from measurements of exhaled air to examination of excised lung samples can be used to estimate how much of a substance reaches and is retained by various regions of the lung.

Analysis of exhaled air—Gas chromatography/mass spectroscopy can be used to measure exhaled air for contaminants that were absorbed by the lung. Breath measurements have been shown to correlate with preceding exposures for selected volatile organic compounds (45).

Analysis of body fluids-Sputum and fluid obtained by nasal lavage can be analyzed for the presence of toxic substances. Blood and urine can be analyzed for the presence of toxicants, but current measurements of these fluids generally yield very little or no

information about the delivered dose of a toxicant to the lung.

Analysis of the whole lung—Invasive and relatively noninvasive techniques can be used to determine the quantity of particles in the lungs of living subjects. Whole-lung scanning for particles labeled with radioactive tags (performed on an experimental basis) permits determination of the total concentration in the lung of certain types of inhaled particles and the size of the particles. Open-lung biopsy (an invasive technique requiring strong justification in humans) permits direct counting of particles or determination of fiber burden per gram of lung tissue (29). A noninvasive technique, magnetopneumography (MPG), provides a means of actively monitoring the dust retained in the lungs of people exposed to magnetic or magnetizable dusts. MPG can be used intermittently, for test purposes only, or to monitor individuals (particularly workers) for unacceptable rates of dust accumulation. Only magnetic or magnetizable dusts (e.g., asbestos, coal) can be monitored with MPG.

Analysis of samples of lung tissue-Scanning electron microscopy has been used to determine the deposition site, in rat, mouse, and hamster lungs, of particles small enough to reach the conducting airways. It also has permitted quantification of the particles present at selected deposition sites. Transmission electron microscopy has been used to locate inorganic particles in lung tissue, which can then be analyzed to identify the particle type. The techniques allow determination of the chemical composition and structure of a wide range of particles of varying sizes and elemental composition (31).

Determining Physical Properties of a Toxicant

Determination of the physical properties of a toxicant may allow an investigator to predict how a gas or particle will behave in the environment (how it will be dispersed following emission) and in the lung (how it will be deposited and cleared from the respiratory system). This background paper does not address methods for determining atmospheric dispersion but is concerned with methods for determining regional deposition within the lung.

Toxicants are inhaled as ***gases, vapors,*** or ***aerosols*** (table 3-l). Many factors influence deposition of gases and aerosols in the lung. For instance, exercise-induced oral (as opposed to nasal) breathing increases the amount of gas or particles that bypasses the nasopharyngeal region and reaches the deep airways. Doses of toxicants that overwhelm normal lung defenses (see ch. 2) can also affect the deposition of gases and particles. The most influential factors in deposition are the physical properties of the substances understudy (and the species in which they are tested.

As a general rule, water soluble gases inhaled through the nose will be partially extracted in the upper airways. Less soluble gases will reach the small airways and alveoli. Particle size generally determines the region of the lung affected by particles. Particles with an

Table 3-l—Defining Gases and Aerosols

Gases	Substances that are in the gaseous state at room temperature and pressure.
Vapors	The gaseous phase of a material that is ordinarily a solid or liquid at room temperature and pressure.
Aerosols	Relatively stable suspensions of solid particles or liquid droplets in air.
Dusts	Solid particles formed by grinding, milling, or blasting.
Fumes	Vaporized material formed by combustion, sublimation, or condensation.
Smoke	Aerosol produced by combustion of organic material.
Mist	Aerosols of liquid droplets formed by condensation of liquid on particulate nuclei in the air or by the uptake of liquid by hydroscopic particles.
Fog	See mist.
Smog	Complex mixture of particles and gases formed in the atmosphere by sunlight's effects on nitrogen oxides and volatile organic compounds.

SOURCE: T. Gordon and M. Amdur, "Responses of the Respiratory System to Toxic Agents," in *Cassarett and Doull's Toxicology: The Basic Science of Poisons, 4th edition* (Elmsford, NY: Pergamon Press, 1991), pp. 383-406.

aerodynamic size greater than 10 micrometers are mostly removed in the upper airways, while smaller particles penetrate deeper. Extremely small or extremely thin particles can cross the alveolar epithelial barrier and cause interstitial lung injury (see ch. 2).

Knowledge of the physical properties of a toxicant, coupled with the resultant knowledge of the probable site of deposition, points an investigator to the likely site and type of toxic injury. Actual behavior often differs from predicted behavior, but differences are generally revealed during the investigation.

Determining Species Differences

Toxicologists often try to predict the human health effects of toxicants by first studying them in animals. Animals provide useful models for studying toxicant exposure, but differences in anatomy, biochemistry, physiology, cell biology, and pathology affect the way species respond to airborne toxicants (8,46). Risk assessments of toxic substances generally depend on experimental data obtained from a variety of species, and it is essential to consider and study species differences before selecting the appropriate animal for study and making judgments about whether an exposure/dose administered to an animal has relevance for human health (23).

Respiratory tract anatomy differs significantly among species. Although most mammals have similar respiratory tract components, the structure of those components—which affects how substances behave in the lung—varies (e.g., humans differ from most other animals in the size and shape of the nasal airways, in the pattern of tracheobronchial tree branching, and in alveolar size). In addition to direct study of differences, computer modeling techniques now permit three-dimensional reconstructions of the lung, based on tissue samples, that improve the ability to extrapolate from test results in animals to likely health effects in humans (25).

Breathing patterns and lung defense mechanisms also affect the fate of toxicants in the lung. For instance, humans often breathe through their noses and their mouths, while some other animals (notably the rodents used in toxicology) can only breathe through their noses. These differences have a major impact on deposition of particles. Also, scientists now know that alveolar clearance mechanisms, which are designed to clean out the lung and prevent the type of damage that derives from prolonged exposure, are much faster in some species than in others.

Relative distribution of lung cell types differs among species, which affects the type of damage toxicants can inflict. Similarly, the mechanism of injury may differ among species and by exposure type (e.g., chronic exposure to beryllium causes an immune reaction in human lungs, but beryllium acts through direct cytotoxicity in rat and human lungs at acute exposure levels (19)). Only certain species can be used to test disease states of interest to scientists. For example, rats are generally the species of choice for pulmonary toxicologists, but toxicologists often use guinea pigs when asthmatic responses are in question because guinea pigs have airways more sensitive to bronchoconstriction than most species, and hence share a similarity with human asthmatics.

No single species makes a perfect physical surrogate for humans in studying the health effects of airborne toxicants. In addition to scientific issues, toxicologists must also consider the availability and expense of different species, especially when the effects of chronic, rather than acute, exposure are being studied. Much has been learned about species differences, and scientists are beginning to account for those differences when extrapolating from effects in animals to humans. Most experts agree, however, that increased interspecies comparisons and studies of the mechanisms of injury would increase the utility of animal tests in the risk assessment process.

Summary of Exposure Assessment and Dosimetry Technologies

Technologies that measure the presence of gases and aerosols in the ambient air and at target sites within the body play an essential role in risk assessment of airborne toxicants. These technologies have evolved rapidly and continue to improve estimates of human exposure to toxic substances. Scientists point to important gaps in the “exposure assessment knowledge base,” however (29,35). These include, generally:

. Lack of knowledge about the disposition of inhaled gases, vapors, and extremely small particles within the respiratory tract;

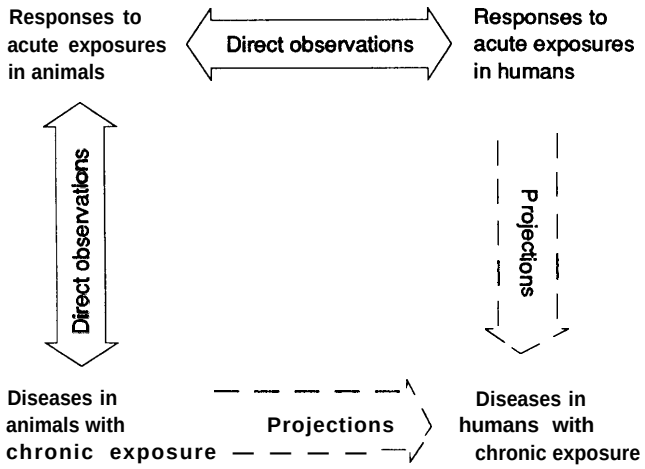
.Lack of knowledge about the disposition of gases and aerosols within the respiratory tracts of sensitive individuals and groups within the population;
.Inadequate knowledge of the species differences that may affect interpretation of test results; and
.Lack of a sound basis for extrapolating the effects of exposure from high to low concentrations.

The gaps present no insurmountable barriers to effective risk assessment but will require time and resources to fill.

HEALTH EFFECTS ASSESSMENT

A health effects assessment completes two steps of the risk assessment process: hazard identification, by determining whether a substance causes damage, and dose-response assessment, by determining the damage caused by a specific dose. Health effects assessments utilize controlled exposure conditions, as with laboratory and clinical studies, or uncontrolled exposure conditions, as with epidemiologic studies. Each type of study has advantages and disadvantages. To compensate, scientists try to integrate the results of multiple studies in their conclusions (figure 3-2). For instance, laboratory, clinical, and epidemiologic studies have contributed to the decision-making process on permissible ozone exposure levels. The following subsections describe the types of tests that can help investigators reach conclusions about the pulmonary effects of airborne toxicants.

Figure 3-2—Integrated Approach to Identifying Pulmonary Toxicants



SOURCE: Office of Technology Assessment, 1992, based on M. McClellan, R. O., "Reflections on the Symposium: Susceptibility to Inhaled Pollutants," American Society for Testing and Materials Special Technical Publication 1024,1989.

Laboratory and Clinical Studies

Laboratory studies and human clinical studies control exposure conditions as closely as possible to limit the influence of extraneous factors on the study. This means, in part, investigators must understand "host factors"—the physical conditions, activity level, and personal habits of the test subject-and other factors, such as time of day or season of the year, that may affect the outcome of a study. It also means investigators choose not only the health effect to study but the amount of toxicant to which tissues and cells, animals, or human volunteers are exposed.

There are drawbacks to studies involving controlled exposure conditions. The fundamental limitation of experiments involving whole animals lies in extrapolating results to humans. As discussed above, techniques are progressing, but scientists are not yet satisfied with their ability to account for the differences in human and animal lungs when predicting the effects of a toxicant. In addition, studies using whole animals involve considerable expense and, in some instances, problems with the public's views on animal experimentation. Ethical restraints on human clinical studies—investigators may not inflict harm—place inherent limitations on their use for predicting a toxicant's likelihood of causing a deadly or disabling condition. The intentional simplicity of experiments involving controlled exposures also limits their value for revealing the effects of exposure under "real world" conditions. To isolate the effects of one substance, investigators eliminate other potential toxicants from the air, which may alter the effect the test substance has on the lung.

Technologies to Measure Exposure

Toxicologists performing animal experiments must have the capability to generate the types of chemicals and particles they want to test and the capability to expose the animals to fixed amounts of the test substance(s). Technologies to generate gases and aerosols are well developed (2,26). However, the aerosols generated tend to be far more homogeneous than those encountered in typical urban environments. Exposure occurs in whole-body chambers and in apparatuses permitting head-only and nose-only exposures. Existing systems provide for adequate exposure control and measurement and do not constrain evaluation of test results when system limitations are properly accounted for in the analysis (6). However, continued research is important to understand the implications of the differ-

ences in complex, naturally occurring aerosols and those generated for experimental purposes.

Participants in human clinical studies can be exposed to the test substance in exposure chambers (whole-body systems) or through systems using either a facemask, hood, or mouthpiece. Each system has advantages (e.g., exposure chambers permit unencumbered breathing and most accurately simulate normal conditions; mouthpieces are very simple) and disadvantages (e.g., facemasks are difficult to seal; exposure chambers can be expensive to construct and maintain, part of the reason why only four chambers in the United States are effectively operating (37)). It is possible to calibrate the limitations of each system sufficiently to permit adequate evaluation of test results (17). Development of portable exposure chambers could enhance use of that exposure system (37).

Measurements of Effects

Toxicologists can identify pulmonary toxicants through physiologic assays of living subjects, structural analysis of tissues and cells removed from animals or humans, and tests of biochemical responses in removed fluids and cells. The following subsections describe various testing measures.

Physiologic tests—Assays of physiologic function fall into four major categories: measures of ventilatory or gas-exchange functions; measures of increased airway reactivity; measures of particle clearance from the

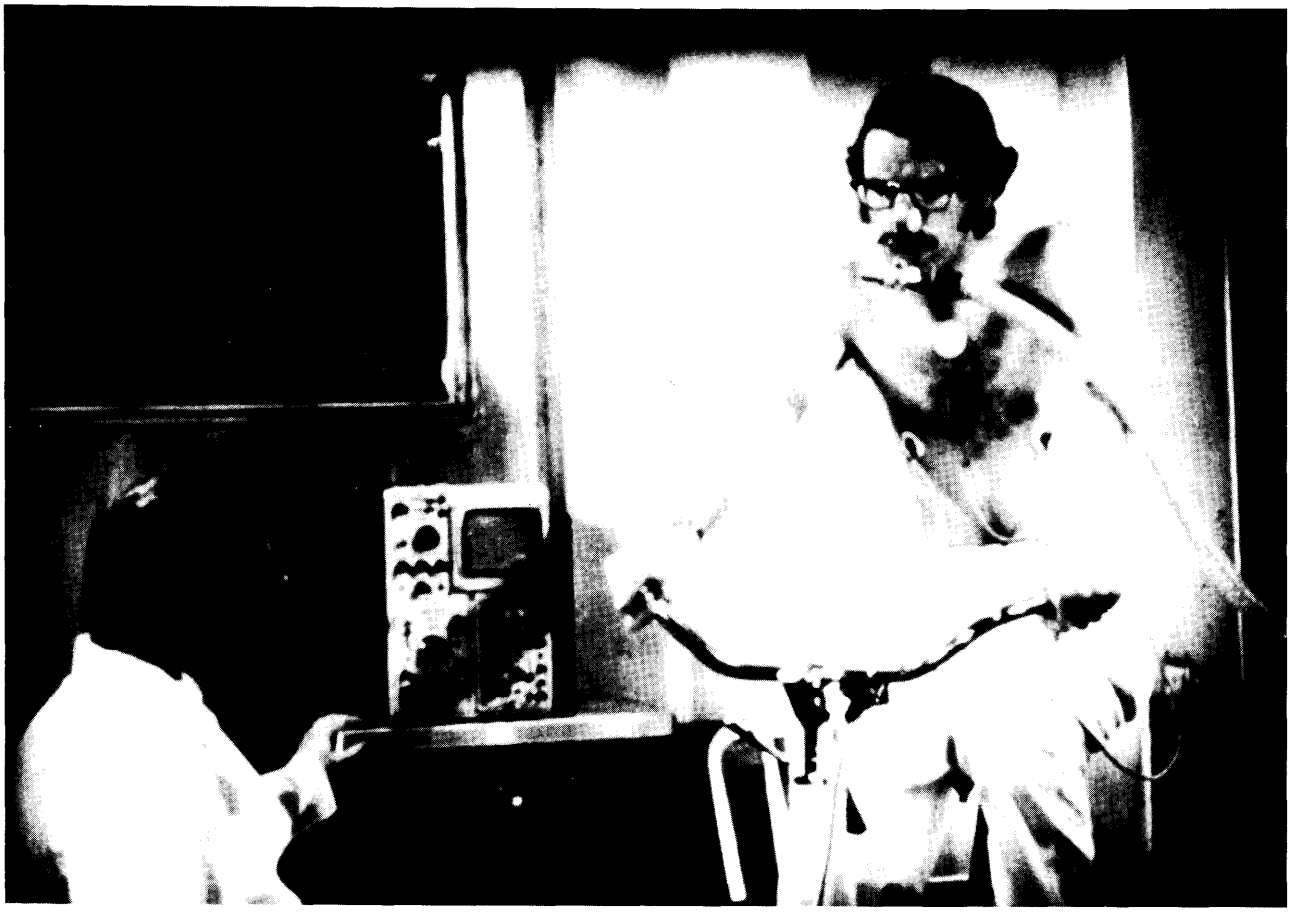


Photo credit: South Coast Air Quality Management District, El Monte, CA

A volunteer undergoes lung function tests while exercising.

lung; and measures of increased permeability of the air-blood barrier. These assays can be used to demonstrate transient and lasting changes in lung function. Functional assays are applied to animals and humans, though specific tests performed vary by species.

Spirometry, which includes various measures of how much and how quickly air can be expelled following a deep breath, constitutes the most common group of respiratory function tests performed in humans. A very frequently used spirometric test measures the amount of air that can be forcibly expelled in 1 second and is referred to as FEV_1 (forced expiratory volume in 1 second). Physicians agree that an FEV_1 below 80 percent of the predicted value (which varies with age, height, and sex) indicates an adverse health effect. EPA has used evidence of decrements in FEV_1 greater than or equal to 10 percent as the basis for regulations. Some studies show that a decrease in FEV_1 following short-term exposure correlates with development of obstructive disease following chronic exposure, but much research remains to be done.

The total amount of air that can be expelled following a deep breath is referred to as forced vital capacity (FVC), and this measure is also a commonly used test. Practitioners of spirometry can chart the air flow rate after 50 percent or 75 percent of the volume has been forcibly expelled (forced expiatory flow of 50 or 75 percent, or FEF_{50} or FEF_{75}). Alternatively, the flow rate between 25 percent and 75 percent of the FVC (maximal midexpiratory flow, or MMEF) can be measured. Some researchers believe that FEF_{50}, FEF_{75}, and MMEF may identify early and subtle damage to airways, which maybe the first stage of the type of severe or irreversible damage reflected in the more common measures of FEV_1 or FVC.

Ventilator function tests that do not require voluntary exhalation maneuvers can be performed in experimental animals. Such tests include measures of the mechanical properties of the lung, i.e., the amount of work required to stretch the lung (inhale) and the work required to push air out of the lung (exhale). Traditional measures of lung mechanics have been used widely in animal studies of toxicants that are pulmonary irritants (7).

The distribution of gases and particles within the lung can also be measured with ventilator function tests. Single- and multi-breath nitrogen washout tests determine the point in exhalation when the airways begin to close (as evinced by an increase in the nitrogen content of exhaled air). A transient increase in the amount of air left in the lung when the limits of forced exhalation are reached appears to correlate with exposure to pulmonary toxicants. Particle distribution can be examined by measuring the number of particles, administered as an aerosol, in exhaled air and with radioimaging techniques. Efforts are underway to validate such tests, which are not yet in widespread use.

Tests of how well gas diffuses from the lung into the blood system—the diffusing capacity of the lung for carbon monoxide (DL_{CO})-are sometimes included among ventilator function tests. The tests use carbon monoxide (at harmless dose levels) because it is readily absorbed by the hemoglobin in the blood. The most common DL_{CO} test requires the subject to inhale a mixture of inert gas and carbon monoxide. Changes in the ratio of inert gas to carbon monoxide, as measured in air captured at the end of exhalation, can indicate changes in the lung's diffusing capacity (e.g., if unusually high levels of carbon monoxide remain in the exhaled air, it indicates alterations in the transfer of CO from the lung to the bloodstream).

Measures of airway hyper-reactivity are another type of physiologic test of pulmonary toxicity. These tests assess whether the bronchoconstrictor response to stimuli increases (i.e., whether the airways become hyper-reactive and resist air flow) during or following exposure to inhaled toxicants. In nonspecific airway hyper-reactivity tests, the stimulus for the bronchoconstrictor response may be cold air, exercise, or various pharmacologic agents. This type of testing has proved useful for measuring airway responses to low concentrations of environmental pollutants. In specific airway hyper-reactivity testing, the stimulus is often a common antigen. In nonspecific and specific tests of airway hyper-reactivity, the tests applied following stimulation of the airways involve an airway resistance measurement and a pulmonary function test, usually FEV_1. Airway hyper-reactivity is characteristic of asthma, although it can occur in nonasthmatics. Some researchers suggest airway hyper-reactivity may play an important role in the development of chronic lung diseases.

Particle clearance assessments also provide physiologic evidence of pulmonary toxicity. These assays determine how exposure to toxicants alters the lung's ability to clean itself out. Though several tests are under development, their utility is hindered by the fact

that as yet there is no generally accepted range of normal clearance performance. Most tests trace the transport and removal of radiolabeled particles following exposure to a toxicant.

The final, major type of physiologic assay of pulmonary toxicity attempts to measure injury to the air-blood barrier, usually equating injury with increased permeability. Permeability Can be determined by measuring ion transport through airway epithelial cells or by measuring the transepithelial transport of molecules into the blood. These tests currently have many drawbacks, and though research appears to be worthwhile, much remains to be done. Permeability of the endothelial cells that line the blood vessels can also be measured, but nondestructive techniques require further validation before they can come into common use.

In 1989, the National Academy of Sciences (NAS) summarized the utility of physiologic assays in identifying pulmonary toxicants (29). A portion of that analysis is reproduced here as table 3-2. The preceding section provides only a cursory overview of the basic types of physiologic tests of pulmonary toxicity, and the reader is referred to the NAS report for detailed descriptions of these and additional tests.

In summary, physiologic function tests provide reasonable measures of response to toxicants but are not particularly specific or sensitive. Changes in function are not unique to individual toxicants (i.e., lung responses to insult are limited). Current tests have limited value in identifying the effects of chronic exposure (which tend to occur insidiously) (44). But when used in tandem with knowledge of exposure, these tests can help identify toxicants.

Structure tests—Injury to lung tissues and cells can, in some instances, be assessed with the naked eye. For instance, in advanced asbestosis the damage asbestos causes to the pleura can be seen unaided in an open chest cavity, and a microscope can provide even greater detail of the damage. Whole lungs or tissues and cells taken from autopsied humans or animals can be directly examined for evidence of toxic effects. X-ray technologies are also useful in structural studies.

Scientists also know that cells of the pulmonary system normally appear in relatively constant numbers and sites within the lung. Examination of tissue from specific regions of the lung can indicate changes in cell populations that are evidence of toxic effects. Morphometry, a technique that employs microscopy to quantify cell populations and structure size using fixed tissue samples, has been widely used to study toxic substances suspected of causing a specific type of injury throughout the lung. Morphometry is more difficult to use to measure toxic effects on small or scattered regions of the lung because tissue samples reflective of the region can be hard to obtain, but improved techniques are under development to assess the gas exchange region of the lung (18). Morphometry can also be used to examine changes in the structure of the pulmonary vasculature. Structural tests may show abnormalities long before changes are detectable by pulmonary function testing. A substantial amount remains to be learned about whether such changes will result in harm, however.

Tests of biochemical and molecular response—Phagocytic pulmonary cells, physiologic mediators, metabolizes, enzymes, and other biochemical substances that can be associated with toxic response can be removed from the system by lavage (washing) and the lavage fluid can be analyzed for cellular and biochemical content (20,21). Pulmonary inflammatory responses and immune responses can be measured by examining bronchoalveolar lavage fluid (BALF).

An inflammatory response to a toxic exposure produces enzymes and cells not normally present in BALF. BALF analysis can reveal the degree of inflammation and corresponding stage of any disease process. Aspects of an immune response, such as increased numbers of lymphocytes, can also be measured in BALF. Importantly, immune system cells recovered from BALF can be tested in vitro to determine whether they respond properly to antigen challenge or if they respond to a particular antigen of interest. The functional characteristics of other cell lines obtained from BALF can also be assessed.

Development of safe lavage techniques has contributed immensely to the prospects for pulmonary toxicology. The ability to measure the presence of biochemical substances in BALF has grown faster than knowledge of how those substances correlate to toxicity, but current research is quite promising.

Summary of Technologies Applicable to Laboratory and Clinical Studies

Effective laboratory and clinical studies require technologies to control the dose of a toxicant adminis-

tered to a test subject and to limit and account for confounding factors. Scientists have developed several exposure technologies and have characterized their potential and drawbacks.

Many established and recently developed technologies exist to measure changes in lung structure and function following exposure to toxic substances. A substantial database exists on the physiological effects of toxicants on animals. Spirometry--as a stand alone measure of ventilator function and as a component of airway reactivity testing—is the most frequently and easily used technique in human health effects assessments of pulmonary toxicity; additional physiologic measures are under development and may eventually improve the predictive powers of clinical studies of pulmonary toxicity. Microscopy continues to play an important role in laboratory studies, particularly as enhanced by morphometric techniques. The importance of biochemical and molecular measurements, as performed on lavage fluids, is increasing (20,47). Each of these technologies performs well as a diagnostic tool when changes in the lung are gross, but many also measure milder changes that may only represent physiologic variability and, as yet, are not well correlated with changes in pulmonary performance.

Health effects assessments can be performed under acute or chronic exposure conditions. The database on acute exposures is much larger than that on chronic exposures. While this background paper focuses on technologies that identify ***whether*** a substance causes a toxic effect, it is important to acknowledge that data regarding ***how*** that effect results in harm should also improve policymakers' ability to deal appropriately with toxic substances.

Epidemiologic Studies

Epidemiology-the study of the distribution and determinants of disease in populations—provides information about the impacts of air pollutants on human populations. It can be used to associate pollutants with disease even before precise mechanisms of cause and effect are understood, although observed associations are often attenuated by serious confounding factors.

Epidemiologic investigations of airborne toxicants share the difficulties inherent in any observational, rather than experimental, studies. For instance, exposure may be hard to assess. Some observers note that knowledge of exposure need not be precise to be meaningful (4), e.g., self-reported exposures to fumes or smoke have correlated well to later measurements, but results based on precise exposure measurements lend themselves more readily to important regulatory decisions. Another problem with epidemiologic studies is that most lung diseases can have more than one cause, and it is difficult to isolate the effects of one airborne substance from another. Finally, it may take studies of quite large populations to reveal small but important effects of airborne toxicants, and such studies can be difficult and costly to undertake. Though hard (sometimes impossible) to conduct, these types of studies can provide evidence of association between exposure and disease that lay and technical people alike find more credible than evidence from laboratory or clinical studies (28).

Epidemiologic studies take many forms. It is possible to study living or deceased populations; diseased populations can be studied for evidence of exposure; healthy populations can be studied for changes in health status following exposure. In all cases, however, some knowledge of exposure and evidence of a defined health effect must be available for results to be meaningful. The following subsections describe the tools available to measure exposure and health effects in epidemiologic studies. Epidemiology uses some of the same technologies as employed in laboratory or clinical studies; some techniques are unique to epidemiology.

Measurements of Exposure

Many of the exposure assessment technologies described previously are applicable to epidemiologic studies. Outdoor, indoor, and personal monitoring devices can be used to provide current exposure information. Records of outdoor measurements collected by public agencies provide historical data of exposure. Population groups can be examined for biological evidence of exposure (e.g., toxic substances found in exhaled air or in autopsied lungs). These measurements typically lack the precision—with regard to exposure to the toxicant under study and to exposure to substances that may alter (confound) the results--obtainable in laboratory and clinical studies, but are used by the scientific community. Moreover, some epidemiologic studies proceed on the basis of self-reported, rather than measured, exposure information.

Measurements of Health Effects

Epidemiologists use many kinds of data to determine health status. Epidemiologic measures include

Table 3-2—Summary of Characteristics of Physiologic Assays

Measure	Characteristics [a] and Ratings [b] A	B	C	D	E	F
Respiratory function						
Spirometry	++	+	++	++	++	+
Lung mechanics						
Dynamic compliance, resistance, and conductance	+	+	++	+	+	+
Oscillation impedance	+-	+	++	++	+-	+-
Static pressure-volume	+	+-	+	+-	+	++
Intrapulmonary distribution						
Single-breath gas washout	+		++	+	+	+-
Particle distribution						
Exhaled particles	+-	++	++	++	0	+-
Particle deposition	+-	+	+	+-	0	+-
Alveolar-capillary gas transfer						
CO diffusing capacity	++	+-	++	+-	+-	+
Exercise gas exchange	++	+	++	+	+	+
Airway reactivity						
Nonspecific reactivity	++	+-	++	++	+	+
Specific reactivity	+	+	+	+-	++	++
Particle clearance						
Radiolabeled aerosol	+	+	+-		+-	+-
Magnetopneumography	-			+	0	+-
Air-blood barrier function						
Conducting-airway permeability						
Clearance of inhaled DTPA	+-	+	+		0	+-
Transepithelial potential	+-	+	+	+-	0	+-
Alveolar permeability by radiolabeled aerosal	+	+	+		+-	+-
Vascular permeability						
Radiolabeled protein leakage	+	+	++		0	+
Chest x-ray for edema	++		++	+		+-
Extravascular lung water by indicator dilution, PET, or NMR	+	+	+	+-	+-	+-
Rebreathing soluble gases	+	+	+	+-	++	+
Endothelial metabolic function	+	+	+		+-	+-

some of the same technologies applied in laboratory and clinical studies (e.g., spirometry) and some unique technologies (e.g., questionnaires, historical records). Health effects assessment technologies useful in epidemiologic studies are described briefly below. Box 3-B provides details of a long-term, epidemiologic study of the effects of air pollution on the respiratory health of residents of the Los Angeles area.

Biological tests--Spirometry is often used in epidemiologic studies because it is noninvasive and relatively simple to perform in the field; many

[a]Characteristics:

A. Current State of Development. Considerations in this category included the number of groups using the technique, the availability of the required equipment, the magnitude of the present data base, and the degree of standardization of procedures.

B. Estimated Potential for Development. This category reflected the current estimate of the potential for substantial development of the assay beyond its present state. Although it was recognized that advancements are possible for any assay, this category was intended to reflect potential for substantial technical refinements, adaptation for use in large populations, or advancements in ability to interpret results.

C. Current Applicability of Assay to Humans. Primary considerations were the invasiveness of the technique and the requirement for radionuclides. All the assays can be applied to animals, but some are less suitable than others for evaluating humans.

D. Suitability for Measuring Large Numbers of Subjects. The focus of this category was the suitability of the assay for use in studies of large populations of people, as might be required for evaluating effects of some environmental exposures. Considerations included adaptability of equipment for mobile use, length and nature of subject interaction (i.e., degree of cooperation required), resources required to analyze samples and data, and subject safety. For example, a low rating might suggest a low suitability for field use in evaluating hundreds of subjects of various ages and both sexes, whereas the assay might be quite suitable for studies of dozens of selected subjects brought to a stationary facility.

E. Reproducibility. This category focuses on the variability of results within and between subjects.

F. Interpretability. This category reflects the current understanding of (and degree of consensus as to) pathophysiologic correlates, anatomic sites of effect, and causative agents. For many of the assays, there is little disagreement on the physiologic function affected, but the specific mechanism or site of change is uncertain. For example, it is agreed that reduced carbon monoxide diffusing capacity reflects reduced efficiency of alveolar-capillary gas transfer, but the test does not distinguish among the effects of a thickened membrane, reduced surface area, and reduced capillary blood volume.

[b]Ratings:

O = Unknown, or information is insufficient.

- = Current information suggests inadequate development, little potential for development, little applicability to humans, poor suitability for large populations, poor reproducibility, or poor interpretability.

+- = Current information suggests some development, some potential for development, limited applicability to humans, limited suitability for large populations, questionable reproducibility, or questionable interpretability.

+ = Current information suggests adequate development, potential for further development applicable to humans, suitability for large populations, reproducibility, and interpretability.

++ = Current information suggests high development or good potential for substantial development, great applicability to humans, great suitability for large populations, reproducibility, or very good interpretability.

SOURCE: National Research Council, Subcommittee on Pulmonary Toxicology, *Biologic Markers in Pulmonary Toxicology* (Washington, DC: National Academy Press, 1989).

epidemiologic studies use FEV_1 as their measure of toxic effect in the large airways. Nitrogen washout tests have been used to measure small airways effects, but the sensitivity and specificity of that test has been called into question (29). Epidemiologists sometimes test for airway reactivity (28).

Bronchoalveolar lavage (BAL) could be performed in epidemiologic studies, either on living subjects or on autopsied lungs. BAL is invasive, however, and requires high-level skills to perform safely, adding to its expense and detracting from its utility in large-scale studies.

Assessments of data-Certain types of epidemiologic studies rely on routinely collected data rather than biological tests performed in the community. Death certificates provide mortality data that can be coupled with historical exposure data to draw some conclusions about the effects of inhaled pollutants on a population. Morbidity data obtained from diverse sources-hospital admissions and discharge records (3,48), emergency room visits (33), hotline phone calls and follow-up interviews (5), reports of days lost from work or school—provide some indications about the effects of airborne toxicants as well. These sources may be affected by error. For instance, cause of death may be listed inaccurately; social and economic factors influence decisions to seek health care or miss work. Some epidemiologists believe that these errors tend to reduce (rather than increase) the possibility of finding a significant effect. Epidemiologists have relied on these types of information in studies that are widely accepted as indicative of a connection between exposure to inhaled substances and lung injury or disease.

Participants in epidemiologic studies of pulmonary toxicity often complete questionnaires to assess respiratory health (16). Quality control measures for standardized questionnaires have been assessed, and

Box 3-B—The UCLA Population Studies of Chronic Obstructive Respiratory Disease

In **the early 1970s,** researched at the University of California at Los Angeles (UCLA) initiated a 10-part epidemiologic study of the respiratory effects of air pollution. By comparing the respiratory health of several communities exposed to different concentrations of common air pollutants, the researched hoped to elucidate the connections between inhaled toxicants and chronic obstructive respiratory disease, The researchers chose Los Angeles as the study area because of the great variation in the types and concentrations of pollutants within a relatively small but highly populated geographical region. The existence of a uniform network of air quality monitoring stations throughout the area ensured the availability of exposure data, which also influenced the decision to perform the studies in the Los Angeles area.

Four Los Angeles area communities with similar demographics-Lancaster, Burbank, Long Beach, and Glendora--were chosen for study. Lancaster residents were exposed to relatively low levels of chemical air pollutants, while residents of Burbank, Long Beach, and Glendora were variously exposed to higher levels of chemical air pollutants including photochemical oxidants, sulfur dioxide, nitrogen dioxide, particulate, hydrocarbons, and sulfates.

For the initial part of the study, the investigators interviewed participants about respiratory symptoms, residence history, environmental and occupational exposures, and smoking history. Participants also performed lung function tests. The interview% and lung function tests were all performed at the same Mobile Lung Function Laboratory for which the reliability was determined and sensitivity and specificity were estimated. Though researchers noted that long-term studies were necessary, initiaI data led to the following hypotheses:

1. Adverse effects of long-term exposure to high concentrations of photochemical/oxidant pollutants may occur primarily in larger airways both among smokers and never smokers (comparisons of Lancaster and Burbank residents).

2. Long-term exposure to high concentrations of photochemical/oxidant pollutants and of sulfur dioxide, hydrocarbons, and particulate pollutants is associated with respiratory impairment, manifested by dysfunction of the large airways (comparisons of Lancaster, Burbank, and Long Beach residents).

3. **Long-term exposure to high concentrations of photochemical oxidants, nitrogen dioxide, sulfates, and particulate pollutants may result in measurable impairment** in lung function in smokers and never smokers (comparisons of Lancaster and Glendora residents).

Extensive follow-up enabled researched to observe the populations from Lancaster, Burbank, Long Beach, and Glendora in long-term studies. Five years after the initial testing, participants still living in the study area (a substantial number) were reinterviewed and retested at the Mobile Lung Function Laboratory. These reexamination lent support to the following hypotheses:

1. Chronic exposures to mixtures of photochemical oxidants, sulfates and particulate are associated with increased loss of lung function, which is especially evident in the small airways (comparison of Lancaster and Glendora residents.)

2. Chronic exposure to mixtures of sulfur dioxide, sulfates, oxides of nitrogen and/or hydrocarbons ultimately adversely affects the large airways as well as small airways (comparison of Lancaster and Long Beach residents).

3. Passive exposure teat least maternal smoking (but not to paternal smoking alone) affects the airways of younger boys (analysis of all four communities).

4+ Smoking cessation leads to relatively early and sustained improvement in indexes of small airway function and other indices of respiratory health (analysis of all four communities).

The UCLA population studies of chronic obstructive respiratory disease add support to certain hypotheses regarding lung function and pollutant exposures. Nonetheless, the data reflect the types of problems that have characterized large epidemiologic studies. Exposure data are crude; experts fault the researchers controls for the effects of migration and self-selection. EPA concluded that the studies could not support standards setting for any of the pollutants involved. The studies do, however, point toward productive avenues for laboratory and clinical research that could clarify the effects of the pollutants found in the Los Angeles area on lung function.

SOURCE: Office of Technology Assessment, 1992, based on chapter 3 references 10,11,12,13,14,15,32,38,39,40,41,42.

though recall bias (sick or highly exposed individuals generally remember exposures or illnesses better than healthy or unexposed individuals) enters into play, questionnaires generally are considered useful. Daily diaries of short-term symptoms have become more prominent in recent years. They avoid the recall bias found in annual questionnaires and appear to be more sensitive (36).

Summary of Technologies Applicable to Epidemiologic Studies

In epidemiologic studies, exposure information is supplied with exposure assessment technologies and self-reported exposure data. Because "free-living" humans have knowing and unknowing encounters with multiple possible toxicants, exposure data in epidemiologic studies are necessarily imprecise. Many investigators believe that when confounding factors are properly accounted for, the ability to gather information on environmentally relevant exposures renders epidemiologic studies worthwhile even given the problems of collecting exposure data.

Biological tests applied in epidemiologic studies have the same advantages and disadvantages they present in laboratory and clinical studies, with the added requirement that they be easy to use in the field or on large populations. Reliance on public health records and population survey is a feature common to all epidemiology, including investigations of respiratory disease.

Summary of Health Effects Assessment Technologies

Each type of study (laboratory, clinical, or epidemiologic) has technological advantages and disadvantages, and individual studies within each type have strengths and weaknesses. Clear evidence of change in lung structure or function is unpersuasive if exposure data are problematic; evidence of health effects in animals under tightly controlled exposure conditions may be unpersuasive if no human data are available. Despite the availability of many testing technologies, certainty about the pulmonary toxicity of many commercial substances has eluded investigators and regulators because of the lack of a full array of information sources. The database on the acute effects of short-term, high-dose exposures to toxicants is relatively large and growing, and forms the basis for existing regulations of pulmonary toxicants. Fewer data are available on the effects of chronic, "environmentally relevant" (i.e., low dose) exposures to suspected toxicants. On one hand, animal data on chronic exposures can be obtained using current testing technologies, but problems remain in extrapolating results from animals to humans. On the other hand, human data may be impractical or impossible to obtain given the ethical constraints of clinical testing and the length of time and large populations necessary to conduct meaningful epidemiologic studies.

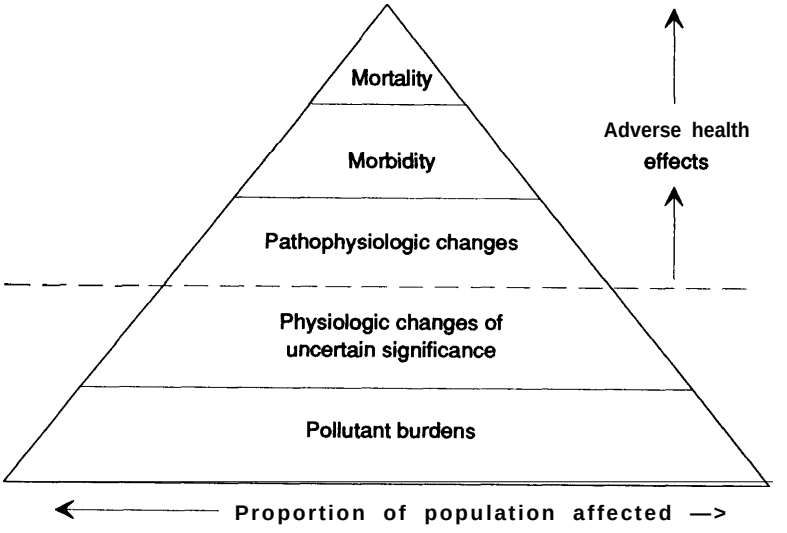
LIMITS OF TECHNOLOGY

The previous sections establish that current technology can measure the biological effects of toxic substances on the lung, but that conclusion begs an important question: Are the measured effects adverse? Humans come equipped to survive in a hostile environment; most organ systems—the lung included-are resilient and operate with a reserve capacity that accommodates some level of change or damage (43). In the case of pulmonary toxicology, it appears science has learned to measure biological effects more quickly than it has learned to correlate those effects with persistent changes in performance or with disease processes. This disjuncture creates problems for regulators.

Most researchers recognize a hierarchy of biological effects of exposure to toxic substances, ranging from mortality (inarguably adverse) to measurable traces of toxicants in tissue (arguably adverse) (figure 3-3). Because some people or populations are more sensitive to toxic effects than others, and because some people or populations are more highly exposed to toxic substances than others, severe, unquestionably adverse effects are likely to occur in a smaller segment of the population than less severe, more questionably adverse effects. Effects may be reversible or irreversible, with a tendency among researchers to concern themselves more with irreversible effects. Concern for reversible effects increases, however, if chronic exposures prevent reversal. Evidence to support a clear demarcation between adverse and nonadverse effects remains elusive. If, as in the case of many suspected pulmonary toxicants, evidence does not exist to associate early changes with later, more extensive or irreversible changes, effects that are measurable may still be adjudged nonadverse.

The American Thoracic Society (ATS) has defined adverse respiratory health effects in humans as "medially significant physiologic or pathologic changes generally evidenced by one or more of the following: (1) interference with the normal activity of the affected person; (2) episodic respiratory illness; (3) incapacitating illness; (4) permanent respiratory injury; or (5) progressive respiratory dysfunction" (l). Most often, however, regulators must use a combination of limited

Figure 3-3—Spectrum of Biological Response to Pollutant Exposure



SOURCE: American Thoricic Society, "Guidelines as to What Constitutes an Adverse Respiratory Health Effect, with Special Reference to Epidemiologic Studies of Air Pollution," *Am. Rev. Respir. ilk.* 131:666-668, 1985.

animal data and limited human data to reach conclusions about existing substances, and always must rely on animal data or extrapolations based on knowledge of chemical structures to predict the potential effects of new substances. Decisions about regulations most often are made in the absence of data that would enable a determination of adversity as precise as that found in the ATS definition.

Researchers and regulators agree that the integrated results of laboratory, clinical, and epidemiologic studies of short-term exposures can yield conclusive information about the acute effects of pulmonary toxicants. Current regulations generally are designed to prevent acute effects. Researchers and regulators generally are not satisfied that current technologies or current data provide them with a sufficient basis to regulate exposure to airborne toxicants because of the potential effects on the lung of chronic exposures. Much research is directed at developing improved methods for studying chronic exposures, but many questions remain. Chapter 4 provides more detail on regulations based on pulmonary toxicity and describes current Federal efforts to improve the basis for decision making with regard to chronic, low-dose exposure to inhaled toxics.

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