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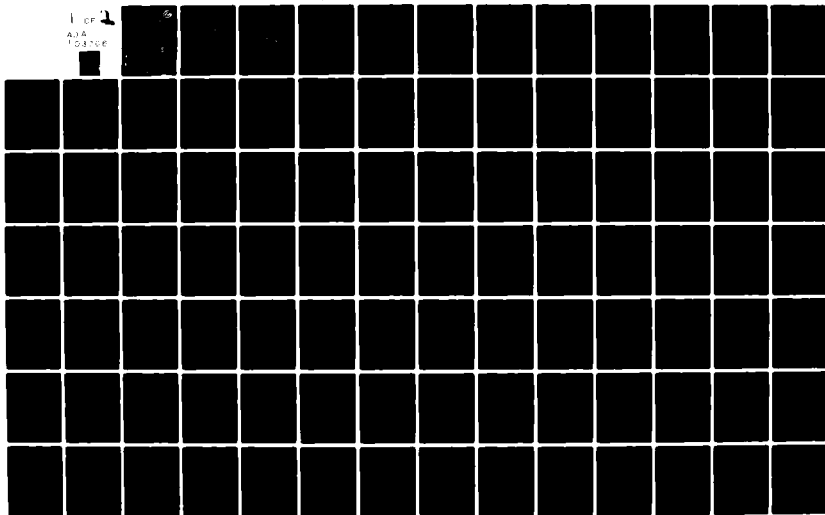
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EVALUATION OF SHORT-TERM BIOASSAYS TO PREDICT FUNCTIONAL IMPAIRMENT

Selected Short-Term Pulmonary Toxicity Tests Final Report

Steve Drill, Richard Thomas

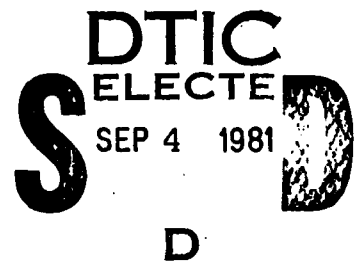
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EVALUATION OF SHORT-TERM BIOASSAYS TO PREDICT FUNCTIONAL IMPAIRMENT
Selected Short-Term Pulmonary Toxicity Tests Final Report

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test procedures are the test's state of development and whether it (1) has been performed in small animals and conscious animals; (2) is terminal; (3) is sensitive, accurate and reproducible; and (4) is relatively easy to perform. The tests that satisfy these criteria are compliance and resistance tests, lung volume and capacity tests, the distribution of ventilation by nitrogen washout test, arterial blood gas measurements and the carbon monoxide diffusing capacity test. Several other tests merit consideration for use in specific screening programs but do not satisfy all the selection criteria. Some tests are not currently recommended for a pulmonary toxicity screening program.

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EXECUTIVE SUMMARY

The Metrek Division of the MITRE Corporation, under contract to the United States Army Medical Bioengineering Research and Development Laboratory, is reviewing and recommending short-term tests for evaluating and predicting the functional and/or morphological impairment produced by toxic substances using animal test systems. This document presents information on the available tests for the pulmonary system and recommends those tests which are suitable for use in a screening program.

The pulmonary system may be viewed as having four functions which are discussed in this report under the headings respiratory mechanics, gas exchange, pulmonary circulation and defense mechanisms. In addition, changes which may be observed in morphology or biochemistry as a result of chemical toxicity are described. The current development of toxicity tests for these six aspects of the pulmonary system does not lend itself to the rigid classification of a tiered system.

The available test procedures have been placed in three categories: (1) those recommended for a screening program, (2) those that may warrant consideration for use in a screening program after further development or that have a special application in a screening program, and (3) those specifically not recommended for inclusion in a screening program. Two sets of criteria have been used in this categorization: those of an administrative nature (costs and manpower), and those of a scientific nature (sensitivity, accuracy, significance).

Administrative considerations include the animal-care and holding-space capabilities of support facilities, and the cost of the animals and equipment used. Very little of the equipment is standard or commercially available due to its unique design and construction, so the high cost of equipment may be a major consideration. Scientific considerations include the sensitivity, accuracy and reproducibility of the procedures; the technical skills required; and the necessary supporting facilities, such as histopathology and analytical laboratories. Primary importance is attached to the appropriateness of the procedures, and to the animals used in predicting effects on humans.

Only five functional tests are recommended for incorporation in a pulmonary toxicant screening program. These are:

- (1) Compliance and resistance, which are measures of mechanical flexibility and obstruction as they affect air flow,

- (2) Lung volume and capacity, which are also measures of respiratory mechanics related to the amount of air available,
- (3) Distribution of ventilation, which is a measure of the evenness of ventilated gas distribution throughout the lungs,
- (4) Arterial blood gases, which measures the delivery of oxygen to the tissues and the removal of carbon dioxide, and
- (5) Carbon monoxide diffusion, which measures the movement of gases between the lung and the blood stream.

Three test procedures are suggested for consideration. Morphologic examinations by light microscopy and electron microscopy provide information on the mechanisms for changes in the functional tests described above. Scanning electron microscopy is excellent for viewing changes in the gas-exchanging surfaces, but is expensive and requires well-trained personnel. The other two test procedures warranting consideration measure the pulmonary defense function. One quantifies the removal of inert particles, such as iron oxide, or of bacteria from the lung. The other examines the viability of the macrophages and their ability to remove foreign material (microbiological or xenobiotic particulates) from the respiratory tract.

Nine techniques for pulmonary toxicants were evaluated and excluded from short-term screening of toxicants because of the difficulty or time involved in performing them, their requirement for large, more expensive animals, and their lack of direct applicability to predicting effects in humans.

Some experimental procedures currently in the research and development stage are briefly discussed for their future potential as screening tests.

FOREWORD

The authors express their appreciation to Dr. Mary Henry, Project Officer of the U.S. Army Medical Bioengineering Research and Development Laboratory, for the support and guidance that she provided during the course of the project. The expert contributions by Jeffrey Drazen, M.D. and Jerry R. Gillespie, D.V.M., Ph.D., who submitted critical reviews of this report in its draft form, is gratefully acknowledged. Leadership and advice by Dr. Paul Clifford and Dr. Barbara Fuller throughout the course of the project have been of great value. The editorial assistance by Ms. Lee Johnson and the technical assistance by Ms. Yasuko Anglin and Ms. Terry Zimmerman is sincerely appreciated.

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1.0 INTRODUCTION

One method of systematically describing the damage produced by exposure to a toxic agent is to describe the damage manifested in each individual organ or system, and to further divide the damage into components which relate to individual aspects of function or structure within an organ or system. Utilizing this approach, toxic effects can be evaluated by assessing each aspect of function separately and combining these individual determinations. This analytical method underlies the medical approach to diagnosis and treatment of human poisonings. It has also been widely used in experimental animal toxicology.

The Metrek Division of The MITRE Corporation, under contract to the United States Army Medical Bioengineering Research and Development Laboratory, is recommending short-term (i.e., hours to days) tests for evaluating and predicting the functional or morphological impairment produced by toxic substances using animal test systems. Effects in four organ systems--pulmonary, hepatic, renal and cardiovascular--are being considered. This document presents information on the suitable, available tests for the pulmonary system and recommends specific tests that would be appropriate for inclusion in a screening program.

Only those tests which have a demonstrated ability to detect the damage produced by toxicant exposure have been considered. Selection of tests which satisfy this criterion has been based on an

operational definition of damage. According to this definition, damage to the pulmonary system consists of alteration of one or more of the following: (1) morphology at the cell organelle level or higher; (2) respiratory gas exchange, including gas exchange in the tissues, gas transport in the blood, and gas exchange in the pulmonary system; and (3) defense system activity at the cellular level or higher.

Alterations of function not explicitly covered by the definition of damage have been considered to be damage if they have been adequately correlated with morphological damage as described herein. A change in measurement of some of these parameters may be considered predictive, as well as indicative, of damage in the animal test system, if an abnormality in one parameter regularly precedes or accompanies that in another (i.e., an abnormal reading in test A is generally associated with a concurrent or subsequent abnormal reading in test B). Although this operational definition may be considered somewhat arbitrary, the selection of proven indicators of damage, based on the definition, then becomes nonarbitrary. Had a definition of damage not been explicitly stated, it would have been implicitly applied in the selection of tests.

The information contained in this report has been compiled from published and unpublished reports, and communications with individuals active in the development or application of measurements of pulmonary damage. A companion directory of individuals and organizations involved in pulmonary testing in animals was compiled solely

from personal communications, so that only current activities of the organization and researcher(s) would be represented.

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2.0 MEASUREMENTS OF PULMONARY SYSTEM DAMAGE

Measurements describing the structure and function of the lungs have been used as indices of pulmonary system damage in animals. Many techniques used in man have been adapted for both large and small animals. Much of the information necessary for evaluating the reliability, sensitivity and capability of specific tests to detect and characterize pulmonary damage is not yet available. Many experts in the field of pulmonary function measurements are currently working on the refinement of specific test protocols.

Information on pulmonary testing has been categorized according to the functional or structural alteration which each test measures, and further divided, in some cases, by major differences in the techniques used to evaluate these changes. The measurements used to reflect damage to the pulmonary system have been grouped into the following six broad categories:

Morphological

Respiratory Mechanics

Gas Exchange

Circulation

Defense Mechanisms

Biochemical

Although there are specific tests which could conceivably be included in more than one category, the tests have been allocated to the category that best describes the aspect of function or structure they

measure. No one test measurement or category of measurements will give a comprehensive evaluation of damage to the pulmonary system. Any given measurement may be useful for reflecting damage caused by one compound, yet the action of another compound may be entirely different and indicated only through use of an alternate measurement.

The operational characteristics compiled for each damage measurement include:

- The complexities of the technique and the extent to which it has been used
- Any peculiarities in technique which separate it from other similar tests
- The species in which the test has been performed
- The compounds which have been tested for toxicity or used to elicit a toxic response (this category also includes the purpose of the study when the test was used for something other than toxicity testing)
- The use of anesthesia
- Whether or not the procedure, as reported, entailed death of the animals

In compiling the species utilized for a particular test, only those in which the test has actually been performed are included. This information is summarized in Table 2-1. This list does not imply that the test can only be used in these animals, but rather that use of the test has only been documented in certain animal models.

When used, anesthesia has been described as either "initial" or "sustained." "Initial" anesthesia is that used during preparation of an animal subject for testing (e.g., surgical manipulation, insertion

of a cannula) and implies that the animal was conscious during the measurement procedure. "Sustained" means that the animal was anesthetized throughout the procedure. Where both initial and sustained anesthesia are indicated, the animal was prepared for testing under anesthesia, allowed to regain consciousness and recover, and anesthetized again during the test. When anesthesia is used, its effect on the results of the test should be considered.

Most researchers have used a battery of tests to evaluate pulmonary function. In some instances, the use of anesthesia and/or terminating the animals during or after the study depended more on other testing procedures used by the investigator than upon the particular test being evaluated. Cases of animal termination clearly unrelated to the performance of a test have been so noted in the text and in appendix tables.

In almost all instances in which a test was used to evaluate the pulmonary toxicity of a substance, the route of administration of the substance being tested was by inhalation. Therefore, this has not been specified on the tables. In some cases, another route of administration was used, and this has been specified on the table in parentheses after the name of the substance to which it applies.

In the following sections, measurements of pulmonary damage are discussed, each within one of the six previously mentioned categories. The information in each of these subsections is augmented by material which has been tabulated in an appendix. Not all

measurements applicable to a category are discussed in detail. The majority of the text is devoted to description and evaluation of those tests or measurements which appeared to have the greatest applicability to and potential for pulmonary toxicant screening.

2.1 Morphological Techniques

Morphological indices of pulmonary damage include both quantitative and qualitative comparisons of total tissue, tissue components, cells and cellular components. Quantitative, or morphometric, techniques basically involve (1) measuring volume and applying standard stereological counting procedures to either selected cell types or cellular components; and (2) calculating volumes, surface areas, lengths and/or numbers of lung structures, including cells. Several stereological techniques have been automated, which increases their value in assessing pulmonary damage. They are best suited for detailed descriptions of subtle toxicant damage. Any microscopic morphometric evaluation is tedious and time-consuming. The advent of quantitative morphological techniques will limit the chance for observer error inherent in earlier microscopic descriptive studies.

Morphological studies are essential to establish the presence of subtle pulmonary damage, and selected morphological studies provide information that enable researchers to understand the mechanism of damage by specific toxins. It is generally accepted that morphological studies detect damage before other pulmonary measurements do. In this section, and in Table A-1 in Appendix A, those few specific

morphological measurements and one broad category of morphological measurements that may reflect pulmonary damage are discussed. The specific measurements include those relating to Type 1 cell damage or death, Type 2 cell proliferation, mean alveolar intercept and diffusing capacity.

Type 1 alveolar cells are squamous or flattened epithelial cells and are characterized by extensive cytoplasm containing few inclusions except for many pinocytotic vesicles and a few small mitochondria. Type 2 cells have cytologic features suggestive of either secretory or phagocytic function. They are characterized by their regular cuboidal shape, large nuclei, vacuolated cytoplasm, large numbers of lamellar bodies and multivesicular bodies. The mitochondria are prominent and may be either plump or rodlike.

Type 2 cell proliferation follows or accompanies Type 1 cell death and these reflections of pulmonary damage may be measured in tandem or separately. These indices of pulmonary damage may be measured by standard microscopic observation of $1\mu\text{m}$ thick sections; by histochemical methods using lactate dehydrogenase stain, which is thought to be specific for Type 2 cells; and by autoradiographic analysis of radiolabelled thymidine uptake. Lactate dehydrogenase is also in pulmonary alveolar macrophages and may be in other pulmonary cells, which complicates the use of this enzyme as a specific cellular marker.

Demonstration of tissue and/or cellular changes remains the most accepted evidence of pulmonary injury. The extent of the injury and the specificity of the cellular target of a toxic agent within the respiratory system requires examination of upper airways (nostrils, nasal passage, pharynx, larynx and cervical trachea); lower airways (thoracic trachea, mainstream bronchi, large, small and terminal airways); and exchange surface (alveolar ducts, acini, alveoli and associated vascular structures). Gross, light microscopic, scanning and transmission electron microscopic techniques have all been valuable in determining the presence of injury, its extent and its duration. Histochemical and quantitative methods have greatly enhanced the investigators' ability to specify the tissue or cellular types involved in the pathogenic state, the nature of the response and a more precise measure of the extent of the damage. Changes in the histochemistry of the collagen in the interstitial space may not be detectable with conventional histological techniques. If the histochemical changes correlate with the biochemical assessment of collagen and with the functional behavior of the lungs, there is convincing evidence that significant biological changes are occurring in the lung.

Hence, in addition to providing detailed information concerning the pathogenesis and pathophysiological changes associated with inhaled toxics, morphological, biochemical, and functional techniques are vital to the early detection of pulmonary damage.

To date, there are meager data relating pulmonary structure and function. In general, major aspects of function relate well with structural observations, and the correlation of properly done quantitative morphologic studies with functional values are surprisingly good. Despite the tedious and time-consuming nature of quantitative morphometric studies, they are necessary for providing convincing (state of the art) evidence of pulmonary injury.

Total lung histopathology has been classified as one category in Table A-1 due to the multiple and sometimes arbitrary choices which are made regarding fixation, sampling and microscopic methods. A gross examination could be performed first, followed by a closer--although subjective--microscopic observation of possible alterations of lung structures, cells and cellular components. The appearance of abnormalities would clearly indicate a damaged lung, and further observations by morphometric and other techniques could be performed to quantify damage, if desired.

2.2 Respiratory Mechanics

Measurements of respiratory mechanics which have been performed in animals are outlined in Appendix B, Tables B-1 through B-7. The simplest measurements, respiratory rate and tidal volume are complemented by tests of increasing complexity. Respiratory mechanics can be usefully divided into static and dynamic mechanics. Static mechanics describe and quantify the mechanics of the respiratory system under conditions of no respiratory gas flow. This includes

measures of lung-volume subdivisions and chest wall and lung compliances.

Dynamic mechanics describe and quantify the mechanics of the respiratory system under conditions of respiratory gas flow. This includes measures of pulmonary and airway resistance, flow-volume relationships, and frequency dependence of compliance and resistance. In general, dynamic mechanics measurements are technically more demanding and are particularly difficult to measure in small laboratory mammals.

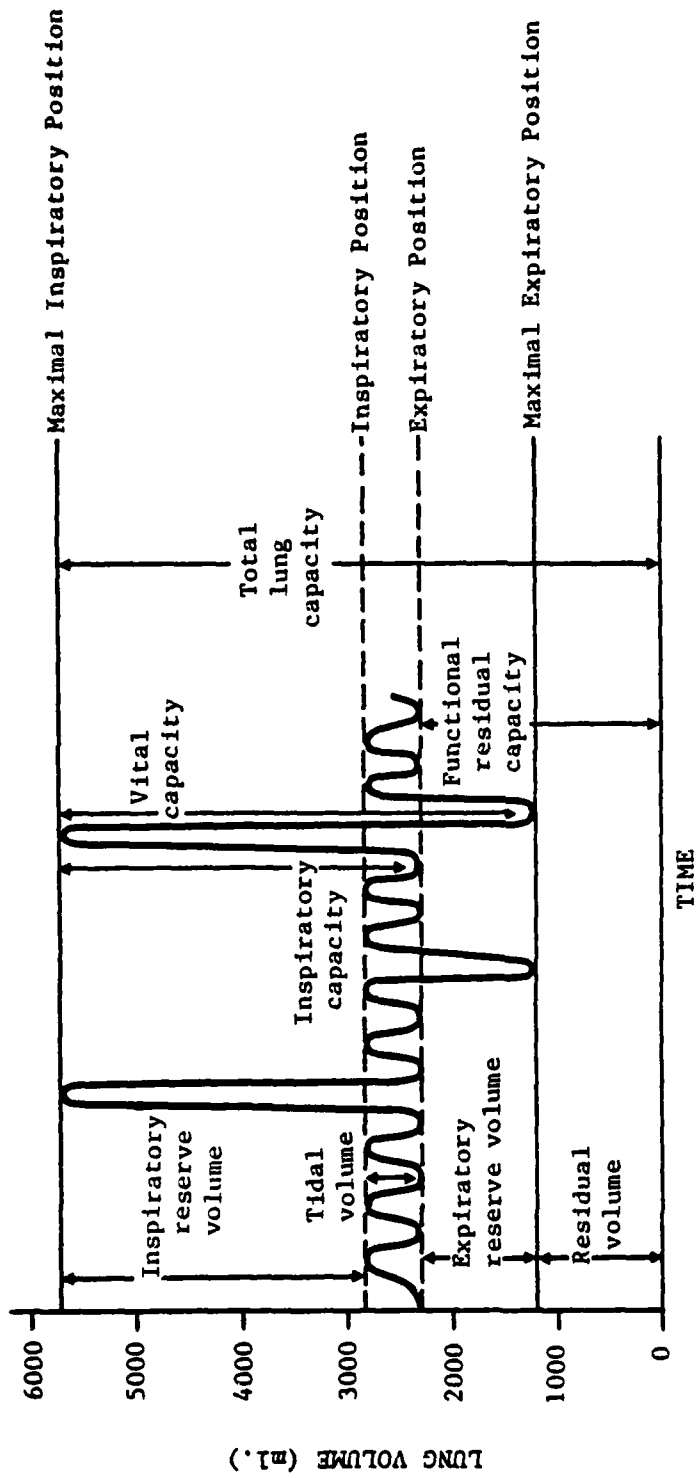
The same basic equipment is used for many of the measurements of respiratory mechanics, and it is relatively simple to set up this equipment so that a battery of tests can be performed with only minor rearrangement (accomplished, for example, by opening and closing valves). This approach is highly labor-efficient and is almost uniformly practiced by pulmonary researchers. As is the case with many measurements used to indicate damage to the pulmonary system, the precise interpretation and significance of alterations in measurements of respiratory mechanics are subject to debate. In the following paragraphs, various lung volumes and capacities are defined and their interrelationships indicated.

In humans, the volume of the lung has been divided into four component volumes, which are defined by the functional limits of the lung (see Figure 2-1). The tidal volume (V_T) is the volume of air inhaled or exhaled during normal breathing. The additional volume of

air that can be inhaled after inhalation of a normal tidal volume by a maximal inspiratory effort is termed the inspiratory reserve volume (IRV). Similarly, the amount of air that can be exhaled after a normal tidal exhalation by a maximal effort is called the expiratory reserve volume (ERV). The volume of air remaining in the lungs after a maximum exhalation is the residual volume (RV).

Combinations of the lung volumes are called lung capacities (see Figure 2-1). The maximum volume to which the lungs can be expanded is the sum of the four "lung volumes" and is called the total lung capacity (TLC). The inspiratory capacity (IC) is the sum of the tidal volume and the inspiratory reserve volume. The vital capacity (VC) is the sum of the inspiratory capacity and the expiratory reserve volume (i.e., it is the maximum size of a single breath which can be breathed in or out of the lung by an individual). The volume of air in the lungs after a normal tidal expiration is termed the functional residual capacity (FRC) and is the sum of the expiratory reserve volume (ERV) and the residual volume (RV). Changes from control lung volumes or capacities are associated with (a) airway obstruction, (b) alteration of lung recoil force and/or (c) destruction of pulmonary parenchyma. The relationships between pulmonary volumes and capacities are summarized in Figure 2-2.

In laboratory animals, lung volumes and capacities have been defined in terms of inflation and deflation pressure limits and FRC.



Adapted From Guyton, 1976

FIGURE 2-1
LUNG VOLUME COMPARTMENTS DURING NORMAL BREATHING AND
DURING MAXIMAL INSPIRATION AND MAXIMAL EXPIRATION

$$\text{TLC} = \text{TV} + \text{IRV} + \text{ERV} + \text{RV} = \text{IC} + \text{FRC}$$

$$\text{VC} = \text{TV} + \text{IRV} + \text{ERV} = \text{TLC} - \text{RV} = \text{IC} + \text{ERV}$$

$$\text{IC} = \text{TV} + \text{IRV} = \text{TLC} - \text{FRC}$$

$$\text{FRC} = \text{ERV} + \text{RV} = \text{TLC} - \text{IC}$$

KEY: ERV - Expiratory Reserve Volume
FRC - Functional Residual Capacity
IC - Inspiratory Capacity
IRV - Inspiratory Reserve Volume
RV - Residual Volume
TLC - Total Lung Capacity
TV - Tidal Volume
VC - Vital Capacity

FIGURE 2-2
RELATIONSHIPS BETWEEN PULMONARY VOLUMES AND CAPACITIES

Leith (1976) has discussed these definitions in animals. The following definitions are generally accepted by workers studying laboratory mammals: TLC is the volume in the respiratory system when the transpulmonary pressure (P_{TP}) is 30 cm H₂O. P_{TP} is defined as the pressure inside the lung relative to that outside the lung (i.e., the airway opening pressure minus the pressure in the pleural space). RV is the volume in the lung when P_{TP} is equal to -30 cm H₂O. FRC is the volume in the lung at the end of expiration. The remainder of the volumes and capacities are calculated as described above.

In the following paragraphs, various aspects of respiratory mechanics and techniques applicable to their measurement in animals are discussed. The aspects described are: respiratory rate and tidal volume; residual volume; vital capacity; total lung capacity; inspiratory capacity; expiratory reserve volume; functional residual capacity; resistance; distribution of ventilation; closing volume; and chest wall, lung and respiratory system pressure-volume curves.

Respiratory rate and tidal volume are the simplest and most widely utilized measurements of pulmonary mechanics. Measurement of respiratory frequency (f) and tidal volume (V_T) are relatively simple and are frequently used as a measure of pulmonary function. The product of f and V_T is minute ventilation (V_E) or total ventilation. As a result of inhaled air pollutants, V_T and f may be changed early as a result of airway-receptor irritation and reflex stimulation. These responses are often only transient in duration.

More sustained changes in f and V_T are indicative of more severe and lasting pulmonary injury. Ventilation will be discussed in more detail in the section on dynamic mechanics. Table B-1 in Appendix B outlines the basic techniques which have been used for determining respiratory rate and tidal volume in animals. These measurements (f and V_T) are usually made together (1) by collecting expired gas in a recording spirometer or gas bag during a specified time and recording or counting f ; (2) by recording the integration of the respiratory flow signal from a pneumotachograph--which gives a signal equivalent to volume--and counting the f from the volume trace; or (3) by recording V_T and f from signals from a body plethysmograph (either pressure, flow or volume plethysmograph). Any of these three techniques may be used as part of the measurement of other aspects of pulmonary mechanics, such as volume-pressure, volume-flow relationships or resistance measurements (Appendix B, Tables B-4 and B-5).

Functional residual capacity (FRC) and residual volume (RV) are measured in animals with one of two general types of techniques: gas dilution or gas volume-pressure relationships with a body plethysmograph. Gas dilution techniques are of two general types: gas equilibration and gas washout. In the first, a known concentration and volume of an inert gas like helium or neon, or a respiratory gas like oxygen, is exchanged with the gas in the lung until equilibration of gas concentration is achieved between the test gas volume and the volume in the lung. Knowing the original test gas volume--the

concentration of the test gas in the test volume and within the lung, and the concentration of the test gas after equilibration--one can calculate the original volume in the lung (either FRC or RV) with the following relationship:

$$C_{s1} \cdot V_{s1} + C_{L1} \cdot \text{FRC (or RV)} = C_E \cdot V_{s2} + C_E \cdot \text{FRC (or RV)}$$

where C_{s1} is the concentration of the test gas in the test volume at the beginning of the test. V_{s1} is the volume of test gas mixture at the beginning of the test; V_{s2} is the volume of test gas mixture at equilibration; and C_{L1} is the concentration of test gas after equilibration with the test gas volume, and in the volume in the lung. C_E is the concentration of test gas at the end of the determination. Either FRC or RV can be determined, depending upon whether the determination is begun at the expiratory position or at the maximum expiratory position, respectively (see Figure 2-1).

In the second, the test gas is oxygen, which is breathed into the lung via a T-valve, and the expired gas is collected. The procedure is continued until nearly all of the nitrogen in the lung has been replaced with oxygen. By knowing the original concentration of nitrogen in the lung (C_{LN2}), and by measuring the expired volume (V) and the nitrogen concentration in the expired volume (C_{VN2}), one can calculate the original volume in the lung (FRC or RV) with the relationship:

$$C_{LN2} \cdot \text{FRC (or RV)} = C_{VN2} \cdot V$$

The plethysmographic technique requires a pressure plethysmograph (PP) which can be calibrated for volume by the relationship between pressure changes in the PP and the volume changes in the animal's lungs (or body). In this technique the animal is placed within the closed PP and breathes through an airway to the outside. The pressure changes of the airway opening (ΔP_{a0}) and the PP (ΔP_{pp}) are recorded. The airway of the animal is obstructed at RV or FRC depending upon which volume is to be measured. The animal inspires against a closed airway and ΔP_{a0} and ΔP_{pp} are recorded. Using the volume-pressure relationship of Boyle's Law, we are able to calculate FRC (also called thoracic gas volume when measured this way) or RV by the following relationship:

$$\text{FRC (or RV)} = \frac{P_{a02} V}{P_{a01} - P_{a02}}$$

where: P_{a01} is the pressure at the airway opening prior to the animal inspiring against a closed airway. P_{a02} is the pressure at the airway opening at the end of inspiration against a closed airway. V is the volume change that occurs with the inspiratory effort against a closed airway. This is determined from the calibration of the PP and ΔP_{pp} during the inspiration against a closed airway.

The mechanisms that determine FRC and RV in most small laboratory mammals differ from those in human beings and larger mammals. The size of the FRC and RV values in animals can be changed by body position, state of excitement and level of general anesthesia.

Hence, great care must be exercised in setting up conditions for measuring them, in interpreting their values compared to controls, and in comparing their values to adjusted values in other species. Under the same conditions of measurement, thoracic gas volume measured with the PP is usually larger than FRC measured with the test-gas equilibration technique. If there is a large difference, it is interpreted to mean there is a large volume of gas trapped in slowly ventilated or obstructed airways.

FRC has also been determined by application of Boyle's Law, which states that the volume of a gas varies in inverse proportion to the pressure to which it is subjected. Application of this principle may involve occlusion of an animal's airway opening at the end of expiration, and as the animal expands the pulmonary air (during inspiratory efforts), the changes in pressure and volume within a plethysmograph are recorded. FRC can then be calculated as the absolute value of the product of the final pressure and the ratio of the change in volume to the change in pressure (DuBois et al. 1956).

Changes in TLC, FRC and/or RV values from normal are difficult to interpret and values may actually be normal in the presence of serious problems. Some suggest that these measurements may be of considerable value, particularly in detecting changes in lung structure at the parenchymal level (O'Neil 1978). In general, decreased values are indicative of restrictive lung diseases or damage, whereas abnormally high values are indicative of airway obstruction. Other

functional and morphological techniques can help to determine which general type of injury is responsible for the change in TLC, FRC or RV values.

The other major group of static mechanical measurements are the volume-pressure relationships of the respiratory system. The volume-pressure relationships are measured under conditions of no gas flow in or out of the respiratory system and provide a means of assessing the elastic recoil of the system. Compliance of the system is the slope of the line relating the volume and pressure over a specified volume range and is expressed as volume (ml) per unit pressure (cmH₂O). Compliance of the respiratory system (C_{RS}) includes that of the chest wall (C_w) and lung (C_L) and is related by $1/C_{RS} = 1/C_w + 1/C_L$. The elastic recoil of the lung does not vary significantly in different animal species; however, the elastic recoil of the chest wall varies considerably. In general, small mammals like rodents have relatively low elastic recoil values of their chest wall (high C_w values) and large mammals like humans have relatively stiff chest walls (low C_w values); C_w values dominate C_{RS} in larger mammals and C_L values dominate C_{RS} values in small mammals. It is therefore important to assess C_L and C_w when examining the elastic recoil of the system. C_w values change with the size of the animal and within an animal species. C_L is determined largely by the elastic recoil of the lung parenchyma or exchange area. Elastic recoil of the lung parenchyma is the sum of the recoil

forces of the lung tissues in the alveolar walls and smallest airways, and the surface tension forces at the liquid-air interface lining the exchange area. Some gaseous toxic agents cause pulmonary edema, which will effect the pulmonary surfactant lining, and this will change the lung recoil force and compliance. C_L values measured from volume-pressure curves of lungs filled with liquid are a measure of tissue elastic forces, whereas those measured from gas-filled lungs are a measure of tissue and surface-tension recoil forces.

Volume-pressure relationships of the respiratory system, chest wall and lungs are done in living animals and in excised lungs. In living animals, static, quasi-static and dynamic compliance measurements are made. In each, transrespiratory (P_{RS}), transthoracic (P_W) and/or transpulmonary (P_{TP}) pressure are measured. P_{RS} is the difference between airway pressure (P_{ao}) and the pressure around the body surface (P_B); P_W is the difference between pleural pressure (P_{Pl}) and P_B ; and P_{TP} is the difference between P_{ao} and P_{Pl} . P_{Pl} is estimated in the living animal with a low-volume, balloon-tipped catheter placed in the intrathoracic esophagus. Lung volume is usually measured with a large syringe, spirometer, pneumotactograph or plethysmograph. Volume and pressure are recorded simultaneously on an X-Y recorder with volume on the Y-axis.

Static volume-pressure curves result from a series of volume-pressure points identified on an X-Y graph as the result of stepwise

inflation and/or deflation of the lungs. At specified volume points, and with no gas flow, pressure is measured. Under conditions of no gas flow, only elastic recoil pressures are measured at any particular lung volume. During gas flow, there are elastic recoil (P_E), resistance (P_R), inertial (P_I) and tissue viscosity pressures (P_{TV}). P_R , P_I and P_{TV} approach zero as gas flow is reduced to low levels. Under conditions at very low gas flows, quasi-state, volume-pressure measurements are made. This is a more convenient technique than static volume-pressure measurements and is used frequently in studies on laboratory animals.

Dynamic compliance measurements are made on the spontaneously breathing animal or an animal ventilated at high frequencies and will be discussed in the next section of dynamic mechanics.

Since the volume-pressure curve changes slope greatly at the extremes of lung volume, it is important to measure volume-pressure relationships at known volume histories and at specified lung volumes. As indicated above, the volume-pressure relationships of the lung are used to define the limits of vital capacity.

Volume-pressure relationships and resistance are nearly always determined in tandem, as both require measurement of P_{p1} and P_{a0} . The variations in techniques outlined in Appendix B, Tables B-4 and B-5, reflect differences in methods used for measurement of P_{p1} (direct by intrapleural catheter or approximation by esophageal balloon catheter) and volume changes (by plethysmograph or pneumotachograph).

Although, theoretically, dynamic pulmonary mechanics measurements should detect early pulmonary injury, there is no reliable way in which to measure dynamic pulmonary mechanics in mammals to date. Koo and coworkers (1976) have made tedious measures of maximum expiratory flow volume curves and other dynamic respiratory functions in small mammals, and have identified and acknowledged the technical difficulties in measuring these relatively fast flow events in small mammals. The major technical difficulties are associated with: (1) the relatively small dimensions of small mammalian airways; (2) the relatively large dead space of conventional measuring equipment; (3) the relatively high frequencies at which small mammals breathe; and (4) the relatively poor frequency response characteristics of equipment measuring flow, volume and pressure in their respiratory systems.

The technical limitations have prohibited acceptable measure of dynamic pulmonary function and have made it impossible to measure these events in small mammals with early or mild pulmonary injury. The state of the art has only recently made it possible to begin to evaluate techniques for measuring dynamic pulmonary mechanics in small mammals.

Dynamic respiratory mechanics include measurement of mechanical features of respiration during gas flow into or out of the lungs. Total ventilation (V_E), as described earlier, is a basic measure of the overall effectiveness of the mechanical function of the lung.

It is the product of f and V_T and the sum of dead space ventilation (V_D) and alveolar ventilation (V_A).

V_D is that portion of the total ventilation that enters or leaves the anatomical and physiological dead space. In terms of respiratory gas exchange, it is "wasted ventilation" and it increases with certain lung disorders. "Anatomical dead space" includes all the conducting airways where there is no gas exchange between the air spaces and pulmonary capillary blood. "Physiological dead space" includes all the anatomical dead space, and the volume of gas in the alveoli that are not perfused--or poorly so--with pulmonary capillary blood, and in which gas exchange is slow or does not occur.

V_A is the effective ventilation that takes part in respiratory gas exchange. Hyperventilation is greater alveolar ventilation than is required by the animal's metabolic level, and hypoventilation is less alveolar ventilation than is required by the animal's metabolic level. Hyperventilation may be seen in the early stages of respiratory stress and hypoventilation is usually found in advanced and/or severe respiratory disorders.

The levels of V_A and V_D are calculated from values of V_E , alveolar carbon dioxide partial pressure (P_{ACO_2}) and the output of carbon dioxide V_{CO_2} with the relationships:

$$V_A \text{ (ml/min)} = \frac{V_{CO_2} \times 0.863}{P_{ACO_2}}$$

$$V_D \text{ (ml/min)} = V_E - V_A$$

Respiratory system resistance (R_{RS}) is a measure of the pressure (cm H_2O) per unit flow (ml/sec) and is determined from simultaneous records of transrespiratory pressure (P_{RS}) and respiratory gas flow. It includes resistance in the chest wall and lung (pulmonary resistance, R_L). Since R_{RS} is a measure of a number of factors, it is rarely measured alone but is commonly determined concurrently with pulmonary and airway resistance.

Pulmonary resistance (R_L) is a measure of the frictional resistance in the airways (R_A) and the tissue viscosity resistance (R_T). It is relatively low in healthy animals and contributes a relatively small amount to the work of breathing.

It can change substantially with alterations in the cross-sectional area of large airways, as occurs with bronchoconstriction or large amounts of mucous production in the airways. It is determined from measures of transpulmonary pressure (P_{TP}) and respiratory gas flow during spontaneous or forced ventilation.

R_A is an estimate of frictional airway resistance and requires an estimate of alveolar pressure versus airway opening pressure while small oscillating volumes of gas are measured at simultaneously recorded flow rates in and out of the respiratory system. In many laboratory animals, R_A dominates R_{RS} . R_A can be substantially changed following large airway injury. The test has not been particularly sensitive in detecting small airway injury which occurs with many air pollutants.

In healthy small animals, the distribution of ventilation is nearly even in the respiratory system. However, in large healthy animals, there is measurable maldistribution of gas volume, ventilation and blood perfusion associated with the effects of gravity upon the lung tissue and pulmonary blood supply. This maldistribution worsens with a wide variety of pulmonary disorders. If R_A and compliance (C_L) of all the units of the lung were equal, ventilation to all regions of the lung would be equal and there would be no maldistribution of ventilation. Insult by inhaled toxicants affects the lung, usually causing greater or lesser changes in R_A and/or C_L in different regions. The consequence of uneven changes in R_A and C_L is an exaggeration of the maldistribution of ventilation within the injured lung.

Several techniques are being employed in an attempt to detect maldistribution of ventilation following mild and/or early injury to small airways by inhaled toxins: (1) high frequency oscillations to measure frequency dependence of compliance and impedance; (2) single-breath nitrogen washout; (3) closing volume measurements; and (4) radioisotopic-labeled gas distribution in the lung.

In the healthy individual, impedance and dynamic compliance of the lung do not change as frequency of breathing or oscillation of gas flow in and out of the lung are increased to very high rates.

In humans, in which there are unevenly distributed lesions in the small airways, the distribution of resistance in the various

regions of the lung have been shown to be different. Under these circumstances, the impedance to flow of gas in and out of the lung increases as the frequency of breathing increases. There is technology now that permits the use of this test on laboratory animals. It may well be the most sensitive functional test for mild, small-airway lesions.

The measure of the dependence of dynamic pulmonary compliance on high frequencies may enable one to detect early lesions in the alveolar walls. This test is complex in interpretation and technically very demanding and difficult.

The single-breath nitrogen washout gives an index of the evenness of ventilated gas distribution in the lung and has been sensitive to the detection of substantial airway disease.

The distribution of ventilation is determined by having an animal inhale 100 percent oxygen, and either constructing a curve of nitrogen concentrations in the exhaled gas until a steady state is reached (multiple breath); or holding the pure oxygen for a period of time, and monitoring the nitrogen concentration continuously as the animal expires (single breath). A steady washout of nitrogen from the lungs in the multiple breath test, or a steady level of nitrogen in the final portions of the expirate of the single breath test, will indicate even distribution of ventilation. Nitrogen-washout techniques for measuring the distribution of ventilation have been successfully applied to animals (Alarie 1978).

Of the many techniques for measuring closing volume, although useful in humans as an indicator of ventilation distribution, few have been widely applied in pulmonary testing in small animals. Closing volume has been defined as the lung volume at which dependent (lower) lung zones cease to ventilate, presumably because of airway closure. Premature airway closure or increased closing volume may reflect obstruction in the small airways or loss of lung elastic recoil. However, care must be exercised in interpreting results of closing volume measurements in animals since the distribution of lung volume and ventilation may be quite different from that of humans.

Two closing volume measurement techniques have been applied to animals: nitrogen washout and bolus inhalation. These techniques are outlined in Appendix B, Table B-6. Closing volume is represented by the final steep slope of the nitrogen washout curve, which is due to the emptying of the upper lung. Measurement of the closing volume by bolus inhalation has been applied to animals. At the end of expiration, the animal inhales small amounts of tracer gas, which is presumably distributed to upper lung zones, and then inhales room air or oxygen to fill the lower pulmonary zones. During the next expiration, while lung volume changes are recorded, the marker gas is monitored at the mouth. The concentration of the marker gas increases at the closing volume (Green et al. 1972; Kosch et al. 1979a).

Amis (1979) has refined the original radioisotopic-labeled gas techniques of West and co-workers (1977) to measure the distribution

of ventilation in the lungs of dogs. This technique has not been tested completely in the injured lung, but shows promise as a sensitive test for detecting airway and pulmonary parenchymal injury.

Maximum expiratory flow below 80 percent of vital capacity is independent of effort and dependent upon airway resistance, lung recoil and lung volume. Measure of the relationship of maximum expiratory flow at different lung volumes has been used to examine the relative contribution of small and large airways to pulmonary resistance in healthy and injured lungs. Koo and co-workers (1976) and Kosch and co-workers (1979b) have developed techniques for study of expiratory flow-volume relationships in laboratory animals. The methods are technically demanding and time-consuming, and require general anesthesia and tracheostomies.

2.3 Gas Exchange

Gas exchange is the general function by which there is a net transfer of respiratory gases between the alveolar spaces and the pulmonary capillary blood. Specifically, it is concerned with the net diffusion of oxygen from the alveolar spaces to the pulmonary capillary blood, and of carbon dioxide from the pulmonary capillary blood to the alveolar spaces. These functions are linked to and dependent upon the level of alveolar ventilation, the evenness of distribution of ventilation, the volume and distribution of pulmonary capillary blood flow, and the diffusion membrane between the alveolar space and the pulmonary capillary blood.

Measurement of arterial blood-gas partial pressures (P_{aO_2} and P_{aCO_2}) can give an overview of the respiratory gas exchange and total pulmonary function. Additional tests are necessary to identify mechanisms causing abnormal respiratory gas exchange. Arterial blood-gas partial pressures are frequently measured in laboratory animals as part of the assessment of their pulmonary function. Blood gases have been measured in rats, hamsters, guinea pigs, cats, dogs, monkeys, preterm lambs, and calves, and can be measured in any animal from which an adequate amount of blood (0.1 to 1.5 ml) can be removed anaerobically. Serial blood sampling and analysis has been performed in animals as small as rats. Pulmonary damage affecting the gas "exchanging ability" of the lung may be manifested in the blood by an abnormally low partial pressure or concentration of oxygen, and in some instances by abnormally high concentration and partial pressure of carbon dioxide. Several additional measurements (pH, base excess, HCO_3 , O_2 saturation) have also been made in many cases, as these factors reflect the capacity of the blood for O_2 and CO_2 .

Hypoxemia (low arterial oxygen partial pressure [P_{aO_2}]) can result from: (1) low-inspiratory oxygen, (2) hypoventilation, (3) pulmonary alveolocapillary diffusion impairment, (4) mismatching of ventilation and perfusion and/or (5) right-to-left pulmonary vascular shunt. Hypercapnia (high-arterial carbon dioxide partial pressure [P_{aCO_2}]) can result from hypoventilation, and in very severe circumstances, from mismatching of ventilation and perfusion.

Hypoventilation, described under dynamic pulmonary mechanics, refers to an abnormally low alveolar ventilation. Under this circumstance, the alveolar-gas partial pressure of oxygen (P_{aO_2}) is low, and the carbon dioxide partial pressure (P_{aCO_2}) is high. These changes from expected P_{aO_2} and P_{aCO_2} occur because the transport for O_2 -uptake (V_{O_2}) and CO_2 -output (V_{CO_2}) is diminished. The low P_{aO_2} and high P_{aCO_2} causes an increase in the alveolar-pulmonary capillary partial pressure differences for O_2 and CO_2 , respectively ($P_{aO_2} - P_{cO_2}$ $P_{cCO_2} - P_{aCO_2}$). Nevertheless, the net transfer of O_2 and CO_2 between the alveolus and capillary blood decreases and results in a decrease in P_{aO_2} (hypoxemia) and an increase in P_{aCO_2} (hypercapnia). Pulmonary injuries for which there is inadequate ventilatory compensation lead to hypoventilation. Hypoventilation is the most significant cause of hypercapnia.

Pulmonary alveolocapillary diffusion impairment is rarely a significant pulmonary abnormality because of the large, diffusion-capacity reserve of lungs and the relatively high diffusion coefficients of O_2 and CO_2 . CO_2 is about 20 times more diffusible than oxygen between the pulmonary capillary blood and the alveolar space. It is not retained with alveolocapillary wall changes. However, O_2 transfer can be impaired if there is significant wall damage. Pulmonary diffusion capacity can be estimated with functional tests using carbon monoxide or radioisotopic-labelled O_2 . It is not likely that these tests will detect early or mild pulmonary

injury because of the large diffusing capacity reserve. Techniques for measuring blood gases are well developed and, in most cases, the investigators use commercial blood-gas analyzers. In all other cases, the equipment used operates on the same principle as the commercial analyzers and, therefore, a description of the analytical methodology has not been provided in the tables in Appendix C in the column headed "Specific Technique Employed." What this column does contain is a description of the blood-sampling technique employed, as this appeared to be the only significant difference between techniques. Blood sampling has been accomplished both by percutaneous puncture and by inserting indwelling catheters. Arterial blood has been taken from the caudal, carotid and femoral arteries and the aorta, and mixed venous blood (pulmonary venous return) has been sampled in the pulmonary artery via a catheter inserted into a peripheral vein. Procedures are simple, nonlethal, and have been done without anesthesia.

Three techniques have been used: the single-breath techniques, the rebreathing technique, and the steady-state technique using an end-tidal sample. All of the techniques have been performed without the use of anesthesia. Carbon monoxide is used rather than O_2 for this purpose because it binds strongly to hemoglobin allowing the assumption of zero P_{aCO} in arterial blood.

In the single-breath technique (Ogilvie et al. 1957), the subject makes a maximal inspiration of a gas mixture containing measured

amounts of CO, helium and/or other inert gases and oxygen, holds it for about 10 seconds, and releases it. The CO diffusing capacity (D_{LCO}) is calculated from these concentrations plus the concentrations of CO and helium in the expired end-tidal gas. If diffusing capacity is impaired, a smaller-than-normal quantity of CO will be removed from the inspired air. For the rebreathing technique, the subject rebreathes air in a closed system for a period of time (on the order of 10 minutes) and the CO content of the air is continuously monitored. The steady-state, end-tidal technique involves having the subject breathe a mixture containing a fixed amount of CO, collecting the end-tidal expirate and recording its CO content when it has reached a steady state. Of the three techniques, the single-breath method appears to be the most rapid and most widely used.

By repeating the single-breath carbon monoxide diffusing capacity measurement at 2X or 3X P_{aO_2} greater than 150 mmHg, one can obtain values to calculate the pulmonary capillary blood volume (V_C). V_C can be of value in assessing the presence and magnitude of pulmonary emphysematous changes in which there is destruction of pulmonary parenchyma distal to the terminal airways.

Uneven matching of ventilation and perfusion is the most common cause of hypoxemia. The assessment of ventilation distribution was described in the section on dynamic pulmonary mechanics, and the distribution of pulmonary capillary blood will be described in the section on circulation below.

Right-to-left pulmonary vascular shunts (i.e., diversion of blood flow from the right or pulmonary side of the heart to the left or systemic side through an anomalous opening) cause hypoxemia and are usually indicative of severe, often irreversible pulmonary injury. There are techniques for estimating the magnitude of right-to-left shunts. However, they are rarely used in toxicity studies because the injury must be long-standing and advanced to produce right-to-left shunts. In general, if the PaO_2 is less than 500 mmHg following several minutes of O_2 breathing, one should suspect the existence of a significant right-to-left shunt.

2.4 Pulmonary Circulation

Blood circulation through the lungs is the final step in the pathway of pulmonary gas exchange. As outlined in Appendix D, Table D-1, several indices of pulmonary circulation have been described and measured (i.e., blood pressures and longitudinal distribution of vascular resistance) or calculated (i.e., capillary blood volume, vascular resistance, left-to-right shunt) in cats and dogs, before and after exposure to a number of toxic substances. However, none of these has been widely used to detect damage in the pulmonary system.

Blood pressures at points within the pulmonary circulation are the only directly measured indices of circulatory function. Although the blood pressure in the lungs is highly dependent on nonpulmonary factors, these factors can be effectively eliminated by measuring pressures in several branches of the cardiopulmonary circulation.

Blood pressures are usually measured with an external strain-gauge transducer attached to an appropriately placed catheter. Recently they have been measured with a pressure transducer actually in an indwelling catheter.

Pulmonary vascular resistance (i.e., resistance to blood flow through pulmonary vessels) has been calculated from the blood pressures and rate of blood flow in the pulmonary artery or vena cava. No instances have been found in which this measurement has been used to evaluate damage produced by a pulmonary toxicant.

The final calculated parameter is the left-to-right shunt of pulmonary blood (i.e., the portion of the pulmonary circulation which does not reach the alveoli). In certain types of cardiopulmonary damage, the shunt will be dramatically increased. This has been calculated from the arterial-alveolar oxygen difference during pure oxygen-breathing in dogs.

2.5 Defense Mechanisms

In addition to being an efficient organ of gas exchange, the lung, as a primary target organ, must also function as a protective barrier for the body against harmful agents. The major function of the pulmonary defense mechanisms is the inactivation and/or the removal of inhaled foreign material by: dilution in airway fluid lining, immunologic inactivation, mucociliary transport, phagocytosis by alveolar or blood-origin macrophages, and/or lymphatic drainage to lymph nodes. Impairment of this function results in prolonged contact with the invading material, further invasion into the lung and

body, and increased overall toxic effects upon the host. Techniques for measuring separately the components of the pulmonary defense system (i.e., immunologic, mucociliary and alveolar macrophage functions), have been developed in animal systems. These techniques are outlined in Appendix E, Tables E-1 through E-4, and are discussed below.

The components measured in efforts to describe mucociliary function are the rate of mucociliary transport, the frequency of ciliary beating and the size and distribution of mucous-secreting cells. Techniques for acquiring these measurements are outlined in Appendix E, Table E-1. The most widely used index of mucociliary transport is the rate of movement of inert particles. Radiolabelled or highly visible particles are inhaled or deposited on the trachea. The rate of particle movement up the mucociliary escalator may be measured in vivo by scanning radioactivity or by high-speed filming. The filming can be accomplished either by inserting a bronchofiberscope into the trachea or by exposing it surgically. In vitro techniques have also been used to measure mucociliary transport. These techniques involve stroboscope observations of deposited or previously inhaled graphite particles in excised trachea. Similar in vivo filming and in vitro observation techniques have been used to measure cilia-beating frequency as an index of mucociliary transport function.

An increase in the production of mucus, which is a sensitive and integral part of the mucociliary transport system, is reflected by an

increase in size and distribution of mucus secreting cells. Standard histopathologic techniques and scanning electron microscopy have been used to observe and quantify mucus cells in tissue sections. Counts of mucus cells are used as an index of the lung's defense response to injury. This is an indirect index and is not known to correlate with an effective clearance of inhaled toxicants.

Damage to alveolar macrophages, which would reduce the ability of the system to ingest and remove foreign material, has been measured primarily by in vitro techniques as outlined in Appendix E, Table E-2. Alveolar macrophages are extracted from lung lavage, and damaged macrophages are considered to be an indication of decreased resistance to respiratory infection. Green and Goldstein (1966) have labeled bacteria with an isotope which enables them to estimate the number of bacteria inhaled by an animal, the number cleared by the mucociliary escalator, the number engulfed by macrophages and the number killed with and without air pollution insult.

2.6 Biochemical Techniques

The absence of biochemical indicators of pulmonary damage (based on the definition in Section 1.0) has been documented in the literature (Witschi 1975, 1976; Witschi and Cote 1977) and emphasized during personal communications (Witschi 1979; Henderson 1979; Mustafa 1979). Turino et al. (1974) state that "... At present, we are just beginning to appreciate alterations of structure and the mechanisms which may underlie such structural alterations at the molecular

level." Further emphasizing this point, Mustafa and Tierney (1978) state "...we are...just beginning to understand the biochemical mechanisms by which agents injure the lung...."

The problems inherent in elucidating biochemical indicators of pulmonary damage are due primarily to the complex nature of lung tissue. There are over 40 different pulmonary cell types and their functions are not yet well understood. This cellular heterogeneity of the lung hampers biochemical analyses of lung tissue homogenates. Since conventional biochemical assays only reflect the average change of many different cells, even major changes in one population may go unnoticed (Witschi and Cote 1977; Mustafa and Tierney 1978). Further emphasizing this point, Crystal (1976), in his book, The Biochemical Basis of Pulmonary Function, states "Unless biochemical measurements are made on the local level, changes may be lost in the overall complexity of the lung." Despite the complexity of the cell types and distribution of tissue types in the lung, assessment of the biochemistry of lung regions (e.g., parenchyma vs. airways), cell types (e.g., alveolar macrophages, airway epithelium), and/or tissue types (e.g., collagen), has shown distinctive biochemical features in pulmonary tissue exposed to certain toxic substances. It is likely that biochemical testing will become an important and sensitive indicator of early pulmonary injury.

Pulmonary toxicity studies at the biochemical level have been done to test for indicators of pulmonary damage, and to understand

the injury mechanisms of respiratory toxins. Attempts have been made to correlate biochemical measurements with those indicators of pulmonary damage previously defined. Several approaches have been undertaken to develop biochemical indicators of pulmonary damage. These include techniques which measure:

- Various biochemical parameters correlating to Type 2 alveolar cell proliferation, including RNA to DNA ratios and the rate of synthesis of RNA and DNA
- Specific enzymes and components of lavage
- Lung tissue or serum enzyme activities
- Lipid peroxidative products
- Components of surfactants and collagen
- Secretion of glycoproteins by tracheal slices
- Energy utilization and O₂ consumption of lung cell suspensions, lung slices and homogenates

Type 2 cell proliferation has been studied morphologically and biochemically. It is well understood that Type 1 alveolar cell damage is followed or accompanied by Type 2 cell proliferation. However, Witschi (1976), writing of attempts to correlate changes in measurements of biochemical parameters with proliferation of Type 2 alveolar cells, concludes that the evidence is only indirect and, therefore, inadequate for screening purposes. Autoradiography and other morphological techniques used in measuring Type 2 alveolar cell proliferation are reviewed in Section 2.1.

Due to the complexity of the lung tissue, lung lavage has been used to examine biochemical indicators of pulmonary damage (Henderson et al. 1979). Those biochemical indicators examined, and the significance of their measurements, are outlined in Table 2-2. The lavage techniques described by Henderson and co-workers (1979) is included in Appendix F, Table F-1.

The trachea has either been exposed to toxic substances in vivo, then excised, sliced and incubated in media, or sliced and exposed in vitro to toxic agents where the secretion rates of mucus glycoproteins have been monitored for inhibition. Last and co-workers (1979), in studies of chromate toxicity, found that tracheal slices were sensitive to the effects of chromate; and they also found similar rates of inhibition between slices from the two different exposure methods at approximately the same exposure concentrations, thus validating the in vitro procedure.

The perfused lung technique has been used primarily to study metabolism, not the effects of toxic substances. In this technique, the lungs from a laboratory animal are excised and the pulmonary artery, left atrium and trachea are cannulated. Perfusion media is then pumped through the pulmonary artery and the vessels in the lungs, and collected at the left atrium while the lungs are ventilated with a ventilation pump through the trachea. Slices of excised lungs can also be cultured in perfusion media. The lung slices have been used to study lung metabolism, especially glucose oxidation rates (Rhoades 1974).

TABLE 2-2

BIOCHEMICAL MEASUREMENTS FROM BRONCHOPULMONARY
LAVAGE BY HENDERSON ET AL., 1979

<u>MEASUREMENT</u>	<u>SIGNIFICANCE</u>
lactate dehydrogenase	cytoplasmic enzyme, occurs extracellularly in presence of damaged cells or from serum in the presence of pulmonary edema
glucose - 6P - dehydrogenase	cytoplasmic enzyme shown to occur in Type 1 cells (Vijayaratnam and Corrin, 1972), occurs extracellularly in presence of damaged cells; marker enzyme for hexose - monophosphate shunt pathway of glucose metabolism, which increases during repair process. Precaution: is a marker for leukocytes and RBC's if any hemorrhagic diathesis occurs. (Rossi et al. 1975)
acid phosphatase	lysosomal enzyme, indicating phagocytosis by or damage to alveolar macrophages. Precaution: not specific for pulmonary alveolar macrophages (Unane 1976; Weissman et al. 1971)
β -glucuronidase	lysosomal enzyme, indicating phagocytosis by or damage to alveolar macrophages. Precaution: not specific for pulmonary alveolar macrophages (Unane 1976; Weissman et al. 1971)
alkaline phosphatase	associated with Type 2 epithelial cells but not in Type 1 cells or alveolar macrophages (Witschi 1976; Vijayaratnam and Corrin, 1972); increased levels in lavage might reflect Type 2 damage; transudation of serum proteins will also increase levels
trypsin inhibitory capacity	important in repair process; therefore, an increase may suggest response to damage or be indication of transudation; difficult to measure in edematous lungs and is difficult to interpret
collagen	reduction in quantity or presence of excess catabolites considered indication of injury to connective tissue

The most commonly cultured mammalian lung cells are the alveolar macrophages, since they are easy to obtain from lung lavage samples; however, their contribution to overall lung metabolism and function is probably small (Tierney 1974). Because the lung contains such a wide variety of distinct cell types, the dispersal and culturing of viable cell types has been difficult (Clements et al. 1972). Those cell types that have been successfully cultured, such as Type 2 cells, have been mainly used in the study of cellular metabolism and have not been used to investigate the effects of toxic substances.

These in vitro techniques have the same major disadvantage as other in vitro techniques, in that the properties of the organs, tissues or cells used probably differ from those in the intact animal. All of these techniques are in the developmental stage and are not sufficiently established to be useful for screening purposes.

These techniques have been utilized to a limited extent in efforts to define the mechanism(s) of injury of a few known respiratory toxicants; however, the measurements made have not been conclusively correlated to define the indices of damage. The specific indices examined with respect to the toxic action of three widely studied oxidants (i.e., O₂, O₃, NO₂), as well as selected other compounds, are listed in Table 2-3.

The use of biochemical techniques to determine damage induced by those compounds which might be expected to have similar toxic actions (e.g., oxidants) has not proved satisfactory, since biochemical

TABLE 2-3

BIOCHEMICAL INDICES OF PULMONARY FUNCTION WIDELY STUDIED DURING
DESCRIPTION OF MECHANISMS OF TOXICANT INJURY

<u>INDICATOR MEASURED</u>	<u>COMPOUNDS TESTED</u>	<u>REFERENCES</u>
glucose-6-phosphate dehydrogenase	O ₃	Chow and Tappel, 1972 and 1973; DeLucia et al., 1972
	paraquat	Witschi and Kacew, 1974
	CdCl ₂	Hayes et al., 1976; Omaye et al., 1976
lactate dehydrogenase	NO ₂	Witschi, 1975; Buckley and Balchum, 1965 and 1967
	beryllium	Reeves, 1966; Reeves and Vorwald, 1967
	paraquat	Witschi and Kacew, 1974
	O ₃	Chow and Tappel, 1973
	CdCl ₂	Hayes et al., 1976
aldolase	O ₃	Chow and Tappel, 1973
	NO ₂	Witschi, 1975; Ramazzotto and Rappaport, 1971; Buckley and Balchum, 1965 and 1967
benzpyrene hydroxylase	Ni(CO) ₄	Sunderman, 1967
	cannabis	Witschi and Saint-Francois, 1972
	O ₃	Palmer et al., 1971 and 1972
aryl hydrocarbon hydroxylase	cigarette smoke	Akin and Benner, 1976
	beryllium	Jacques and Witschi, 1973
	paraquat	Witschi and Kacew, 1974

TABLE 2-3 (Continued)

<u>INDICATOR MEASURED</u>	<u>COMPOUNDS TESTED</u>	<u>REFERENCES</u>
glutathione reductase	O ₃	Chow and Tappel, 1972 and 1973; DeLucia et al., 1972
	CdCl ₂	Omaye et al., 1976
glutathione peroxidase	O ₃	Chow and Tappel, 1972 and 1973
	CdCl ₂	Omaye et al., 1976
isocitric dehydrogenase	paraquat	Witschi and Kacew, 1974
	CdCl ₂	Hayes et al., 1976
pyruvate kinase	O ₃	Chow and Tappel, 1973
	paraquat	Witschi and Kacew, 1974
uridine kinase	paraquat	Witschi and Kacew, 1974
	butylated hydroxytoluene	Adamson et al., 1977
thymidine kinase	butylated hydroxytoluene	Adamson et al., 1977
monoamine oxidase	O ₂	Mustafa and Tierney, 1978
	paraquat	Witschi and Kacew, 1974
cytochrome C reductase	paraquat	Witschi and Kacew, 1974
	O ₃	Mustafa and Tierney, 1978; DeLucia et al., 1972
cytochrome C oxidase	NO ₂	Ramazzotto and Rappaport, 1971
succinic dehydrogenase	NO ₂	Ramazzotto and Rappaport, 1971

TABLE 2-3 (Continued)

<u>INDICATOR MEASURED</u>	<u>COMPOUNDS TESTED</u>	<u>REFERENCES</u>
malate dehydrogenase	CdCl ₂	Hayes et al., 1976
malic enzyme	O ₃	Chow and Tappel, 1973
phosphofructokinase	O ₃	Chow and Tappel, 1973
lysozyme	CdCl ₂	Omaye et al., 1976
cathepsins C & D	CdCl ₂	Omaye et al., 1976
β-N-acetyl glucosaminidase	CdCl ₂	Omaye et al., 1976
acid phosphatase	CdCl ₂	Omaye et al., 1976
alkaline phosphatase	O ₃	Scheel et al., 1959
5' nucleotidase	O ₃	Scheel et al., 1959
cytochrome P-450	O ₃	Mustafa and Tierney, 1978
6-phosphogluconate dehydrogenase	O ₃	Chow and Tappel, 1973
ATPase	paraquat	Witschi and Kacew, 1974
malonaldehyde (product of lipid peroxidation)	O ₃	Chow and Tappel, 1972
	NO ₂	Thomas et al., 1968
	O ₃	Fletcher and Tappel, 1973
conjugated dienes (products of lipid peroxidation)	NO ₂	Thomas et al., 1968
thioarbituric acid reactive products (malonaldehyde analysis)	CdCl ₂	Omaye et al., 1976
dipalmitoyl phosphatidylcholine (surfactant component)	O ₂	Mustafa and Tierney, 1978

TABLE 2-3 (Concluded)

<u>INDICATOR MEASURED</u>	<u>COMPOUNDS TESTED</u>	<u>REFERENCES</u>
palmitic acid (surfactant component)	NO ₂	Arner and Rhoades, 1973
	O ₂	Morgan et al., 1965
hydroxyproline (collagen component)	NO ₂	Drozdz et al., 1977
collagen breakdown products	CdCl ₂	Henderson et al., 1979
O ₂ consumption	O ₃	Mustafa and Tierney, 1978
	NO ₂	Buckley and Balchum, 1965
metabolism of glucose	paraquat	Mustafa and Tierney, 1978
	O ₂	Mustafa and Tierney, 1978
	NO ₂	Mustafa and Tierney, 1978
zinc	paraquat	Hollinger et al., 1978
	O ₃	Dixon et al., 1966
copper	O ₃	Dixon et al., 1966
molybdenum	O ₃	Dixon et al., 1966

response has been shown to vary with compounds. This problem is compounded by the ability of many metabolic pathways to adapt to stressed conditions (e.g., an increase or decrease in the level of an enzyme may appear only for a limited time, followed by normal levels after adaptation). The substances listed in Table 2-3 have been compiled to illustrate the multitude of indicators studied in efforts to define toxic mechanisms of injury for selected compounds. However, considering all the difficulties enumerated in this section concerning the ability of biochemical measurements to consistently reflect pulmonary damage, it is unlikely that the inclusion of any of these in a screening program for potential pulmonary toxicants would be advantageous. Nevertheless, it is likely that biochemical testing will become important and sensitive in detecting pulmonary damage, and will have considerable application in future screening efforts.

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3.0 CONCLUSIONS AND RECOMMENDATIONS

The available short-term protocols for assessment of pulmonary system damage have been assigned to six categories. Four of the categories--respiratory mechanics, gas exchange, pulmonary circulation, and defense mechanisms--are defined on the basis of pulmonary function, while the remaining two--morphology and biochemistry--are defined on the basis of structure. The structural and biochemical activities of the lung influence the mechanical, gas-exchange and lung-defense functions, and for that reason are an inseparable part of function. The advantage of the division of the categories is to enable one to define specific measurements more precisely and to attempt to associate specific functional, structural or biochemical features with specific regions and/or cell types of the lungs. Evaluation for pulmonary damage caused by inhaled substances should include several measures of function, structure and biochemistry.

An alternate approach would be to determine the structural basis of alterations in the pulmonary function measurements and to devise a battery of tests which encompass all of the distinct aspects of damage. Much additional research is necessary before this approach can be successfully utilized.

Within each of the categories of function, there is essentially only one level of test sophistication and measurement interpretation, as there is practically no reliable information about which tests are more sensitive, accurate, etc.; therefore, the available tests are

not readily adaptable at this time to a tiered screening program, although there are certain measurements which would be best performed as the last in the battery of appropriate tests. The only possible hierarchicalization is likely to be one based on species utilized.

Evaluation of individual tests within a category is based on a myriad of considerations. These considerations pertain primarily to: (a) costs of the measurement (defined broadly to include monetary costs of the equipment, animals and labor; the time required to perform the experiment; and the skills necessary to perform the measurement), (b) validity of the measurement, including its sensitivity, accuracy and reproducibility, and (c) significance with regard to reflecting human pulmonary damage. At the current stage of pulmonary testing development, many important considerations can be applied only subjectively to the evaluation of individual tests, while others cannot be addressed at all due to a lack of data. Those which can be applied with sufficient confidence to be used as selection criteria are described in the following section.

3.1 Criteria Used in Evaluating Pulmonary System Tests

To evaluate the available tests defensibly, a number of pertinent selection criteria have been identified. These criteria are: the test's state of development and whether it (a) has been performed in small animals and conscious animals; (b) is terminal; (c) is sensitive, accurate and reproducible; and (d) is relatively easy to perform. Other criteria are useful in theory, but their

application is prevented by the unavailability of the requisite data. For example, the cost of equipment required to perform the test is important, but extremely difficult to ascertain, because much of the equipment utilized at present is of unique design and construction. The rationale for selection of each of the criteria employed is presented in the following paragraphs.

The selection of the animal species is of utmost importance and influenced largely by the following three major considerations:

- (1) Appropriateness of the animal as a biomedical model. If the aim is to predict the response of human beings from study of an animal's response to a pulmonary toxicant, one must know if the animal model is capable of responding as a human being might respond.
- (2) Initial costs of animals (rare and large animals are generally costly) and costs of maintaining the animals. Large or unusual laboratory animals are usually more costly to maintain than conventional, small, laboratory mammals.
- (3) Ease with which the pulmonary function tests are adapted to small laboratory animals. Small laboratory animals have many advantages in testing (e.g., being readily available; generally less costly to purchase and maintain; available in uniform breed types; and in general better known in terms of their husbandry, diseases, and biology). However, with the exception of restraint requirements, large animals present fewer technical problems in respiratory function tests. This is particularly true when measurements of dynamic pulmonary mechanics are to be done.

Benefits and drawbacks have both been ascribed to the use of anesthesia. The major drawbacks are (1) the toxic and depressing effects of the anesthetic agent on the pulmonary system and (2) the dose-dependent and variable changes in respiratory function caused by

the anesthetic agents. Because of the anxiety produced by the testing situation, conscious animals may change aspects of their cardiopulmonary function during testing. Anesthesia has the advantage of significantly reducing the time and difficulty involved in restraining uncooperative animal test subjects. Some pulmonary function tests can only be done on animals while under general anesthesia.

Whether or not the measurement procedure entails animal termination is an important consideration if the intention is to perform serial or multiple measurements during a single experiment, or to use the animal for more than one experiment. In the first two cases, however, the measurement techniques themselves may damage the lungs and affect subsequent measurements, while in the latter, there is also the possibility that exposure sustained in one experiment may alter the sensitivity of the lungs to additional challenges. Reuse of animals is probably a realistic consideration only with larger animals, with which it may be an economic necessity as well. All of the measurements of respiratory mechanics and gas exchange, and four of the five measurements of pulmonary circulation, do not entail animal death. Pulmonary clearance and studies on lung lavage material can be done repeatedly on living animals.

Two of the criteria employed for evaluating pulmonary function tests are subjective. The first is a judgment about the test's state of development and its refinement for toxicity screening. This judgment is based in part on comments made by researchers in telephone

contacts and in their publications, and in part on the number of published experiments in which the measurement was utilized to indicate toxicity.

The ease with which a test is performed is also a highly subjective evaluation encompassing the physical difficulty of performing the test and the conceptual difficulty involved in interpreting the results. Judgements about the ease of a test's performance must be recognized as being more-or-less equivocal.

3.2 Tests Recommended for Inclusion in a Pulmonary Toxicant Screening Program

Several pulmonary function tests satisfy all of the specified selection criteria and are, therefore, recommended for inclusion in a pulmonary toxicant screening program. These are briefly discussed in the following paragraphs.

Compliance and Resistance Tests

These measurements have been made in many species using several techniques. Some of these techniques are not terminal and do not require sustained anesthesia. The value of these measurements has been confirmed by a number of researchers under a variety of conditions.

Lung Volume and Capacity Tests

These measurements have also been made in many species using several techniques. As indicators of toxicity, they have not been as widely employed as compliance and resistance, but they are considered

by some to be quite sensitive (O'Neil 1978), and may be more sensitive indicators of restrictive or obstructive conditions.

Distribution of Ventilation by Nitrogen Washout Test

This measurement has been made by two separate techniques in a number of species. The test is considered sensitive (Coate 1978; Alarie 1978), particularly to damage along the peripheral airways that do not affect lung volumes, capacities, resistance or compliance; however, the technique is demanding to perform.

Arterial Blood Gas Measurements

Blood gas measurements are valuable indices of the overall function of the lung. They are best done on conscious animals with chronic, indwelling catheters so that blood collection does not disturb the animal. Anesthesia will itself change blood-gas values from the control (conscious) state and make interpretation of results difficult.

Carbon Monoxide Diffusing Capacity Test

This measurement has been widely made in animals. Both the single-breath and rebreathing techniques have been used in small animals, but only under sustained anesthesia. One advantage of diffusing capacity is that the measurement techniques require some of the same equipment used to perform several of the recommended measurements of respiratory mechanics.

3.3 Tests Warranting Consideration for Inclusion in a Pulmonary Toxicant Screening Program

There are several tests which do not satisfy all the selection criteria listed in Section 3.1, but do warrant consideration for inclusion in a pulmonary toxicant screening program.

Morphological Measurements

Although these measurements are terminal in nature, they can provide important information and usually detect pulmonary damage sooner than alterations are detected in other pulmonary tests. Observations of general morphology, and gross and microscopic histopathology may be appropriate for inclusion in a screening program after other nonterminal tests have been performed. For screening purposes, these tests might also be performed only for those substances for which no indications of damage were obtained using other techniques. Scanning electron microscopy is a relatively fast and informative test for surface injury in the lung. It is expensive, but may be the most effective means of detecting lesions in small airways and parenchyma.

Pulmonary Clearance of Inert Particles or Bacteria

Although these measurements are terminal, as are most defense mechanism measurements performed in small animals, consideration should be given to inclusion in a screening program of at least one pulmonary defense measurement. Pulmonary clearance has been widely demonstrated in small animals and provides an overall analysis of the functioning of the pulmonary defense system.

Viability of Alveolar Macrophages and Alveolar Macrophage Function of Phagocytosis

Although these measurements are terminal in small animals, consideration should be given to including at least one measurement of pulmonary defense in a screening program. Tests of viability and function of alveolar macrophages have been well developed, are easy to perform, and have been used and are recommended for general toxicity screening (Waters 1979).

3.4 Tests Not Recommended for Inclusion in a Pulmonary Toxicant Screening Program

There are tests, originally reviewed, which for varying reasons fail most of the selection criteria and, therefore, should not be included in a pulmonary toxicant screening program. These are briefly reviewed below.

Morphometric Measurements

These measurements are not recommended for inclusion in a screening program for pulmonary toxicants, since they necessitate termination of the animals, and are difficult to perform, time-consuming, and more useful for quantification of damage than detection of damage.

Distribution of Ventilation by Closing Volume

This technique is not recommended for inclusion in a toxic substances screening program because its applicability has not been widely documented. The technique for measuring closing volume is not well developed and its use in small animals has not been reported.

O₂ Uptake, CO₂ Output, Specific Ventilation, Alveolar Gas Pressures

These measurements of gas exchange are not recommended for inclusion in a pulmonary toxicant screening program in small mammals, because no documentation was found in the literature concerning the successful application of these procedures in animals smaller than dogs.

Circulatory Measurements

These measurements are not recommended for inclusion in a screening program for pulmonary toxicants, because no documentation was found in the literature concerning the successful application of these procedures in animals smaller than cats. Even those tests performed in cats and dogs have not been widely used or, as is the case with pulmonary vascular resistance, have not been used to evaluate damage produced by a pulmonary toxicant.

Mucociliary Transport of Inert Particles

This measurement is not recommended for inclusion in a pulmonary toxicant screening program because it has not been performed in conscious animals other than dogs, the use of small animals has been minimal, and, when used, small animals must be terminated before or after observation. In addition, pulmonary clearance tests, which have been more widely demonstrated to be useful, can provide an indication of mucociliary transport and are more likely to be applicable for screening purposes.

Cilia Beating Measurements

These measurements are not recommended for inclusion in a screening program for pulmonary toxicants because they can only be performed in a terminated animal or in an anesthetized animal, which must be terminated upon test completion. Neither technique has been widely employed.

Respiration and ATPase Activity of Alveolar Macrophage

These measurements are not recommended for inclusion in a screening program for pulmonary toxicants because they are time-consuming and have not been widely demonstrated to be useful.

Resistance to Respiratory Infection

This measurement is not recommended for inclusion in a pulmonary toxicant screening program because the test is not only terminal in nature but requires a large number of animals in order to obtain meaningful results.

Biochemical Measurements

These measurements are not recommended for inclusion in a toxic substances screening program because many of the measurements are terminal and have not been sufficiently developed to be useful in a screening program.

3.5 New Pulmonary Tests

A few new tests are currently being developed which may show promise in detecting pulmonary damage. These are not currently

recommended for inclusion in a screening program; however, with further development they may prove useful in screening toxic substances for pulmonary effects.

New and developing tests:

1. Oscillatory airway resistance.
2. Maximum expiratory flow-volume.
3. Frequency dependence of resistance.
4. Frequency dependence of compliance.
5. Frequency dependence of impedance.
6. Pulmonary mechanical reflexes.

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APPENDIX A

MORPHOLOGICAL MEASUREMENTS OF PULMONARY
SYSTEM DAMAGE

TABLE A-1
MORPHOLOGICAL MEASUREMENTS OF PULMONARY SYSTEM DAMAGE

TEST SYSTEM PARAMETER(S); TISSUE AREA OR CELLULAR EMPHASIS	OBSERVATION TECHNIQUE(S)	QUANTITATIVE MEASUREMENT	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	REFERENCES	COMMENTS
TYPE 2 CELL PROLIFERATION	AUTORADIOGRAPHY, LIGHT AND ELEC- TRON MICROSCOPY	PERCENTAGE OF [³ H] THYR LABELLED CELLS IN TOTAL CELL COUNT	MOUSE, RAT	NO ₂ , O ₃	EVANS ET AL., 1971, 1975 AND 1977	AN INDICATION OF TYPE 1 CELL DAMAGE: LABELLED CELLS INDI- CATE CELL MULTIPLICATION
	AUTORADIOGRAPHY	PERCENTAGE OF [³ H] THYR LABELLED CELLS IN TOTAL CELL COUNT	MOUSE	URETHANE (IN DRINKING WATER)	KAUFFMAN, 1976	
	ELECTRON PHOTO- MICROGRAPHS, AUTOMATED IMAGE ANALYZER, ION STAIN	PERCENTAGE OF TYPE 2 CELLS AMONG TOTAL CELLS	GUINEA PIG	NO ₂	TUEN AND SHERWIN, 1971; AND SHERWIN ET AL., 1972	
TYPE 1 CELL DAMAGE	ELECTRON MICRO- SCOPY	MEASUREMENT OF AREA OF BASE- MENT MEMBRANE NOT COVERED BY TYPE 1 CELL	RAT	NO ₂	EVANS ET AL., 1978	
TYPE 1 CELL DAMAGE, TYPE 2 CELL PROLIFERATION	LIGHT AND ELEC- TRON MICROSCOPY	NOT QUANTITATIVE	RAT	CaCl ₂	STRAUSS ET AL., 1976	
CYTOPLASMIC COMPONENTS OF TYPE 2 CELLS	ELECTRON MICRO- SCOPY	VOLUME DENSITY OF LAMELLAR BODIES, MITOCHONDRIA AND CYTOSOL; SURFACE DENSITY OF LAMELLAR BODIES AND MITO- CHONDRIA	RAT	(STARVATION)	SAHEJAH ET AL., 1978	TYPE 2 CELL LAMELLAR BODIES ARE THOUGHT TO BE SITE OF SURFACTANT STORAGE, HAS BEEN CORRELATED WITH CHANGES IN SURFACE ELASTIC FORCES

TABLE A-1 (Continued)

MORPHOLOGICAL MEASUREMENTS OF PULMONARY SYSTEM DAMAGE

TEST SYSTEM PARAMETER(S); TISSUE AREA OR CELLULAR EMPHASIS	OBSERVATION TECHNIQUE(S)	QUANTITATIVE MEASUREMENT	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	REFERENCES	COMMENTS
SIZE AND DISTRIBUTION OF MUCUS SECRETING CELLS	SCANNING ELEC- TRON MICROSCOPY	COMPARISONS OF SIZE AND NUMBERS OF MUCOUS CELLS	RABBIT	CIGARETTE SMOKE	BASSUR AND BASSUR, 1976	AN INDICATION OF THE MUCOCILIARY RESPONSE TO IRRITANTS (ALSO INCLUDED IN SECTION ON DEFENSE MECHU- LISH RESPONSES)
MEAN ALVEOLAR INTERCEPT	LIGHT MICROSCOPY	DIAMETER OF ALVEOLI	RAT, DOG	PAPAIN, $CaCl_2$ N-NITROSO-N- METHYLNITRENE	JONANSON AND PIERCE, 1973; SWIDER ET AL., 1973 AND NYAN ET AL., 1978	VERY TEDIOUS AND TIME CON- SUMING
DIFFUSING CAPACITY	LIGHT AND ELEC- TRON MICROSCOPY	VOLUME DENSITY OF: AIR SPACES, SURFACE LINING LAYER, TISSUE AND CAPIL- LARIES; DENSITY OF SURFACE ALVEOLI, DENSITY OF CAPIL- LARIES; AND MEAN THICKNESS OF AIR/BLOOD BARRIER	RAT, RABBIT, SHREW, DOG, MONKEY	O_2 (TECHNIQUE DEVELOPMENT)	WEIBEL, 1970 AND 1972; WEIBEL ET AL., 1975	VERY TEDIOUS, TIME CONSUMING, NOT WELL CORRELATED WITH MECHANICAL MEASUREMENTS OF DIFFUSING CAPACITY
TOTAL HISTOPATHOLOGY OF THE LUNG	LIGHT MICROSCOPY, TRANSMISSION AND SCANNING ELEC- TRON MICROSCOPY	QUALITATIVE: PATHOLOGIC OBSERVATIONS; QUANTITATIVE: MEASUREMENTS OR COUNTING OF SELECTED CELLS, CELLULAR COMPONENTS OR TISSUE VOLUMES OR WEIGHTS	RAT, RABBIT, HOUSE, GUINEA PIG, R. BIT, DOG, CAT, MONKEY	MAJOR EMPHASIS HAS BEEN ON KNOWN RESPIR- ATORY TOXINS SUCH AS PARA- QUAT, O_3 , SO_2 , NO_2 , $CaCl_2$, CIGARETTE SMOKE	BERKHEISER, 1963; BOATHMAN AND FRANK, 1974; KIR- BROUGH AND GAINES, 1969; KIMBROUGH AND LINDER, 1972; SCHWARTZ ET AL., 1976; AND SPICER ET AL., 1976	MANY VARIATIONS EXIST FOR FIXATION, SAMPLING AND STAINING; BROAD APPROACH NEEDS TO BE UNDERTAKEN TO BE CONCLUSIVE IN RESULTS, MUCH SUBJECTIVITY IN QUALITA- TIVE OBSERVATIONS AND CONSER- VATION IN QUANTITATIVE MEASUREMENTS

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APPENDIX B

RESPIRATORY MECHANICS MEASUREMENTS OF
PULMONARY SYSTEM DAMAGE

TABLE B-1
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - RESPIRATORY RATE AND TIDAL VOLUME

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
RESPIRATORY RATE, TIDAL VOLUME	NON-REBREATHING VALVE	HAMSTER, RAT, CHIMP, PIG, RABBIT, DOG	TECHNIQUE DEVELOPMENT	NONE	NO	MAINDIFFY AND TESARCK, 1975	NON-OPERATING VALVE IS A SIMPLE APPARATUS WHICH CAN BE SCALDED TO SEVERAL SPECIES
	SPIROMETER	CAT	DIEETHYL ETHER	SUSTAINED	NO	KATZ AND MCAL, 1962	
	PLETHYSMOGRAPH	SMALL ANIMALS	EQUIPMENT & TECHNIQUE DEVELOPMENT	NONE	NO	JACKY, 1978	
	CAPACITANCE RESPIROMETER	RABBIT, HENIA-TONE SWINE	TECHNIQUE DEVELOPMENT, CORALIT METAL POWDER	INITIAL	NO	BARROW ET AL., 1971; KERFOOT ET AL., 1975	

TABLE B-2
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - RESIDUAL VOLUME

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
RESIDUAL VOLUME	GAS DILUTION (HELIUM)	MANSTER, RAT, COTINGA PIG, RABBIT	TECHNIQUE DEVELOPMENT	SUSTAINED	NO	TAKEZAWA ET AL., 1979; O'NEIL, 1978; COSTA, 1979	MAY BE COMPLICATED BY EXCHANGE OF GASES AT MOUTH
	GAS DILUTION (HELIUM)	MURKIN, DOG	1,2,4-TRICHLOROETHANE, AUTO EXHAUST	SUSTAINED	NO	COMTE ET AL., 1977; LEWIS ET AL., 1974	MAY BE COMPLICATED BY EXCHANGE OF GASES AT MOUTH

TABLE B-3
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - LUNG CAPACITIES

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
VITAL CAPACITY	PLETHYSMOGRAPH WITH REGULATED TRANSPULMONARY PRESSURE	MANSTER, GUINEA PIG, RAT, RABBIT	TECHNIQUE DEVELOPMENT, CIGARETTE SMOKE, COAL DUST	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	LUCEY ET AL., 1978; COSTA, 1979; O'NEIL, 1978; DALREY, 1979; MOORMAN ET AL., 1975; KOO ET AL., 1976	MAY BE COMPLICATED BY EXCHANGE OF GASES AT MOUTH, RIDGING OF EXHALED CO ₂
	AIR INJECTION	MANSTER, GUINEA PIG, RAT, RABBIT	TECHNIQUE DEVELOPMENT	SUSTAINED	NO	HAUDERLY, IN PRESS; O'NEIL, 1978	
TOTAL LUNG CAPACITY	PLETHYSMOGRAPH WITH REGULATED TRANSPULMONARY PRESSURE	MANSTER, RAT, GUINEA PIG, RABBIT	TECHNIQUE DEVELOPMENT	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	LUCEY ET AL., 1978; COSTA, 1979; O'NEIL, 1978	MAY BE COMPLICATED BY EXCHANGE OF GASES AT MOUTH, RIDGING OF EXHALED CO ₂
	GAS DILUTION (HEON)	MANSTER, RAT, GUINEA PIG, RABBIT	TECHNIQUE DEVELOPMENT	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	TAKEZANA ET AL., 1979; O'NEIL, 1978; COSTA, 1979	
	GAS DILUTION (HELIUM)	MONKEY, DOG	COAL DUST, AUTO EXHAUST	SUSTAINED	NO	MOORMAN ET AL., 1975; LEWIS ET AL., 1974	

TABLE B-3 (Continued)
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - LUNG CAPACITIES

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
INSPIRATORY CAPACITY	PLETHYMOGRAPHS WITH ISOLATED TRANSPULMONARY PRESSURE	HAMSTER, BAT, GUINEA PIG, RABBIT	TECHNIQUE DEVELOPMENT, COAL DUST	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	LUCY ET AL., 1978; COSTA, 1979; O'NEILL, 1978	
	AIR INJECTION	HAMSTER, BAT, GUINEA PIG, RABBIT	TECHNIQUE DEVELOPMENT	SUSTAINED	NO	O'NEILL, 1978	MAY BE COMPLICATED BY EXCHANGE OF GASES AT MOUTH, RIPPING OF EXHALED CO ₂
EXPIRATORY RESERVE VOLUME	PLETHYMOGRAPHS WITH ISOLATED TRANSPULMONARY PRESSURE	HAMSTER, BAT, GUINEA PIG, RABBIT	TECHNIQUE DEVELOPMENT, BLENDICIN (LUNG-TRACHEAL)	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	LUCY ET AL., 1978; COSTA, 1979; SWIDER ET AL., 1978	
	FORCED MANUAL EXPIRATION (SPYROMETER)	MONKEY	1,2,4-TRICHLOROACETENE	SUSTAINED	NO	COATE ET AL., 1977	

TABLE B-3 (CONCLUDED)
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - LUNG CAPACITIES

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
FUNCTIONAL RESIDUAL CAPACITY	BOTLE'S LAW WITH PLETHYSMOGRAPH	BAT, HAMSTER, MONKEY	TECHNIQUE DEVELOPMENT, BLEOMYCIN (ENDOTRACHEAL)	SUSTAINED	RESEARCHER DEPENDENT	MAUDENLY, IN PRESS; SHIDER ET AL., 1978; LAI AND HILDEBRANDT, 1978; PARK ET AL., 1978; KOSCH ET AL., 1979b; DIAMOND AND O'DONNELL, 1977; KOO ET AL., 1976	
	WASH DILUTION	GUINEA PIG, RAT, HAMSTER, RABBIT, PRENATURE LAMB	PAPAIN (INTRAVENOUS), DESCRIPTIVE RESEARCH	SUSTAINED	RESEARCHER DEPENDENT	JOHANSON AND PIERCE, 1973; AND SHAFER ET AL., 1976	GAS DILUTION TECHNIQUES ARE GENERALLY MORE TIME CONSUMING AND CUMBERSOME THAN PLETHYSMOGRAPHIC TECHNIQUES
	HELIUM DILUTION	MONKEY, DOG	TECHNIQUE DEVELOPMENT, AUTO EXHAUST, NITAMINE, EPOXY	SUSTAINED	NO	LIU AND DELAUNER, 1977; LEWIS ET AL., 1974; NYDE, 1978	THIS TEST CAN EASILY BE PERFORMED DURING CO DIFFUSING CAPACITY (SEE TABLE C-1)
	NITROGEN DILUTION	DOG, PONY	DESCRIPTIVE RESEARCH, 30% FUSED CLAY AEROSOL, CIGARETTE SMOKE	INITIAL	RESEARCHER DEPENDENT	MAUDENLY, 1974a & b; AND PARK ET AL., 1977	
	NITROGEN DILUTION	BAT, MOUSE, DOG	CIGARETTE SMOKE, BLEOMYCIN (INTRAVENOUS), CADMIUM AND NO ₂	SUSTAINED	RESEARCHER DEPENDENT	AVIADO AND HATANABE, 1974; VALICENTI ET AL., 1971; DALBET, 1979	

TABLE B-4
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - COMPLIANCE AND RESISTANCE (WITH PLETHYSMOGRAPH)

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
COMPLIANCE, RESISTANCE	PLETHYSMOGRAPH WITH FLUID FILLED INTRA-FLURAL CATHETER AS FIRST PERFORMED BY AMOUR AND HEAD	GUINEA PIG, CAT, DOG	SO ₂ , FORMALDEHYDE, FORMIC ACID, ACETIC ACID, IODINE VAPOR, SODIUM CHLORIDE VAPOR, OZONE, ACROLEIN, BLEPHITICIN	INITIAL	RESEARCHER INDEPENDENT	AMOUR AND HEAD, 1955 AND 1958; AMOUR, 1960 AND 1978; MURPHY ET AL., 1963 AND 1964; COSTA, 1979; SILBAUGH ET AL., 1979	PROBLEMS ASSOCIATED WITH INVASIVE CATHETER: DIFFICULTIES IN INSERTION, STRESSFUL, CAUSE POSSIBLE EXCESS MUCUS SECRETION, STABILITY OVER SEVERAL HOURS NOT RELIABLE
	AMOUR AND HEAD TECHNIQUE PLUS ON-LINE COMPUTER	GUINEA PIG, MONKEY	TECHNIQUE DEVELOPMENT, NITAMINE, SO ₂ , NO ₂ , ISOPROTERENOL, SALBUTAMOL, PROSTAGLANDINS, ACETYLCHOLINE, HYDROCORTISONE, ASCORBIC ACID, TRANS-PORTATION-RELATED EMISSIONS	INITIAL	NO	DENNIS ET AL., 1969; ALARIE ET AL., 1970a AND 1971a & b; WEISSBERG ET AL., 1976 AND 1978; POPA ET AL., 1974; DONGLAS ET AL., 1977a AND 1977b; BRINK ET AL., 1975, 1976 AND 1977; WEISTER, 1978; ULRICH ET AL., 1977	USE OF COMPUTER GREATLY FACILITATES CALCULATIONS AND ALLOWS FOR A BREATH BY BREATH ANALYSIS FOR PRECISE DETERMINATION OF WHEN AIRWAY RESPONSES BEGIN
	PLETHYSMOGRAPH WITH ESOPHAGEAL CATHETERIZATION	RAT, MICE	TECHNIQUE DEVELOPMENT, CIGARETTE SMOKE, CARBONUM, NO ₂	SUSTAINED	RESEARCHER DEPENDENT	PALECKE, 1969; AVIADO AND WATANABE, 1974; HAUDELLI, IM PRESS; DIAMOND AND O'DONNELL, 1977; DALREY, 1979	ESOPHAGEAL CATHETER APPROXIMATES PLEURAL PRESSURE, YET NON-INVASIVE. SMALL ANIMALS REQUIRE FLUID FILLED CATHETER WHICH MAY REDUCE FREQUENCY RESPONSE
	PLETHYSMOGRAPH WITH ESOPHAGEAL CATHETERIZATION PLUS ONLINE COMPUTER	GUINEA PIG, MONKEY	TECHNIQUE DEVELOPMENT, NITAMINE, ISOPROTERENOL, SALBUTAMOL, PROSTAGLANDINS	INITIAL	NO	WEISSBERG ET AL., 1978; SKONNIK ET AL., 1979	

TABLE B-4 (Concluded)
 RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - COMPLIANCE AND RESISTANCE (WITH PLETHYSMOGRAPH)

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
COMPLIANCE, RESISTANCE (CONCLUDED)	PLETHYSMOGRAPH WITH ENDOTRACHEAL CANNULATION AND RESPIRATOR PLUS ON-LINE COMPUTER	GUINEA PIG, RABBIT, DOG	VARIOUS DRUGS (INTRAVENOUS), AUTO EXHAUST	SUSTAINED	YES	WEISSBURG ET AL., 1978	
	PLETHYSMOGRAPH WITH FORCED OSCILLATIONS	GUINEA PIG, RABBIT, MONKEY, CAT, DOG	ASBESTOS, DRUGS, ENZYME DETURGENT DUST, VARIOUS DRUGS (INTRAVENOUS), HISTAMINE, EPONY, TECHNIQUE DEVELOPMENT	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	RIETT, 1974 AND 1978; COATE ET AL., 1978; MURPHY AND ULRICH, 1964; DIAMOND ET AL., 1975; DIAMOND, 1978; KOSCH ET AL., 1979 a,b HYDE, 1978	

TABLE B-5
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - COMPLIANCE AND RESISTANCE (WITHOUT PLETHYMOGRAPH)

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
COMPLIANCE, RESISTANCE	FORCED OSCILLATIONS WITH RESPIRATOR AND ENDOTRACHEAL CANNULATION	DOG	PARAIN	SUSTAINED	NO	MAADDAD ET AL., 1979	
	PNEUMOTACHOGRAPH WITH ENDOTRACHEAL CANNULATION AND RESPIRATOR, AND INTRA-PLEURAL CATETERIZATION	CAT	SO ₂ , SO ₂ - NaCl	SUSTAINED	YES; BUT NOT NECESSARY	JOHN ET AL., 1972	
	PNEUMOTACHOGRAPH WITH ENDOTRACHEAL CANNULATION, INTRAPLEURAL CATETERIZATION AND ON-LINE COMPUTER	DOG	TECHNIQUE DEVELOPMENT	SUSTAINED	NO	STILES ET AL., 1971	
	PNEUMOTACHOGRAPH WITH ESOPHAGEAL CATETERIZATION	DOG, MONKEY	TECHNIQUE DEVELOPMENT, DESCRIPTIVE RESEARCH, RADIOACTIVE AEROSOL, 1,2,4-TRICHLOROBENZENE	RESEARCHER DEPENDENT	NO	HAUDERLY, 1974a; COATE ET AL., 1977; LIU AND DELAUTER, 1977	

TABLE B-5 (Concluded)
 RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - COMPLIANCE AND RESISTANCE (WITHOUT PLETHYMOGRAPH)

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
COMPLIANCE, RESISTANCE (CONCLUDED)	PNEUMOTACHOGRAPH WITH ESOPHAGEAL CATHETERIZATION	DONKEY	H ₂ SO ₄ , (NH ₄) ₂ SO ₄	NONE	NO	SCHLESINGER ET AL., 1978	
	RESPIROMETER PNEUMOTACHOGRAPH PLUS ESOPHAGEAL CATHETERIZATION	RABBIT, MINIATURE SWINE, PRETERM LAMBS, PRETERM MINIATURE SWINE	CHLORINE GAS, COBALT METAL POWDER, TECHNIQUE DEVELOPMENT	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	BARROW AND SMITH, 1975; KERFOOT ET AL., 1975; SHAFER ET AL., 1976 AND 1978	

TABLE B-6
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - DISTRIBUTION OF VENTILATION

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
DISTRIBUTION OF VENTILATION BY NITROGEN-WASHOUT	SINGLE BREATH	DOG, MONKEY	AUTO EXHAUST, 1,2,4-TRICHLORO-BENZENE, TECHNIQUE DEVELOPMENT	SUSTAINED	NO	LEWIS ET AL., 1974; COATE ET AL., 1977; LEHEN ET AL., 1975	THIS TECHNIQUE MAY ALSO BE USED TO INDICATE CLOSING VOLUME
	MULTIPLE BREATH	GUINEA PIG, RAT RANSTER, RABBIT, MONKEY	SO ₂ , O ₃ , NO ₂ , Cd, TRANS-2-BUTENE, FLY ASH, COAL DUST, CIGARETTE SMOKE, FREGON, VARIOUS DRUGS, SULFURIC ACID MIST, DETERGENTS, TECHNIQUE DEVELOPMENT	RESEARCHER DEPENDENT	RESEARCHER DEPENDENT	COATE, 1978; O'NEIL, 1978; DALBEY, 1979; COATE ET AL., 1977; ALARIE ET AL., 1978; ALARIE AND b; ALARIE, 1978; AND ULRICH ET AL., 1977	MUST GET VERY TIGHT CONNECTIONS, NOT EASY ON SMALL MAMMALS, COMPLEX CALCULATION, COMPUTER USE ESSENTIAL, GOOD SINGLE INDICATOR
DISTRIBUTION OF VENTILATION INDICATED BY CLOSING VOLUME	HELIUM BOLUS	MONKEY	TECHNIQUE DEVELOPMENT, PULMONARY DESCRIPTIVE RESEARCH	SUSTAINED	NO	KOSCH ET AL., 1979a	THIS TECHNIQUE IS NOT WELL SUITED FOR SMALL ANIMALS AS CLOSING VOLUME PROBABLY REPRESENTS A VERY SMALL PERCENTAGE OF TOTAL LUNG CAPACITY

TABLE B-7
RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - PRESSURE AND VOLUME RELATIONSHIPS

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
PRESSURE-VOLUME CURVES	PLETHYSMOGRAPH AND RESPIRATOR	RABBIT, MONKEY, RAT	TECHNIQUE DEVELOPMENT	SUSTAINED	RESEARCHER DEPENDENT	CALDWELL AND FRY, 1969; KOSCH ET AL., 1979a; DIAMOND AND O'DONNELL, 1977	
	PLETHYSMOGRAPH PLUS REGULATED TRANS-PULMONARY PRESSURE	HAMSTER	TECHNIQUE DEVELOPMENT	SUSTAINED	RESEARCHER DEPENDENT	LUCY ET AL., 1978 AND PARE ET AL., 1978	
	PLETHYSMOGRAPH PLUS INFLATOR	HAMSTER, RAT, GUINEA PIG	BLEOMYCIN, TECHNIQUE DEVELOPMENT	SUSTAINED	RESEARCHER DEPENDENT	KOD ET AL., 1976; O'NEIL, 1978; COSTA, 1979; SNIDER ET AL., 1978 AND LAI AND HILDEBRANDT, 1978	
	PNEUMOTACHOGRAPHS AND RESPIRATOR	MONKEY	AMACRIDONIC ACID	SUSTAINED	NO	PATTERSON ET AL., 1974 AND 1978	
	SPIROMETER PLUS INFLATOR	DOG, RABBIT,	DESCRIPTIVE RESEARCH	SUSTAINED	RESEARCHER DEPENDENT	DROMBAUGH, 1960 AND VALICENTI ET AL., 1971	
	EXCISED LUNGS - AIR AND SALINE INJECTED	RAT, DOG, HAMSTER	DL-PERCITILLAMINE (IM DIET); N-NITROSO-N-METHYLMETHANES (SUB-CITANUS), CA, O ₂ , CAO, NO ₂ , STARVATION, CIGARETTE SMOKE, BASE-LINE VALUES	NA	YES	HOFFMAN ET AL., 1971; NYAN ET AL., 1978; SAKELJANI ET AL., 1978; DALRY, 1979; KOD ET AL., 1976	TECHNIQUE MUST BE DONE RAPIDLY AND CANNOT BE REPEATED, NO CONTRA.

NA - NOT APPLICABLE

TABLE B-7 (Concluded)
 RESPIRATORY MECHANICS MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - PRESSURE AND VOLUME RELATIONSHIPS

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE(S) EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
MAXIMUM FLOW VOLUME CURVES	PLETHYSMOGRAPH PLUS APPLIED PRESSURE THROUGH TRACHEA	RAT	CIGARETTE SMOKE, CADMIUM, NO ₂	SUSTAINED	YES	DALBET, 1979	PRESSURE APPLIED THROUGH TRACHEA IS CONSIDERED MORE REFLECTIVE OF ACTUAL LUNG MECHANICS
	PLETHYSMOGRAPH PLUS APPLIED PRESSURE TO BODY	MONKEY	COAL DUST	SUSTAINED	NO	MOORMAN ET AL., 1975	

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APPENDIX C

GAS EXCHANGE MEASUREMENTS OF PULMONARY SYSTEM DAMAGE

TABLE C-1 (Continued)
GAS EXCHANGE MEASUREMENTS OF PULMONARY DAMAGE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
CO ₂ OUTPUT (CORRECTED)	FACE MASK, ESOPHAGEAL BALLOON CATHETER, DOUGLAS BAG OR SPIROMETER, NON-REBREATHING VALVE	DOGS	90% FUSED CLAY, BASE-LINE VALUES, SEX, AGE	NONE	NO	MAUDERLY, 1974a,b; MAUDERLY ET AL., 1973	
SPECIFIC VENTILATION	MINUTE VOLUME/OF UPTAKE	DOGS, MONKEYS	90% FUSED CLAY, BASE-LINE VALUES, SEX, AGE	NA	NA	LIU AND DELAUTER, 1977; MAUDERLY, 1974b; MAUDERLY ET AL., 1973	
RESPIRATORY EXCHANGE RATIO	CO ₂ OUTPUT/O ₂ UPTAKE	DOGS, MONKEYS	90% FUSED CLAY, BASE-LINE VALUES, SEX, AGE	NA	NA	LIU AND DELAUTER, 1977; MAUDERLY, 1974b; MAUDERLY ET AL., 1973	
ALVEOLAR GAS PRESSURES (P _A O ₂ , P _A CO ₂)	SAME AS O ₂ UPTAKE, CO ₂ OUTPUT, WITH END-TIDAL SAMPLE	DOGS, PONIES	90% FUSED CLAY, BASE-LINE VALUES	NONE	NO	MAUDERLY 1974a,b; MAUDERLY ET AL., 1973	
ALVEOLAR-ARTERIAL DIFFERENCE	P _A O ₂ -P _a O ₂ , P _A CO ₂ -P _a CO ₂	DOGS, PONIES	90% FUSED CLAY, BASE-LINE VALUES	NA	NA	MAUDERLY 1974a,b; MAUDERLY ET AL., 1973	

TABLE C-1 (Continued)
GAS EXCHANGE MEASUREMENTS OF PULMONARY DAMAGE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
O ₂ UPTAKE	TRACHEAL CANNULA, CONSTANT VOLUME CLOSED SYSTEM	CATS	BASILINE VALUES	SUSTAINED	NO	MADRAU AND COLEMAN, 1965	
	TRACHEAL CANNULA, ONE-WAY VALVE, SPINOMETER	HORNETS	BASILINE VALUES	SUSTAINED	NO	LIU AND DELAUTER, 1977	
	HEAD COVER, ONE-WAY VALVE	HORNETS	BASILINE VALUES	NONE	NO	LIU AND DELAUTER, 1977	
	FACE MASK, ESOPHAGEAL BALLOON CATHETER, DOUGLAS BAG OR SPINOMETER, NON-PNEUMATIC VALVE	DOGS, POWIES	90% FUSED CLAY, BASILINE VALUES, SEI, ACE	NONE	NO	HAUBERLY, 1974a, b; HAUBERLY ET AL., 1973	
		HORNETS	BASILINE VALUES	SUSTAINED	NO	LIU AND DELAUTER, 1977	
CO ₂ OUTPUT	TRACHEAL CANNULA, ONE-WAY VALVE	HORNETS	BASILINE VALUES	SUSTAINED	NO	LIU AND DELAUTER, 1977	

TABLE C-1 (Concluded)
GAS EXCHANGE MEASUREMENTS OF PULMONARY DAMAGE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
CARBON MONOXIDE DIFFUSING CAPACITY	SINGLE BREATH POSITIVE PRESSURE INFLATION	RATS, DOGS, MONKEYS, CATS, RABBITS, GUINEA PIGS, HAMSTERS	NUCLEAR AND FOSSIL FUEL EFFLUENTS, SO ₂ , O ₃ , TRANS-2-BUTENE, NO, FLY ASH, COAL DUST, CIGARETTE SMOKE, 1,2,4-TRICHLOROETHENE, H ₂ SO ₄ , AUTO EXHAUST, BLEOMYCIN, PHARMACEUTICALS, AIR POLLUTANTS	SUSTAINED	NO	COATE ET AL., 1977; CREE ET AL., 1968; DIAMOND, 1978; LEWIS ET AL., 1974; MAUDERLY, IN PRESS; O'NEIL, 1978; ROBINSON AND GILLESPIE, 1975; VALICENTI ET AL., 1971; WIENER, 1979; YOUNG ET AL., 1963.	RAPID SERIAL MEASUREMENTS POSSIBLE (CREE ET AL., 1968)
	FORCED INSPIRATION VIA EXTERNAL PRESSURE RESPIRATOR	DOGS	SO ₂ , H ₂ SO ₄ , AUTO EXHAUST (CO, HYDROCARBONS, NO ₂ , NO, O ₃)	SUSTAINED OR NONE	NO	BRASHEAR ET AL., 1966; LEWIS ET AL., 1974	
	REBREATHING	RATS, GUINEA PIGS, MONKEYS, HAMSTERS	SO ₂ , H ₂ SO ₄ , FLY ASH, AUTO, BLEOMYCIN, FATIGUE, ELASTASE	NONE OR SUSTAINED	NO	AJARIE ET AL., 1975; JOHANSON AND PIERCE, 1973; GOLDSTEIN ET AL., 1977; SWIDER ET AL., 1978; WIENER, 1979	
	STEADY-STATE END-TIDAL	DOGS, PONIES	CIGARETTE SMOKE, BASELINE VALUES	NONE	NO	MAUDERLY, 1972, 1974a, b; MAUDERLY ET AL., 1973; PARK ET AL., 1977	

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APPENDIX D

CIRCULATORY MEASUREMENTS OF PULMONARY SYSTEM DAMAGE

TABLE D-1
CIRCULATORY MEASUREMENTS OF PULMONARY DAMAGE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
CAPILLARY BLOOD VOLUME (V_c)	CALCULATED FROM θ AND VALUES OF P_{aO_2} , P_{vO_2} AT DIFFERENT P_{aO_2}	DOGS	5-HYDROXYTRYPTAMINE, BASELINE VALUES, EFFECT OF AGING	NA	NA	CHEN ET AL., 1968; ROBINSON AND GILLESPIE, 1975; YOUNG ET AL., 1963	θ VALUES WERE BASED ON MEAN λ FOR DOG BLOOD (DELLAND, 1969). CONSTANT OF λ BETWEEN INDIVIDUALS IS QUESTIONABLE AS IS APPLICABILITY OF MEAN λ TO THE DOG. IF λ IS CONSTANT WITHIN A SPECIES THEN RELATIVE VALUES OF V_c CAN BE DETERMINED, REGARDLESS OF λ UTILIZED.
PULMONARY VASCULAR RESISTANCE	CALCULATED FROM PULMONARY ARTERIAL OR VEIN CAVA PRESSURE AND BLOOD FLOW	CATS, DOGS	BASELINE VALUES, SPLANCHNIC ARTERIAL OCCLUSION SHOCK	NA	NA	MADEAU AND COLEMAN, 1965; WILKERSON, 1977	
LONGITUDINAL DISTRIBUTION OF VASCULAR RESISTANCE	LOW-VISCOSITY BOLUS	ISOLATED CAT LUNG	TECHNIQUE DEVELOPMENT	NA	YES	GRIMM ET AL., 1977	
BLOOD PRESSURES	PULMONARY ARTERY, PERONAAL ARTERY, CAROTID ARTERY, LEFT ARTERY, VEIN CAVA	CATS, DOGS, ISOLATED CAT LUNG	PROGRESSIVE, H_2SO_4 , BASELINE VALUES, CALCULATION OF V_c , SPLANCHNIC ARTERIAL OCCLUSION SHOCK	SUSTAINED *	NO	GIBSON ET AL., 1948; GRIMM ET AL., 1977; MADEAU AND COLEMAN, 1965; SACKNER ET AL., 1978; WILKERSON, 1977	

TABLE D-1 (Concluded)
CIRCULATORY MEASUREMENTS OF PULMONARY DAMAGE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
LEFT-TO-RIGHT SHUNT	CALCULATED FROM ALVEOLAR-ARTERIAL O ₂ DIFFERENCE USING OXYGEN BREATHING	DOGS	BLERONICIN	NA	NA	VALICOURT ET AL., 1971	

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APPENDIX E

DEFENSE MECHANISM MEASUREMENTS OF
PULMONARY SYSTEM DAMAGE

TABLE B-1
DEFENSE MECHANISM MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - MUCOCILIARY FUNCTION

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
MUCOCILIARY TRANSPORT OF INERT PARTICLES	INHALED RADIO-LABELLED FERRIC OXIDE SCANNED <u>IN VIVO</u>	DONKEY	TECHNIQUE DEVELOPMENT, TRICHLOROFLUOROMETHANE, DICHLORODIFLUOROMETHANE, CIGARETTE SMOKE, HCl, SO ₂ , ATROPINE SULFATE (INTRAMUSCULAR), METHACHOLINE CHLORIDE (SUBCUTANEOUS)	NONE	NO	SPITZELMAN ET AL., 1968; ALBERT ET AL., 1968 AND 1974; BOWRING ET AL., 1975; LITTMAN ET AL., 1977; BERGER ET AL., 1978	NORMAL CLEARANCE RATE IN DONKEYS IS HIGHLY REPRODUCIBLE; HARD TO PERFORM IN SMALL ANIMALS
	DEPOSITED RADIO-LABELLED ALBUMIN MICRO-SPHERE SCANNED <u>IN VIVO</u>	DOG	ATROPINE (INTRAVENOUS)	SUSTAINED	NO	CHOPRA, 1979	LARGE DEPOSITED PARTICLES ARE REFLECTIVE OF SURFACE (VS. TOTAL) MUCOCILIARY TRANSPORT
	DEPOSITED RADIO-LABELLED FOR EXCHANGE PARTICLES SCANNED <u>IN VIVO</u>	DOG	TECHNIQUE DEVELOPMENT	SUSTAINED	NO	CONNOLLY ET AL., 1978	
	DEPOSITED TEF-LON DISKS FILLED THROUGH BRONCHOPROFIBER-SCOPE	DOG	SO ₂	SUSTAINED	NO	HITSON ET AL., 1975; SACKNER ET AL., 1973; WANNER ET AL., 1973	THIS TECHNIQUE CAN MEASURE BOTH AVERAGE AND MAXIMAL CLEARANCE RATES, WHEREAS RADIIOPASIVE ONLY MEASURE CLEARANCE OR CLEARANCE OF ONE PARTICLE
	TRACHEOTOMY PLUS OBSERVATION AND RECORDING OF MUCUS FLOW ON HIGH SPEED FILM	RABBIT, RAT	SO ₂	SUSTAINED	YES	DALLMAN, 1961	

TABLE B-1 (Concluded)
 DEFENSE MECHANISM MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - MICROVILLIARY FUNCTION

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
MICROVILLIARY TRANSPORT OF INHIBITORY PARTICLES (CONCLUDED)	IN VITRO DEPOSITED GRANULITE PARTICLE TRANSFER IN EXCISED TRACHEA	RAT, GUINEA PIG, MOUSE	DIESEL EXHAUST (IN VIVO)	NA	YES	BATTICELLI ET AL., 1966	
	INHALED GRANULITE PARTICLE TRANSFER IN EXCISED TRACHEA	RAT	SO ₂	NA	YES	FRASER ET AL., 1968	
CILIA BEATING FREQUENCY (IN VIVO)	ISOLATED TRACHEAL RINGS WITH ELECTRONIC STROBOSCOPE	HAMSTER	CaCl ₂ (IN VIVO AND IN VITRO)	NA	YES	ADALIS, 1977	
CILIA BEATING FREQUENCY (IN VIVO)	TRACHEOTOMY PLUS OBSERVATION AND RECORDING ON HIGH SPEED FILM	RABBIT, RAT	SO ₂ , SO ₂ AND CARBON PARTICLES	SUSTAINED	YES	DALMAN, 1956; DALMAN AND STANGROSC, 1961	
SIZE AND DISTRIBUTION OF MUCUS SECRETING CELLS	SEM OBSERVATIONS IN EXCISED TISSUE SECTIONS	HAMSTER	CIGARETTE SMOKE	NA	YES	BASRI AND BASRI, 1976	

TABLE B-2
DEFENSE MECHANISM MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - ALVEOLAR MACROPHAGE FUNCTION

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
PERCENT VIABILITY OF ALVEOLAR MACROPHAGES	ALVEOLAR MACROPHAGES EXPOSED IN <u>VITRO</u>	RABBIT, BAT, HAMSTER	CaCl ₂ , NiCl ₂ , NiCl ₂ ·6H ₂ O, NiO, Ni ₂ O ₃ , VANADIUM OXIDES, FLY ASH PARTICLES AND PbO, NiO OR NiO ₂ . TECHNIQUE DEVELOPMENT	NA	YES	WATERS ET AL., 1974 AND 1975; ABARTI ET AL., 1977; KAVET AND BRAIN, 1977; RYERIK AND EVANS, 1967; GRAHAM ET AL., 1975	A WELL DEVELOPED TECHNIQUE
ALVEOLAR MACROPHAGE FUNCTION (PHAGOCYTOSIS OF BACTERIA)	ALVEOLAR MACROPHAGES EXPOSED IN <u>VITRO</u>	RABBIT	NO ₂ , NO ₂	NA	YES	MURPHY AND EVANS, 1967; VASSALLO ET AL., 1973	
ALVEOLAR MACROPHAGE FUNCTION (PHAGOCYTOSIS OF PLASTIC MICROSPHERES)	ALVEOLAR MACROPHAGES EXPOSED IN <u>VITRO</u>	BAT, HAMSTER, RABBIT	NiCl ₂ , Ni ²⁺ , Ca ²⁺ , Cr ³⁺ , Mn ²⁺ . TECHNIQUE DEVELOPMENT	NA	YES	GRAHAM AND GARDNER, 1977; KAVET AND BRAIN, 1977; GRAHAM ET AL., 1975	A WELL DEVELOPED TECHNIQUE
ALVEOLAR MACROPHAGE FUNCTION (PHAGOCYTOSIS OF RADIO-LABELLED GOLD)	INTRATRACHEAL INSTILLATION OF LABELLED GOLD; COUNTING OF LABELLED MACROPHAGES IN <u>VITRO</u>	HAMSTER	FERRIC OXIDE, COLLOIDAL CARBON (INTRATRACHEAL), COAL DUST (INTRATRACHEAL)	NONE	YES	BRAIN AND CORBERT, 1977	
RESPIRATION AND ATPASE ACTIVITY OF ALVEOLAR MACROPHAGE	ALVEOLAR MACROPHAGES EXPOSED IN <u>VITRO</u>	BAT, SHEEP	NiCl ₂ , Cr ²⁺	NA	YES	GRAHAM AND GARDNER, 1977; CROSS ET AL., 1970	

TABLE E-3
DEFENSE MECHANISM MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - PULMONARY CLEARANCE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
PULMONARY CLEARANCE OF INERT PARTICLES	LUNG BURDEN OF IRRADIATED TiO_2 DETERMINED IN LUNG HOMOGENATE	RAT	SO_2 , PAPAIN (INHALED AND INTRATRACHEAL)	NA	YES	FESIN AND LEACH, 1973; FERIN, 1971	
	LUNG BURDEN OF IRRADIATED MgO DETERMINED IN LUNG HOMOGENATE	GUINEA PIG	(BACTERIAL CHAL LENCE)	NA	YES	BERGSTRON AND NYLANDER, 1976	
PULMONARY CLEARANCE OF BACTERIA	IRRADIATED RADIO-LABELLED BACTERIA COUNTED IN LUNG HOMOGENATE	MOUSE, GUINEA PIG	H_2SO_4 , OZONE, CIGARETTE SMOKE	NA	YES	FAIRCHILD ET AL., 1975; GOLDSTEIN ET AL., 1971; NYLANDER, 1971	
	IRRADIATED VIABLE BACTERIA COUNTED IN LUNG HOMOGENATE	MOUSE, HAMSTER, MONKEY, GUINEA PIG	H_2SO_4 , NO_2 , $CaCl_2$ ETHANOL, CORTICOSTEROID (SUBCUTANEOUS) (STARVATION), MAGNESIUM AND NICKEL SALTS, CIGARETTE SMOKE	NA	YES	FAIRCHILD ET AL., 1975; EHRlich, 1966; GREEN AND KASS, 1964; GARDNER ET AL., 1977; NYLANDER, 1971	

TABLE E-4
 DEFENSE MECHANISM MEASUREMENTS OF PULMONARY SYSTEM DAMAGE - RESISTANCE TO RESPIRATORY INFECTION

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
RESISTANCE TO IN- FUSED RESPIRATORY INFECTION	PERCENT MORTALITY AFTER EXPOSURE OF SUBSTANTI- FIED ANIMALS TO <u>E.</u> <u>COLIFORM</u>	HOUSE, HONEY, MANDRILL	COLORE, NO ₂ , NaO, CaCl ₂ , DIETHYL ET- HYL, SALTS OF CAL- CIUM, NICKEL AND MAGNESIUM	NONE	YES	ERLICH, 1963 AND 1966; HAICHTER, 1976; ROBERT ET AL., 1969 AND 1970; PURVIS ET AL., 1961; PURVIS AND ERLICH, 1963; CAMPBELL, 1978; GARDNER ET AL., 1977; GRAHAM AND GARDNER, 1977	REQUIRES LARGE NUMBER OF ANIMALS

APPENDIX F

BIOCHEMICAL MEASUREMENTS OF PULMONARY
SYSTEM DAMAGE

TABLE F-1
 BIOCHEMICAL MEASUREMENTS OF PULMONARY SYSTEM DAMAGE

TEST SYSTEM PARAMETER	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM(S) UTILIZED	COMPOUND(S) TESTED	ANESTHESIA	TERMINAL	REFERENCE(S)	COMMENTS
ISOMETRIC AND CELLULAR RESPONSE OF AIRWAYS SAMPLED BY BRONCHOPULMONARY LAVAGE	MEASUREMENTS OF LACTATE DEHYDROGENASE, GLUCOSE-6P-DEHYDROGENASE, ACID PHOSPHATASE, 6-GLUCURONIDASE, ALKALINE PHOSPHATASE, TRYPHOSIN INHIBITORY CAPACITY, SIALIC ACID, AND NUCLEATED CELLS	HAMSTER, RAT, GUINEA PIG, RABBIT	CaCl ₂ , CdCl ₂ , TRITON X-100 (LAVAGE), ¹⁴⁴ Ce	NA	YES	HENDERSON ET AL., 1978 a b AND 1979	THIS TECHNIQUE HAS DEVELOPED FOR RAPID SCREENING AND HAS BEEN CORRELATED WITH MORPHOLOGICAL DAMAGE

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