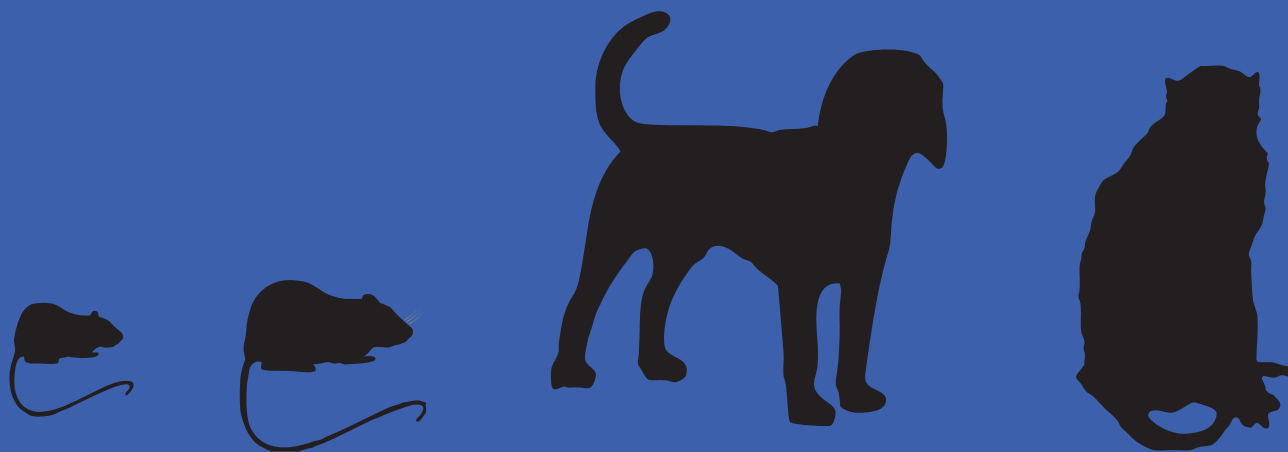




Research Approaches in the Study of Respiratory Diseases



An overview of proven respiratory research models and methods.

Research Approaches in the Study of Respiratory Diseases

Introduction

Some of the leading causes of death worldwide are respiratory related. Today, scientists face many challenges as the cause of respiratory related diseases come in many forms. The study of environmental exposure includes topics such as air quality, climate change, and evolving workplace hazards. Others include infectious disease, genetics, and acute lung injuries.

Scientific research programs across the globe are working on the cause, prevention, and treatment of acute and chronic respiratory diseases. From academic institutions to nationally lead initiatives, each are aggressively trying to find ways to help reduce the staggering numbers of people affected by respiratory disease on an annual basis. Already considered significant public health burdens, it is anticipated that incidences of asthma and chronic obstructive pulmonary disease (COPD) will continue to rise.

This paper provides researchers with the following information: a) a summary of the most commonly researched respiratory diseases, b) references for some of the most relevant and insightful research from peer-reviewed journal articles, c) observations regarding endpoints of interest and, d) the products used to collect these endpoints.

Respiratory Diseases and Research Approaches

COPD

COPD (chronic obstructive pulmonary disease) is a progressive disease that makes it difficult to breathe. The primary contributor to COPD is cigarette smoke. COPD is the third leading cause of death in the U.S., and there is no cure.

The two primary conditions are 1) Emphysema, where damage to the walls between air sacs, causes destruction of the lungs over time, and 2) Chronic bronchitis, which is an inflammation and thickening of airway lining, creating mucus development, and a

chronic cough.

Rodent models are commonly used when studying COPD. Recent publications suggest that cigarette smoke exposure to animals, using a smoke generating device, is the best approximation to human COPD.

Other approaches include long-term lipopolysaccharide (LPS) exposure and the use of genetically modified models.

Primary endpoints of interest include static and dynamic compliance and lung volumes. The following DSI products are often used when studying COPD:

- Smoke Generator
- Resistance and compliance systems
- Pulmonary function testing
- FinePointe software

Suggested COPD Research References

Ramírez-Ramírez E, Torres-Ramírez A, Alquicira-Mireles J, Cañavera-Constantino A, Segura-Medina P, Montaña-Ramírez M, Ramos-Abraham C, Vargas M, Arreola-Ramírez J. "Characteristic plethysmographic findings in a guinea pig model of COPD". *Experimental Lung Research*. 2017 DOI: 10.1080/01902148.2017.1294632.

Li Y, Tian Y, Li J, Dong Y, Wang M, Su-Xiang Feng S, Li L, Mao J, Wang L Luo S. " Bufei Yishen granules combined with acupoint sticking therapy suppress oxidative stress in chronic obstructive pulmonary disease rats: Via regulating peroxisome proliferator-activated receptor-gamma signaling." *Journal of Ethnopharmacology*. 2016;193:354-361.

Kling H, Shipley T, Guyach S, Tarantelli R, Morris A, Norris K. "Trimethoprim-Sulfamethoxazole Treatment Does Not Reverse Obstructive Pulmonary Changes in Pneumocystis-Colonized Non-Human Primates with SHIV Infection." *J Acquir Immune Defic Syndr*. 2014; 65(4):381-389.

ASTHMA

Asthma is a disorder that causes the airways of the lungs to swell and narrow, leading to wheezing,

shortness of breath, chest tightness, and coughing. Asthma affects a staggering 8% of the world's population; and there is no cure.

There are several risk factors for developing asthma such as inhaled substances and particles that provide allergic reactions or irritate the airways. Other factors include genetic predisposition, environmental allergens, and dietary factors.

Rodents are the typical species used when studying asthma. Ovalbumin (OVA) derived from chicken egg is a frequently used allergen that induces a robust, allergic pulmonary inflammation in laboratory rodents. Animals are sensitized to OVA over time and with i.p. injections and later exposed to OVA using an aerosol to induce acute allergic airway inflammation. Airway Hyperresponsiveness (AHR) is assessed using a muscarinic receptor (Methacholine).

Primary endpoints of interest include respiratory rate, peak flows, and resistance; all are contributors to determining levels of bronchoconstriction.

DSI products supporting asthma research:

- Resistance and compliance systems
- Non-invasive airway mechanics systems or rodents
- Whole body plethysmography
- Pulmonary function testing
- FinePointe software

Suggested Asthma Research References

Lunding L, Webering S, Vock C, Behrends J, Wagner C, Holscher C, Fehrenbach H, Wegmann M. "Poly(inosinic-cytidylic) Acid-Triggered Exacerbation of Experimental Asthma Depends on IL-17A Produced by NK Cells." *Journal of Immunology*. 2015;194.

Ezeamuzie C, El-Hashim A, Renno W, Edafiogho I. "Antiallergic and Antiasthmatic Effects of a Novel Enhydrazinone Ester (CEE-1): Inhibition of Activation of Both Mast Cells and Eosinophils." *Journal of Pharmacology and Experimental Therapeutics*. 2014;350:444-454.

Viby N, Isidor M, Buggeskov K, Poulsen S, Hansen J, Kissow K. "Glucagon-Like Peptide-1 (GLP-1) Reduces Mortality and Improves Lung Function in a Model of Experimental Obstructive Lung Disease in Female Mice." *Endocrinology*. 2013;154:4503-4511.

PULMONARY FIBROSIS (PF)

Pulmonary Fibrosis is a disease in which tissue deep in the lungs becomes thick, stiff, and scarred over time. Oxygen supply in the blood is reduced, resulting in perpetual shortness of breath. Over 5 million people are affected by pulmonary fibrosis worldwide; and there is no cure.

There are over 140 known and countless idiopathic causes. However, exposure to environmental pollutants and certain medications can cause the disease.

Mice are the most common species used when studying pulmonary fibrosis. However, several animal models of lung fibrosis exist; ranging from mice to primates.

Bleomycin is a commonly used chemotherapeutic agent that causes an acute lung injury response followed by lung fibrosis. Other approaches include asbestosis, instillation of silica, and transgenic models.

CYSTIC FIBROSIS (CF)

Cystic Fibrosis is an incurable and inherited disorder affecting multiple organ systems; including the lungs. Genetic mutations of a specific protein called cystic fibrosis transmembrane conductance regulator (CFTR) cause abnormally thick mucus to form in the lungs. The mucus blocks the airways, causing lung damage and making it difficult to breathe. Although CF affects many areas of the body, complications and severe lung infections are the primary cause of death in most patients.

Animal models used to study CF typically focus on the use of mice with absent or mutant forms of the CFTR protein. Species including ferrets and pigs are also considered.

Pulmonary fibrosis and cystic fibrosis are two very different diseases. As such, the disease model studied often dictates the endpoints of interest. Although compliance and resistance are two commonly collected parameters, many supplemental endpoints can be calculated at the same time.

DSI products used in fibrosis studies:

- Resistance and compliance systems
- Pulmonary function testing
- FinePointe software

Suggested Lung Fibrosis Research References

Li M, Krishnaveni M, Li C, Zhou B, Xing Y, Banfalvi A, Li A, Lombardi V, Akbari O, Borok Z and Minoo P. "Epithelium-specific deletion of TGF- β receptor type II protects mice from bleomycin-induced pulmonary fibrosis." *Journal of Clinical Investigation*. 2011;121:277-287.

Marcos V, Zhou-Suckow Z, Yildirim A, Bohla A, Hector A, Vitkov L, Krautgartner W, Stoiber W, Giese M, Eickelberg O, Mall M, Hartl D. "Free DNA in Cystic Fibrosis Airway Fluids Correlates with Airflow Obstruction." *Mediators of Inflammation*. 2015.

Acute Respiratory Disorders

Disorders of the respiratory system can be grouped into different categories. Example categories include obstructive versus restrictive or acute versus chronic. Many disorders have similar causes, symptoms, and effects. As such, animal models (and hardware solutions) are often used to study more than one particular disease at a time. These solutions often leverage one of the following platforms:

- Head out plethysmography
- Whole body plethysmography
- Non-invasive airway mechanics
- Resistance and compliance
- Pulmonary function testing
- Inhalation/exposure systems

For more information, see the section titled "Products Used in Respiratory Research".

RESPIRATORY DEPRESSION

Hypoventilation or bradypnea (also known as respiratory depression) occurs when ventilation is inadequate to perform needed gas exchange. Causes of respiratory depression include medications such as opioid narcotics (both prescription and illicit drugs), barbiturates, or sedatives, alcohol, tumors, metabolic disorders, neuromuscular diseases, or airway obstruction. Enhanced funding and research efforts to curtail opioid abuse, addiction, and overdose are at the forefront of government initiatives to address this growing critical health crisis.

Suggested Respiratory Depression Research References:

Kuo A, Wyse B, Meutermans W, Smith T. "In vivo profiling of seven common opioids for antinociception, constipation and respiratory depression: no two opioids have the same profile." *British Journal of Pharmacology*. 2015; 172:532-548.

Zadina JE, Nilges MR, Morgenweck J, Zhang X, Hackler L, Fasold MB. "Endomorphin analog analgesics with reduced abuse liability, respiratory depression, motor impairment, tolerance, and glial activation relative to morphine." *Neuropharmacology*. 2016; 105:215-227.

Morse BL, Vijay N, Morris ME. "-Hydroxybutyrate (GHB)-induced respiratory depression: combined receptor-transporter inhibition therapy for treatment in GHB overdose." *Molecular Pharmacology*. 2012; 82(2):226-235.

RESPIRATORY SYNCYTIAL VIRUS (RSV)

Respiratory syncytial virus (RSV) is a common virus that infects the respiratory tract and lungs. Nearly all children are infected with RSV by the age of 2 years, and it is the leading cause of bronchiolitis and pneumonia in infants. Asthma exacerbations are common with viral infections like RSV, and recent evidence suggests that RSV infection at a young age is linked to recurrent wheezing or asthma in later childhood and adolescence.

Suggested RSV Research References:

Khaitov M, Shilovskiy I, Nikonova A, Shershakova N, Kamyshnikov O, Babakhin A, Zverev V, Johnston S, Khaitov R. "Small interfering RNAs targeted to interleukin-4 and respiratory syncytial virus reduce airway inflammation in a mouse model of virus-induced asthma exacerbation." *Human Gene Therapy*. 2014;25:642-650.

Stark J, Khan A, Chiappetta C, Xue H, Alcorn J, Colasurdo G. "Immune and Functional Role of Nitric Oxide in a Mouse Model of Respiratory Syncytial Virus Infection." *Journal of Infectious Disease*. 2005;191:387-95.

Phipps S, Lam C, Mahalingam S, Newhouse M, Ramirez R, Rosenberg H, Foster P, Matthaei K. "Eosinophils contribute to innate antiviral immunity and promote clearance of respiratory syncytial virus." *Blood*. 2007;110:1578-86.

ACUTE RESPIRATORY DISTRESS SYNDROME

Acute respiratory distress syndrome (ARDS) is a rapidly progressive inflammatory reaction that affects the lungs in the critically ill population. Gas exchange is impaired and the lungs become difficult to inflate, leading to respiratory failure. Risk factors for developing ARDS include cigarette smoking, obesity, recent surgery, oxygen use for pre-existing lung

condition, alcohol abuse, and recent chemotherapy.

Suggested ARDS Research References:

Ortolan L, Sercundes M, Barboza R, Debone D, Murillo O, Hagen S, Russo M, Lima M, Alvarez J, Amaku M, arinho C, Epiphany S. "Predictive Criteria to Study the Pathogenesis of Malaria-Associated ALI/ARDS in Mice." *Mediators of Inflammation*. 2014; Article ID 872464.

Wu N, Wang J. "Curcumin Attenuates Liver Warm Ischemia and Reperfusion-Induced Combined Restrictive and Obstructive Lung Disease by Reducing Matrix metalloproteinase 9 Activity." *Transplantation Proceedings*. 2014; 46(4): 1135-1138

MUCOCILIARY CLEARANCE AND DYSFUNCTION

Mucociliary clearance is an important primary innate defense mechanism that protects the lungs from the effects of inhaled pollutants, allergens, and pathogens. Mucociliary dysfunction can occur in a variety of chronic airway diseases, including cystic fibrosis and primary ciliary dyskinesia. Mucociliary dysfunction can also occur with acute infections such as pertussis (whooping cough). Pulmonary secretions are inadequately cleared and patients develop paroxysmal coughing (numerous, rapid coughing) followed by the characteristic high-pitched "whoop" upon inspiration.

Suggested Mucociliary Dysfunction Research References

Rajavelu P, Chen G, Xu Y, Kitzmiller J, Korfhagen T, and Whitsett J. "Airway epithelial SPDEF integrates goblet cell differentiation and pulmonary Th2 inflammation." *Journal of Clinical Investigation*. 2015;125(5):2012-2031

Marcos V, Zhou-Suckow Z, Yildirim A, Bohla A, Hector A, Vitkov L, Krautgartner W, Stoiber W, Griesse M, Eickelberg O, Mall M, Hartl D. "Free DNA in Cystic Fibrosis Airway Fluids Correlates with Airflow Obstruction." *Mediators of Inflammation*. 2015.

PNEUMONIA

A variety of organisms, including bacteria, viruses and fungi, can cause pneumonia. Globally, it is the leading cause of death in children under 5 years of age with nearly 1 million deaths per year. Risk factors for developing pneumonia include chronic lung diseases such as asthma or COPD, diabetes, heart failure, smoke exposure, dysphagia (difficulty swallowing), alcoholism, neurologic deficits that impair the cough reflex, and long-term use of

acid-suppressing medications such as proton pump inhibitors or H2-blockers.

Suggested Pneumonia Research References:

Zhang ZQ, Wang J, Hoy Z, Keegan A, Bhagwat S, Gigliotti F, Wright TW. "Neither classical nor alternative macrophage activation is required for *Pneumocystis* clearance during immune reconstitution inflammatory syndrome." *Infect Immun*. 2015;83:4594-603.

Kling H, Shipley T, Guyach S, Tarantelli R, Morris A, Norris K. "Trimethoprim-Sulfamethoxazole Treatment Does Not Reverse Obstructive Pulmonary Changes in *Pneumocystis*-Colonized Non-Human Primates with SHIV Infection." *J Acquir Immune Defic Syndr*. 2014; 65(4):381-389

COUGH

A cough is the body's way of removing foreign material or mucus from the respiratory tract or responding when something irritates the throat or airways. Causes of chronic cough (> 8 weeks) include allergies or post-nasal drip, pertussis, asthma, smoking/COPD, cystic fibrosis, gastroesophageal reflux disease, lung tumors, heart failure, or certain medications like ACE inhibitors.

Suggested Cough Research References:

Song K, Shin Y, Lee K, Lee E, Suh Y, Kim K. "Expectorant and Antitussive Effect of *Hedera helix* and *Rhizoma coptidis* Extracts Mixture." *Yonsei Medical Journal*. 2015;56(3):819-824.

Wex E, Bouyssou T. "Olodaterol Attenuates Citric Acid-Induced Cough in Naïve and Ovalbumin-Sensitized and Challenged Guinea Pigs". *PLoS One*. 2015; 10(3).

TUBERCULOSIS (TB)

Tuberculosis (TB) is a highly contagious and potentially fatal infectious disease caused by a bacterium called *Mycobacterium tuberculosis* (M. tuberculosis). Vaccine research relies on the ability to properly expose test subjects to the M. tuberculosis strain. The use of head out plethysmographs, masks, or helmets from DSI enables the aerosol to be delivered simultaneously with the measurement of respiration rate and tidal volume. This helps create a reproducible and more accurate delivery method to the lungs.

Suggested Tuberculosis Research References:

Sibley L, Dennis M, Sarfas C, White A, Clark S, Glee-

son F, McIntyre A, Rayner E, Pearson G, Williams A, Marsh P, Sharpe S. "Route of delivery to the airway influences the distribution of pulmonary disease but not the outcome of Mycobacterium tuberculosis infection in rhesus macaques." *Tuberculosis*. 2016;96:141-149.

Sharpe S, McShane H, Dennis M, Basaraba R, Gleeson F, Hall G, McIntyre A, Gooch K, Clark S, Beveridge N, Nuth E, White A, Marriott A, Dowall S, Hill A, Williams A, Marsh P. "Establishment of an Aerosol Challenge Model of Tuberculosis in Rhesus Macaques and an Evaluation of Endpoints for Vaccine Testing." *Clinical and Vaccine Immunology*. 2010;17:1170-1182.

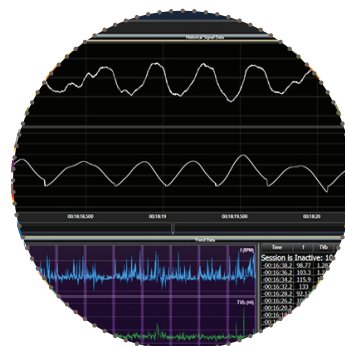
BRONCHIOLITIS

Bronchiolitis is an acute inflammation of the bronchioles (smallest air passages of the lungs) usually caused by respiratory viral infections during the winter and spring months. Some evidence suggests that there is a link between bronchiolitis in infancy and the development of recurrent wheezing or asthma throughout childhood and adolescence. Risk factors for developing bronchiolitis include prematurity, chronic lung disease, congenital heart disease, smoke exposure, daycare attendance, neuromuscular disease, lack of breastfeeding, and low socioeconomic status.

Suggested Bronchiolitis Research References:

Glineur S, Anh D, Sarlet M, Michaux C, Desmecht D. "Characterization of the Resistance of SJL/J Mice to Pneumonia Virus of Mice, a Model for Infantile Bronchiolitis Due to a Respiratory Syncytial Virus." *PLoS ONE*. 2012;7(10): e44581. doi:10.1371/journal.pone.0044581

Products Used in Respiratory Research



FinePointe software is powerful and easy-to-use for collecting, analyzing, and reporting life science data. Fully network-enabled, the smart design minimizes required user interaction while wizards walk users through necessary procedures.



Whole Body Plethysmography - When conscious, unrestrained approaches are desired, FinePointe whole body plethysmography allows for respiratory endpoints to be obtained. This approach is often used as a quick, easy way to screen compounds, run longitudinal studies, and assess the need for further research in a more invasive model.

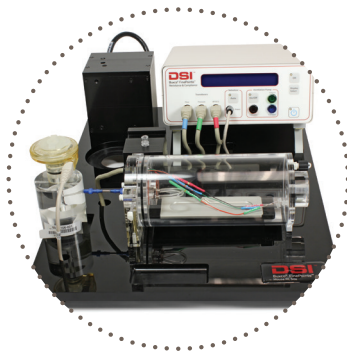
Products Used in Respiratory Research



Non-Invasive Airway Mechanics - FinePointe non-invasive airway mechanics uses specially designed Allay restraint and double chamber plethysmography to monitor nasal and thoracic flow simultaneously. Aerosol administration of a bronchoconstrictor is evaluated on conscious animals deriving specific airway resistance.



Pulmonary Function Testing - The pulmonary function testing system allows for a comprehensive assessment of the lungs. All lung volumes and spirometry equivalent test results are completed in a matter of minutes; allowing for high throughput and quicker results.



Resistance and Compliance - FinePointe resistance and compliance hardware helps researchers measure an animal's airflow and lung pressure. This anesthetized approach allows for direct measurement of an animal's response to a bronchoconstrictor either using an aerosol or I.V. Pulmonary fibrosis models demonstrate a decrease in compliance as a result of stiff lungs. Obstructive cystic fibrosis models affect airway resistance.



Smoke Generator - Completely automated and capable of burning up to 300 cigarettes, the smoke generator captures direct and indirect smoke and delivers it to an exposure apparatus including chambers, towers, or individual exposure tubes.