NTP
National Toxicology Program

NTP Mold Studies Update

Board of Scientific Counselors Meeting
December 6, 2007

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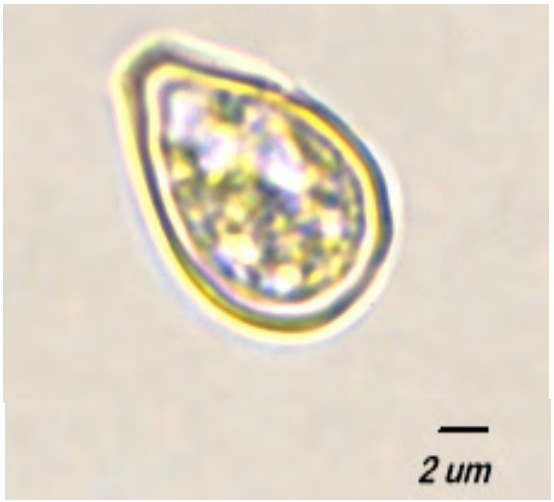
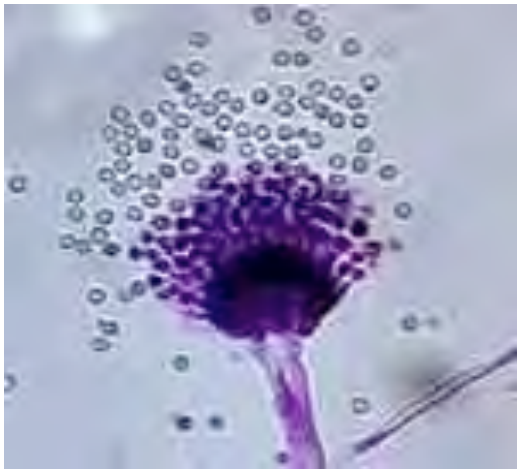
Fungi Are Ubiquitous





images from Airborne Allergens CD

Why Study Mold?

Photos: Dr. Sung-Chul Seo

Why Study Mold?

Exposure to elevated levels of indoor mold has been associated with a number of symptoms:

Allergies, asthma, hives, bleeding lungs, cancer, CNS problems, recurring colds, chronic cough, dandruff (chronic), dermatitis, skin rashes, diarrhea, eye/vision problems, fatigue, general malaise, flu-like symptoms, sudden hair loss, headaches, hemorrhagic pneumonitis, hypersensitivity pneumonitis, irritability, itching, kidney failure, learning difficulties, mental dysfunction, personality changes, memory loss……

Source: PRWeb Vancouver, Feb. 21, 2005

Institute of Medicine Report

- Sufficient evidence of an association between exposure to damp indoor environments and some respiratory health outcomes in sensitized persons.
- Suggestive evidence of an association between damp indoor environments and respiratory illness in otherwise healthy children. Not clear if related to mold, bacteria, dust mites, cockroaches, or a combination thereof.
- Insufficient evidence to determine whether damp environments are related to a variety of health outcomes.
  - Including any association with *Stachybotrys chartarum*
- Many Data Gaps Identified
  - Neurotoxicity
  - Rheumatic diseases
  - Reproductive effects
  - Cardiopulmonary

Damp Indoor Spaces and Health, The National Academy of Sciences (2004)

Clinical Aspects of Fungal Toxicity - Many Uncertainties

- Measurement of exposure to fungal allergens has been restricted to the spores of a select number of fungi
- The potential of different fungi or fragments to cause or aggravate adverse health effects remains unclear
- Relationship between fungal exposures and clinical outcomes often unclear

Nomination History

- Molds were nominated for study by a private individual
  - No specific organisms, endpoints, or health effects were suggested
- Nomination has been through multiple levels of internal and external review
  - NTP BSC and Executive committee - 2004
    - Endorsed study of molds and suggested that the program consider studying organisms commonly found in indoor air (i.e. *Aspergillus* and *Penicillium*) as well as *Stachybotrys*
  - NTP Concept review 2006
    - Solicit expert input on how to conduct large scale "real-life" exposure rodent studies and the toxicological endpoints to be measured
    - Explore the feasibility of conducting studies of "real-life" exposure scenarios to mimic the conditions found in damp or water-damaged buildings

Even early in the process there was considerable debate on what to study and how to study it

NTP Mold Studies Information Group

March 5, 2007

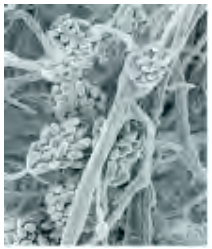
- Our goal for the meeting was to obtain input from scientists with expertise in studying molds to assist the NTP in the design and conduct of animal toxicity studies
- NTP's studies on mold would employ exposure scenarios that closely mimic real world human exposure circumstances to
  - Reveal the range of biological responses in common laboratory animal models
  - Identify potential hazards to human health

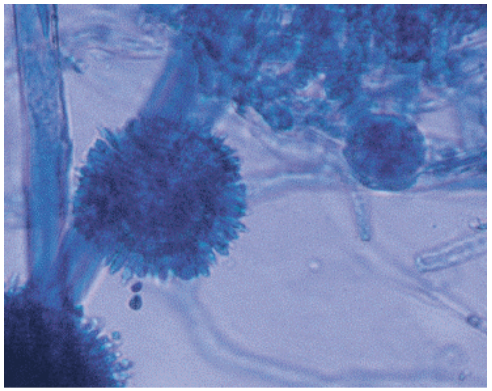
Design Considerations

- Chemical or metabolite to be tested
  - ~ 1.5 million species exist, ~ 80,000 species described
- Route of exposure
  - Mostly inhalation, but also ingestion and dermal
- Species/strain/gender
- Age
  - Susceptible populations

Key Issues - The Complexity of Studying Fungi

- Whole organism versus isolated fractions or toxins
- Growing conditions
  - Temperature, humidity, substrate
- Life stages
  - Spores, age of culture
- Physical and chemical properties vary with life stages and growing conditions



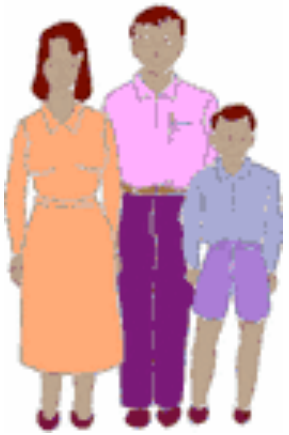


Recommendations

- Use single organisms as well as molds co-cultured on different building materials
  - High humidity scenario, saturation scenario, fresh isolates of single species
- Define growth stage and harvest and dry for use in the entire study
  - Characterize samples with respect to mycotoxins, glucans, allergens, particle size, protease activity, colony forming units, spores.
  - Methods development or validation may be needed for some aspects of characterization
- Evaluate biomarkers appropriate for the strains of fungi being used
  - Host antibodies, fungal products, protein adducts, and metabolites in host tissues
  - Tissue burden and distribution
- Inhalation models are appropriate
  - Ancillary studies can address susceptible populations or specific endpoints

All participants felt that exposing animals to a moldy environment and examining whether there are adverse effects would be an important study to conduct

Path Forward



NTP Research Program

NTP Toxicology Studies of Mold

- Specific Aims
  - Assess organ system toxicity following inhalation exposure to molds
  - Evaluate the available biomarkers of exposure and effect (both general and specific for the organisms to be studied)
  - Evaluate the contribution of different organisms to overall health effects by studying individual isolates as well as mixtures

NTP Toxicology Studies of Mold

- Proposed Approach
  - Conduct subchronic studies in rodents using inhalation as the route of exposure
  - Test two mixtures to simulate real life exposure scenarios
    - Mixed culture of molds from a water damaged building from New Orleans, Louisiana
    - Mixed culture of molds from a damp building with reported health effects (sick-building syndrome)
  - Test four isolates of individual organisms

NTP Toxicology Studies of Mold

- Proposed Approach
  - Test four isolates of individual organisms
    - *Stachybotrys chartarum* isolate 1 (macrocyclic tricothecene chemotype)
    - *Stachybotrys chartarum* isolate 2 (atranone chemotype)
      - Greenish-black fungus found worldwide
      - Colonizes high-cellulose material that becomes chronically moist or water damaged due to excessive humidity, water leaks, condensation or flooding
    - *Aspergillus versicolor*
      - Common on gypsum board, floor, carpet, mattress and upholstered-furniture dust, and damp walls.
    - *Alternaria alternata*
      - Commonly isolated from plants, soil, food, and indoor air environment
      - Important in allergy, infection and asthma severity

NTP Toxicology Studies of Mold

- Endpoints for subchronic toxicity studies
  - Characterize the test materials both prior to and during the studies
    - Evaluate relevant mycotoxins, glucans, allergens, particle size, protease activity, colony-forming units, spores, and endotoxin levels)
    - Develop and validate methods; this may be accomplished using existing analytical chemistry contracts
  - Evaluate neurotoxicity using a functional observation battery, olfactory sensing, and cognitive tests
  - Examine impact on cardiovascular, respiratory, gastrointestinal, and immune systems

Significance and Expected Outcomes

- These studies will provide important information regarding:
  - Which fungal organisms may be causative agents for human health effects
  - Target organs for fungal toxicity
  - Dose-dependent effects with particular emphasis on respiratory, immune, and neurologic endpoints
  - The utility of biomarkers other than IgE as measures of exposure and effect

Bridging the Gaps

- Rodent studies will provide information on additional clinical measures or outcomes that should be examined in epidemiologic studies
- Information from clinical collaborations will be used to develop biomarkers of exposure and effect for the NTP rodent studies

Clinical Collaboration - Heading off Environmental Asthma in Louisiana (HEAL) Study

- Primary objective is to implement and test an Asthma Counselor (AC) program that addresses the multidimensional impact of hurricane Katrina on children with asthma in New Orleans
  - Is there an increase in allergens due to moisture? - mold, cockroaches, dust mites, etc.
  - What is the impact of the disrupted health care system?
  - What is the effect of stress?

HEAL Study Design - 1

- Randomized one-year intervention
- Subjects – 450 children (4-12 years old) with moderate to severe asthma and their caregivers
  - Must have previous asthma diagnosis
- Endpoints
  - Symptoms
    - wheezing, disrupted sleep, slow down or discontinue physical activity due to asthma
  - Biological Measures
    - Spirometry and peak flow measures, quality of life, asthma medication use, unscheduled clinic or ED visits, hospitalizations, biomarkers of exposure
  - Environmental Measures
    - Baseline levels of molds and other allergens
    - Moisture and humidity

HEAL Study Design - 2

- Clinical Evaluation
  - History, physical examination and questionnaire
  - Pulmonary function testing
  - Skin prick allergen testing (expanded mold panel)
  - Blood draw – CBC, total IgE and allergen specific IgE, genetic archiving
- Environmental evaluations
  - Visual inspection, air, dust and HEPA filter sampling
  - Mold, moisture, allergens

HEAL Study - NTP (NIEHS/NIOSH) Collaboration

- Total IgE
- Allergen Specific IgE
  - Specific allergen reactivity in patients with atopic dermatitis - dust mite, cockroach, cat, dog, rat, mouse
  - Mixed mold screening panel - *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigates*, *Candida albicans*, *Alternaria alternata*, and *Helminthosporium halodes*
  - Katrina specific mold evaluation
    - 4 molds from screening panel and 6 identified with increased prevalence post-Katrina (including *Stachybotrys chartarum* and *Aspergillus versicolor*)
    - All samples positive in mold mix screen to be tested
    - 50 mold mix negative
- Specific IgG
  - 4 Common molds

HEAL Study - Preliminary Results

- Total IgE - 60/87 (69%) subjects tested to date have elevated IgE (>100 kU/L)
- Allergen Specific IgE
  - Mixed mold screening panel
    - 39/87 (45%) subjects had a positive ImmunoCap test to the mixed mold screening panel
  - Katrina specific mold evaluation
    - Of the 39 positive samples, reactivity to eight individual molds was high and ranged from 77% to 94% (*Alternaria alternata*). Reactivity to 2 of the molds, *Aspergillus niger* and *Chaetomium globosum* was low at approximately 15%.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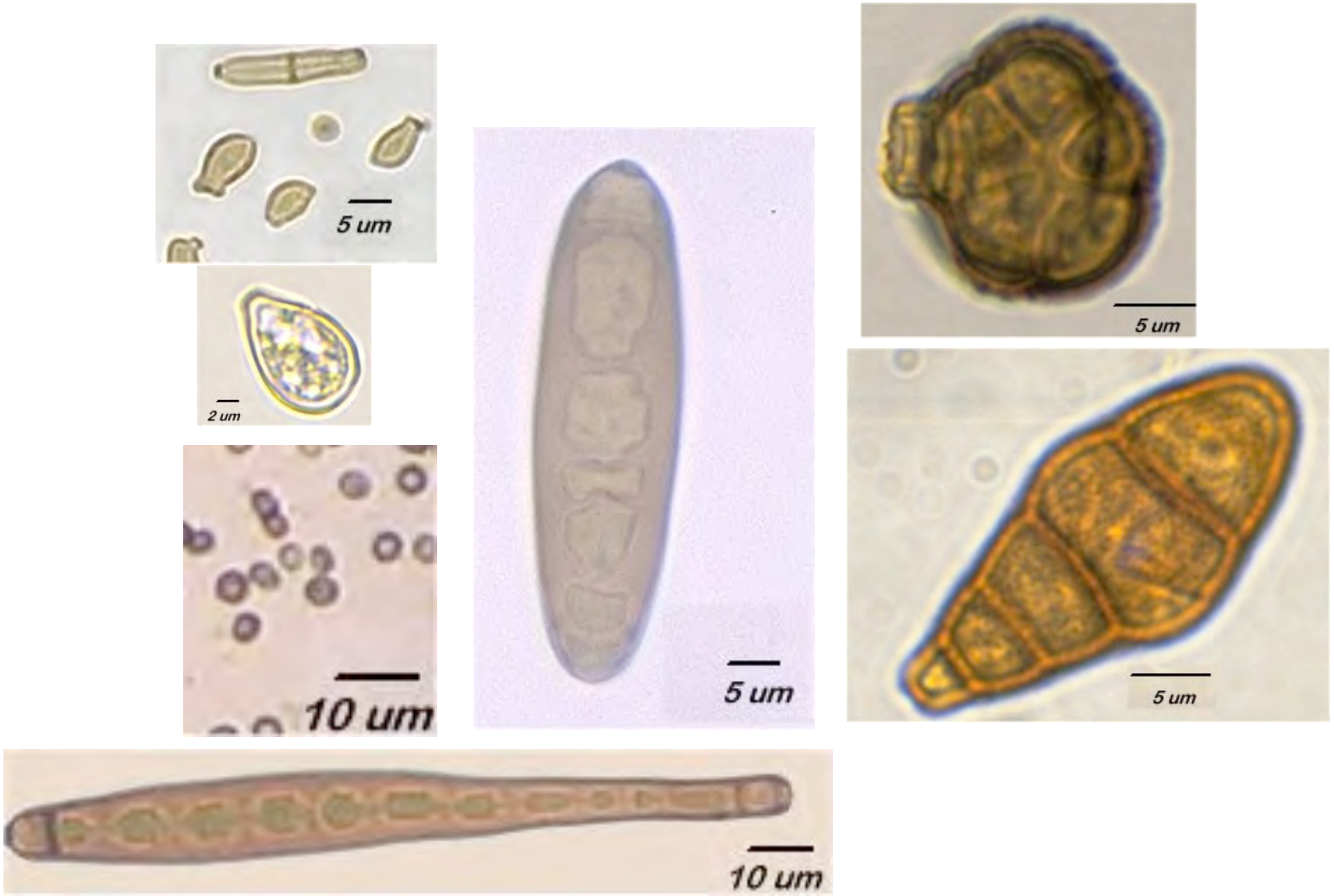
Fungal Spore Morphology



images from: Airborne Allergens CD Hjelmroos, Benyon, Culliver, Jones & Tovey