Immunological Effects of Subchronic Fungal Exposure

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Occupational Allergy Team – ACIB/HELD

Team Mission

1. Bioaerosol hazard identification and diversity characterization.

2. Identification and characterization of emerging occupational high molecular weight allergens and other occupationally relevant antigens.

3. Development of acute, subchronic, and chronic murine models to characterize immunotoxicological mechanisms associated with exposure to occupational hazards.

4. Evaluate the relevance and impact of intervention strategies to effectively prevent exposure to microorganisms in the workplace.

Heightened Awareness of Fungal Exposures

Hurricane Harvey, October 2017

Hurricane Katrina, August 2005

Natural disasters and the accompanying media coverage have heightened awareness of the potential occupational health effects of fungal exposure.

Why are we studying fungi and fungal toxins?

- Increased public interest/concern regarding fungal exposures.
- Fungal related enquiries are frequently received by NIOSH and NIEHS.
- Consensus reports establish fungal exposure associated with adverse health effects.

NAS/IOM, Damp Indoor Spaces and Health, 2004

"*Epidemiologic studies indicate that there is sufficient evidence to conclude that the presence of mold (otherwise unspecified) indoors is associated with upper respiratory symptoms…..in susceptible persons.*"

WHO Guidelines for indoor air quality: dampness and mould, 2009

"*Sufficient epidemiological evidence to show that the occupants of damp or mouldy buildings, both houses and public buildings, are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma.*"

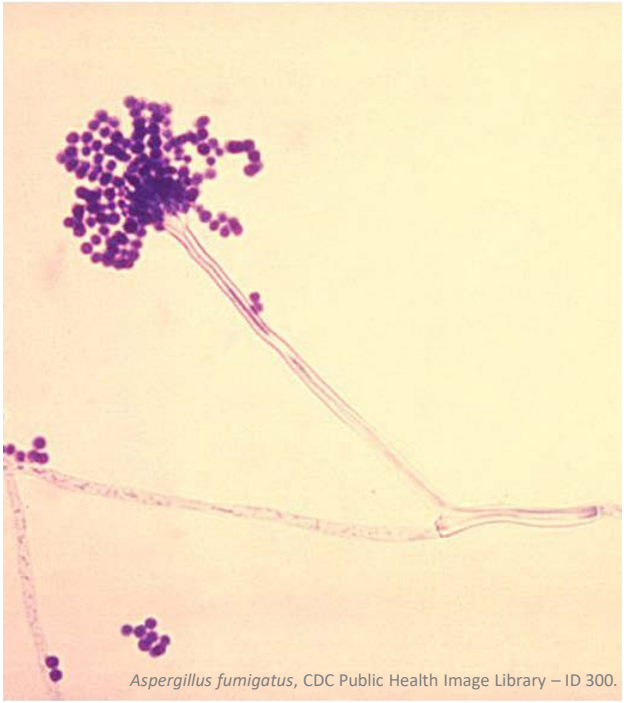
- Knowledge gaps remain.
  - No occupational exposure limits determined for fungi.
  - Assemblage of fungi that contribute to worker exposure remains unknown.
  - Mechanisms that modulate respiratory, neurological and cardiac effects are unknown.

NTP Nomination Background

- Mold was nominated to the NTP in 2004.
- Animal studies to provide “real-life” exposure scenarios to mimic the conditions found in damp or water-damaged buildings.
- In 2007, an expert review panel convened to discuss study design.
- **Key Recommendations**
  - Single organisms/co-cultured on different building materials, production of sufficient quantities of mold, mold co-cultured and harvested at a particular growth stage, characterization of molds – glucans, allergens, particle size protease activity

Key Issues and Challenges

- Fungi is a living organism.
- What is applicable to one organism is not necessarily applicable to others.
- Which organisms to test?
- Appropriate and relevant exposure scenarios (i.e. route of exposure, individual organisms or mixtures).



Aspergillus fumigatus, CDC Public Health Image Library – ID 300.

NIEHS/NTP IAA with NIOSH

- FY12-FY19: IAA (CDC IAG #12-NS12-01) with NIOSH entitled “Immunotoxicity of Workplace Xenobiotics”.

- FY20-FY25: New IAA with NIOSH entitled *“Assessment of Inhalation Exposures to Indoor and Occupational Aerosols”*

Task 1 - <u>Exposure Assessment of Indoor and Occupational Aerosols</u>

- Project 1.1: ITS region sequencing.
- Project 1.2: UPLC-MSMS detection of fungal secondary metabolites.
- Project 1.3: Biomarker discovery.

Task 2 - <u>Murine Models of Repeated Fungal Inhalation Exposure</u>

- *Phase 1*: Pulmonary immunology studies.
- *Phase 2*: NTP subchronic toxicology studies conducted in collaboration with Battelle Memorial Institute.

Task 1. Exposure Assessment of Indoor and Occupational Aerosols

Fungal Hazard Identification

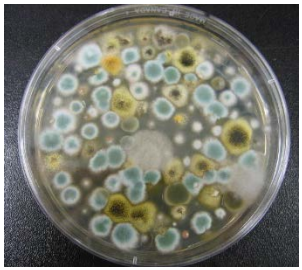
Traditional Methods

Culture

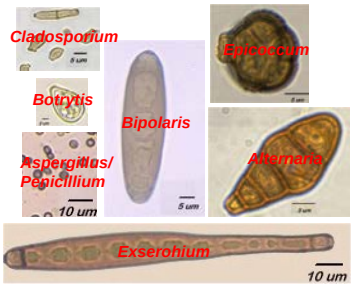
Viable spores or hyphae

Morphology

- Spores
- Hyphae
- Fragments



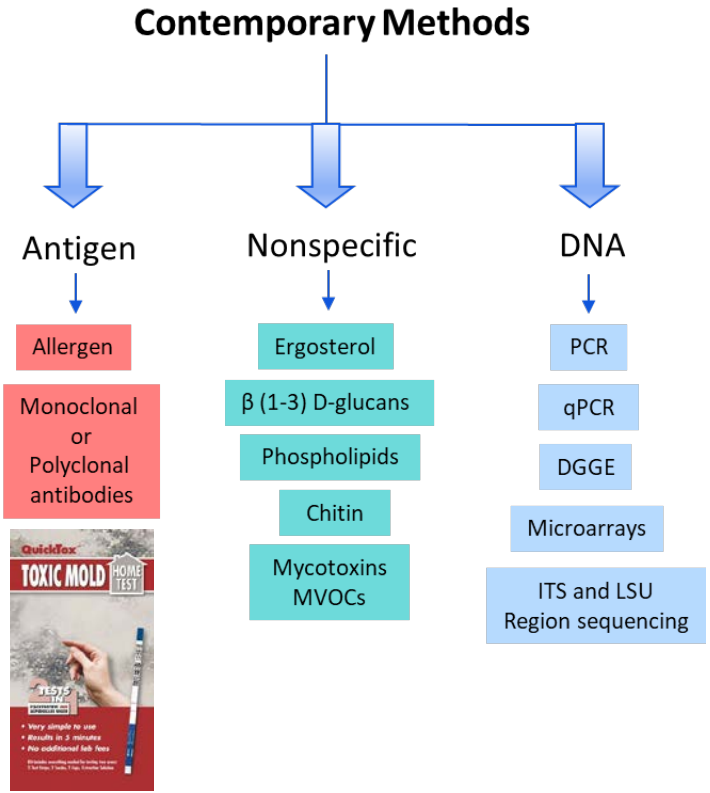
CDC PHIL – ID 20862



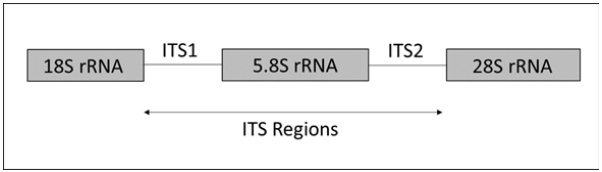
- Paradigm of fungal exposure based on results derived from culture and microscopic assessments.
  - Filamentous fungi/Molds
  - Phylum Ascomycota
  - Other phyla and yeasts predominantly overlooked

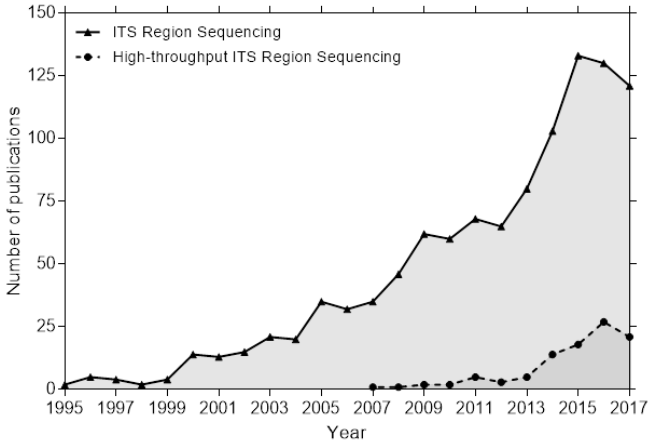
images from: Airborne Allergens CD Hjelmroos, Benyon, Culliver, Jones & Tovey

Contemporary Detection Methods



Internal Transcribed Spacer Region (ITS) "Fungal Barcode"





Green (2018) *Curr Allergy Asthma Rep. 2018 Sep 27;18(11):62*

Evaluation of an Unpleasant Odor at an Aircraft Ejection Seat Manufacturer

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HHE Report No. 2014-0050-3234
April 2015

Evaluation of Potential Hazards during Harvesting and Processing Cannabis at an Outdoor Organic Farm

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HHE Report No. 2015-0111-3271
April 2017

Evaluation of a Medicinal Cannabis Manufacturing Facility with an Indoor and Outdoor Grow Operation

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HHE Report No. 2016-0090-3317
June 2018

Characterizing Exposures During Laser Tattoo Removal in a Hospital Dermatology Center

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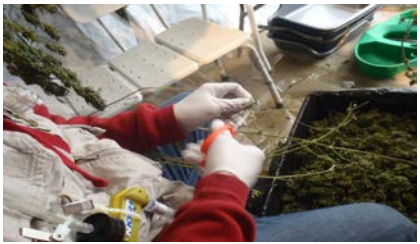
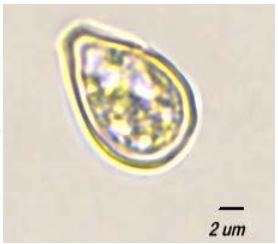
HHE Report No. 2017-0006-3319
May 2018

Task 1 - Highlights

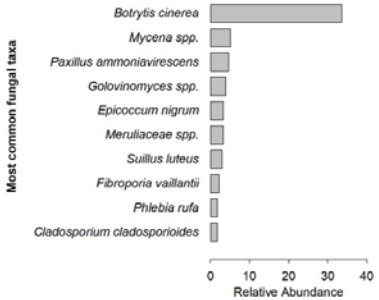
- ITS sequencing resolves an increased assemblage of microbial occupational exposures compared to traditional methods of assessment.

Harvesting and Processing Cannabis





Botrytis cinerea – plant pathogen (grey mold)



Green (2018) *J Occup Environ Hyg* 15 (5): 430-440.

- R2P components – design and development of targeted quantitative assays.
  - NCEZID pathogen surveillance
  - NIOSH HHEs
  - Intramural and Extramural collaboration
- Select previous overlooked species for assessment in Task 2 studies.

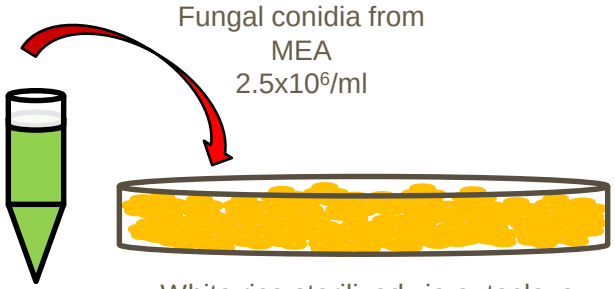
Task 2. Murine Models of Repeated Fungal Inhalation Exposure

Subchronic Fungal Exposure Animal Model

- NTP B6C3F1/N mice.
- Test articles
  - *Aspergillus fumigatus* B-5233/ATCC 13073 conidia
  - *Stachybotrys chartarum* (2 mycotoxin producing strains)
  - *Aspergillus versicolor* (Vuillemin) Tiraboschi ATCC 9577/NRRL 238
- Heat inactivated conidia used as a biological/particulate control.
- Mice exposed twice/week for 13 weeks and then euthanized at 24 or 48 hours after the final exposure.
- Endpoints - pulmonary inflammation, immune and molecular endpoints and histopathology.

JAX Labs

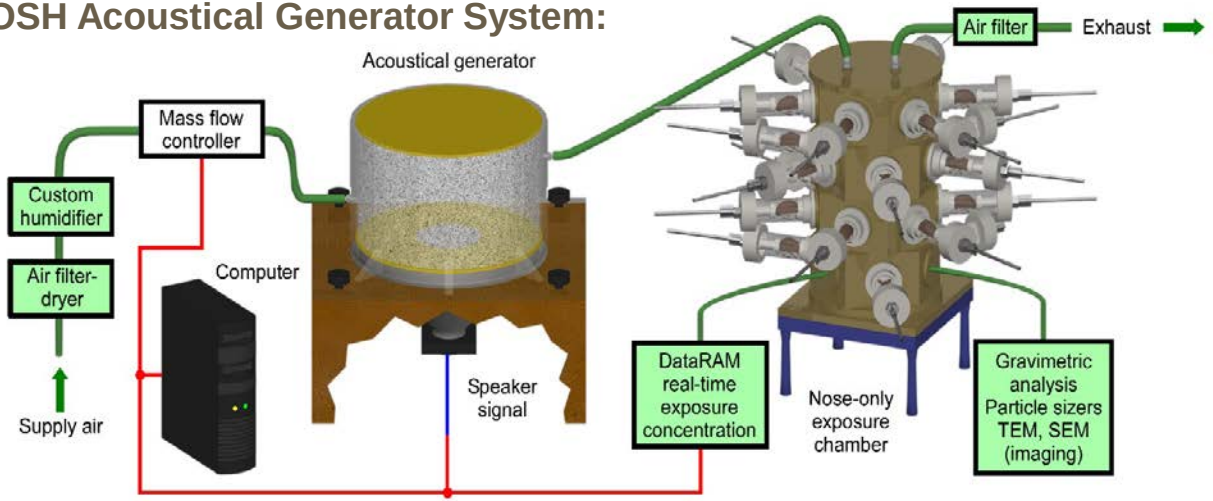
Culture and Aersolization of Fungal Test Articles



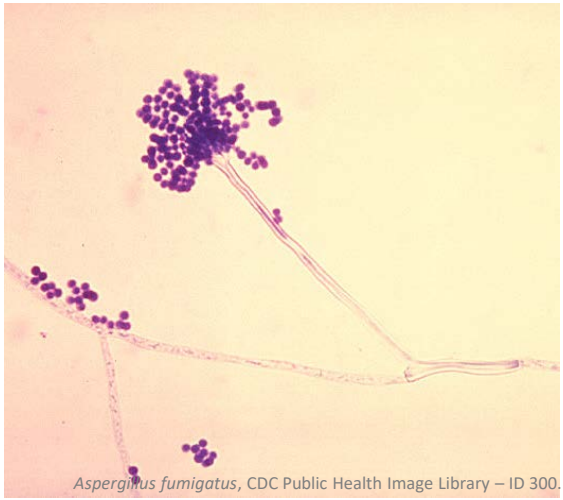
- White rice sterilized via autoclave
- 132˚C 30min

- Rice is incubated in an environmental chamber
- Rice is then desiccated to achieve aerosolizaiton

NIOSH Acoustical Generator System:



Aspergillus fumigatus



Aspergillus fumigatus, CDC Public Health Image Library – ID 300.

Aspergillus fumigatus Fresenius.
CAS No. ASPERGILLUS

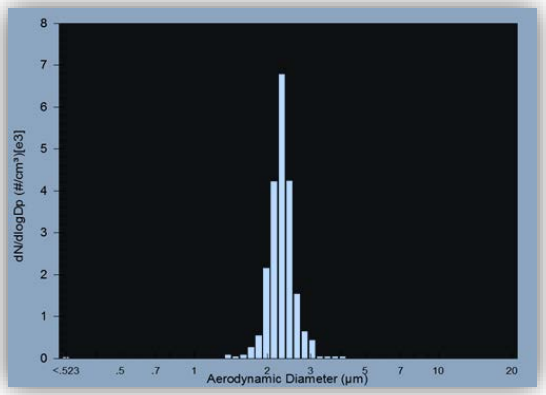
Pulmonary Immunology Studies

Ann Allergy Asthma Immunol, 2018, 121(2): 200-210
Frontiers in Immunology, 2018, 9: 170
Clin Exp Allergy, 2016, 46(10): 1315-1327
Clin Exp Allergy, 2016, 46(6):861-870
PLoS One, 2014, 9 (10): e109855
J Immunotox, 2014, 11 (2): 180-189
PLoS One, 2011, 6 (4): e18777

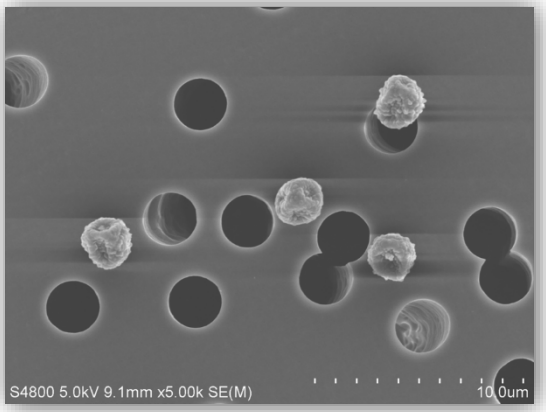
NTP Toxicology Study #C08022

Green BJ, Lemons AR, Goldsmith WT, Croston TL, Nayak AP, Battelli L, Orandle M, Beezhold DH. NTP Technical report on the toxicity studies of Aspergillus fumigatus (CAS No. ASPERGILLUS) administered by inhalation to B6C3F1/N mice. National Toxicology Program Toxicity Report Series. (Submitted)

Fungal Test Article Aerosolization



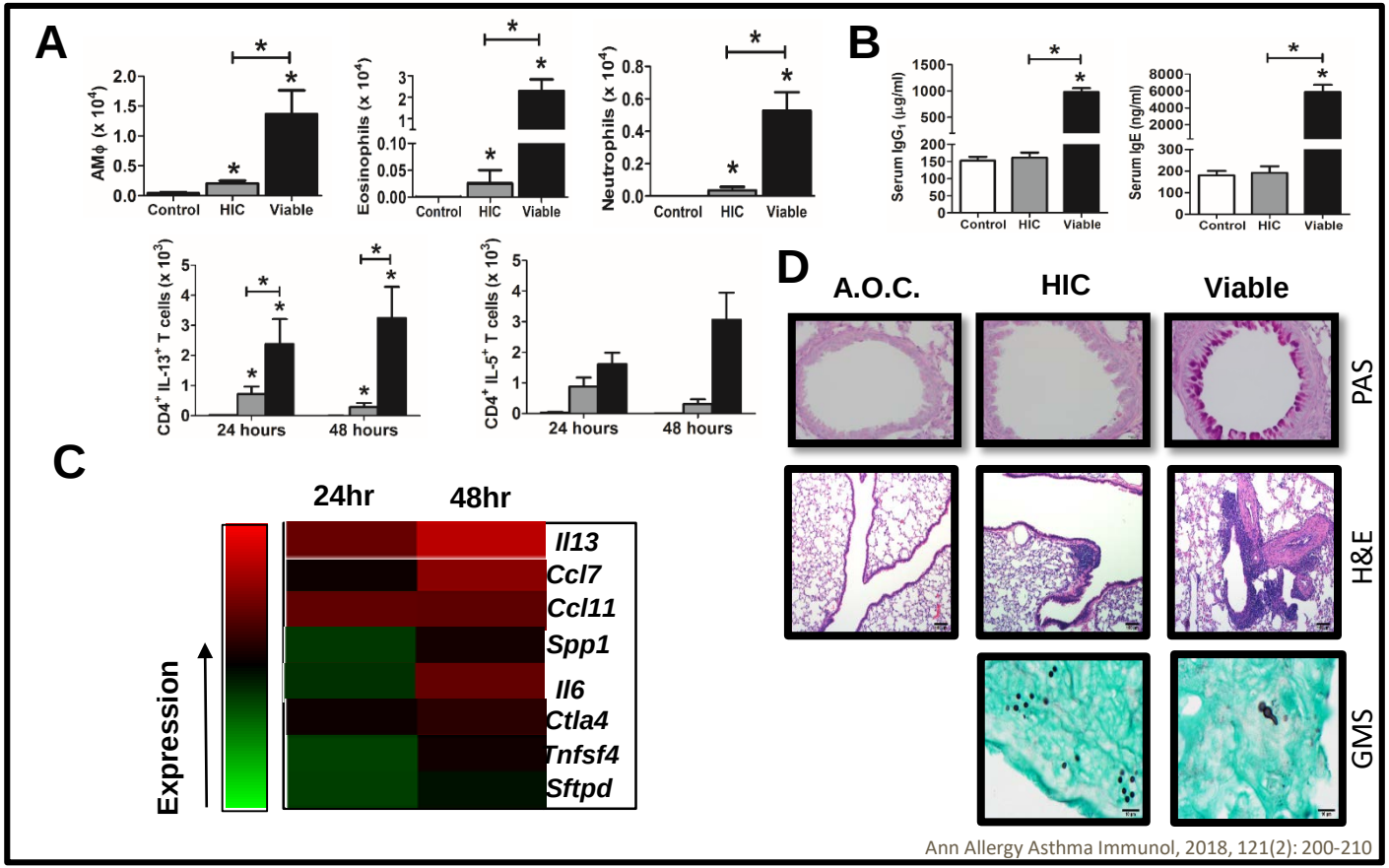
Aerodynamic diameter of *A. fumigatus* test articles aerosolized from cultivated rice cultures.



Homogenous amerospore *A. fumigatus* aerosol with no fragments/rice particulate.

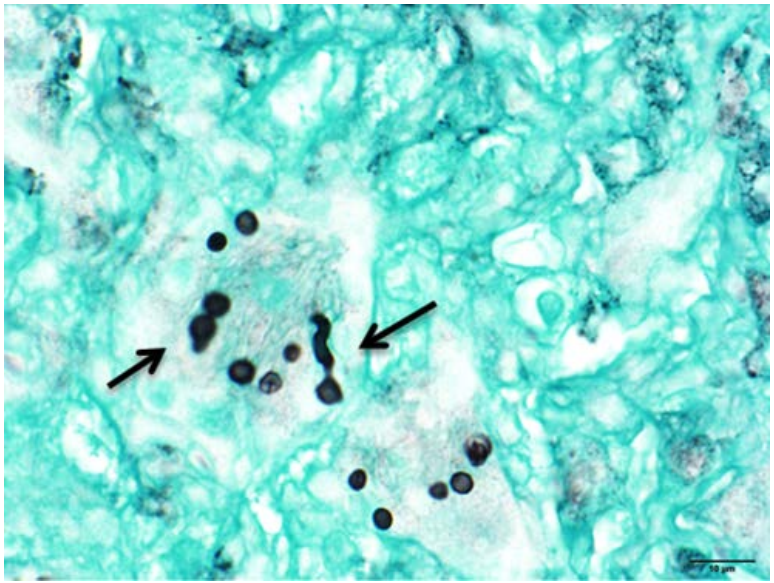
PLoS One, 2014, 9 (10): e109855

Mice exposed to viable *A. fumigatus* demonstrate pulmonary inflammation, pulmonary arterial remodeling and germination of fungal spores *in vivo.*



Ann Allergy Asthma Immunol, 2018, 121(2): 200-210

A. fumigatus spore germination *in vivo* is a significant component for the development of allergic response.



Pulmonary pathology, analysis of cell populations in the BALF, mRNA, proteomics, miRNA and pathway analysis consistently demonstrate that fungal germination *in vivo* is a significant component for the development of allergic response in B6C3F1/N mice exposed to *A. fumigatus* conidia

Ann Allergy Asthma Immunol, 2018, 121(2): 200-210

NTP Toxicology Study #C08022

NIOSH Technical Report on the Toxicity Studies of

Aspergillus fumigatus
(CAS No. ASPERGILLUS)

Administered by Inhalation to B6C3F1/N Mice

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National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
U.S. Department of Health and Human Services

NTP Toxicology Study #C08022

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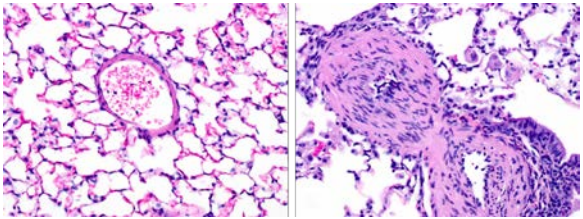
Artery – Hypertrophy, Medial

Air control lung | Viable *Aspergillus fumigatus* lung



- Vascular lesion in the lung was observed only in the viable groups.
- Lesions not observed in the HIC or air control groups.

Stachybotrys chartarum

Stachybotrys chartarum contamination following Hurricane Katrina.

Pulmonary Immunology Studies

Lemons AR, Croston TL, Goldsmith WT, Germolec DR, Beezhold DH, Green BJ. Cultivation and aerosolization of *Stachybotrys chartarum* for modeling pulmonary inhalation exposure. *Inhal Toxicol* (Submitted)

Croston TL, Lemons AR, Barnes MA, Goldsmith WT, Orandle MS, Nayak AP, Rush RE, Germolec DR, Green BJ, Beezhold DH. Inhalation of *Stachybotrys chartarum* fragments induces pulmonary arterial hyperplasia. *Am J Respir Cell Mol Biol* (Submitted)

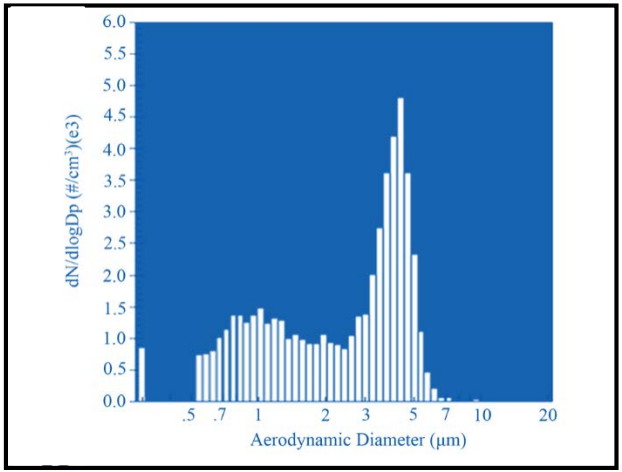
NTP Toxicology Study #C04052

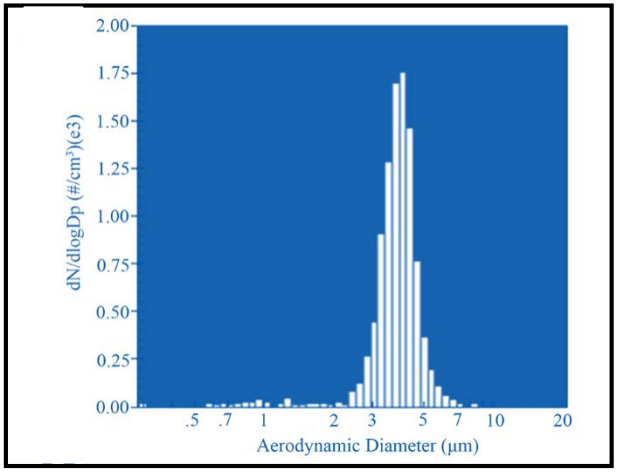
Green BJ, Lemons AR, Goldsmith WT, Croston TL, Nayak AP, Battelli L, Orandle M, Beezhold DH. NTP Technical report on the toxicity studies of *Stachybotrys chartarum* (CAS No. STACHYBOTRYS) administered by inhalation to B6C3F1/N mice. National Toxicology Program Toxicity Report Series. (In preparation)

Fungal Test Article Aerosolization

- Strain A
- Higher levels of trichothecene
- Higher amount of fragmentation

- Strain B
- Lower levels of trichothecene
- Lower amount of fragmentation





Fungal Test Article Aerosolization

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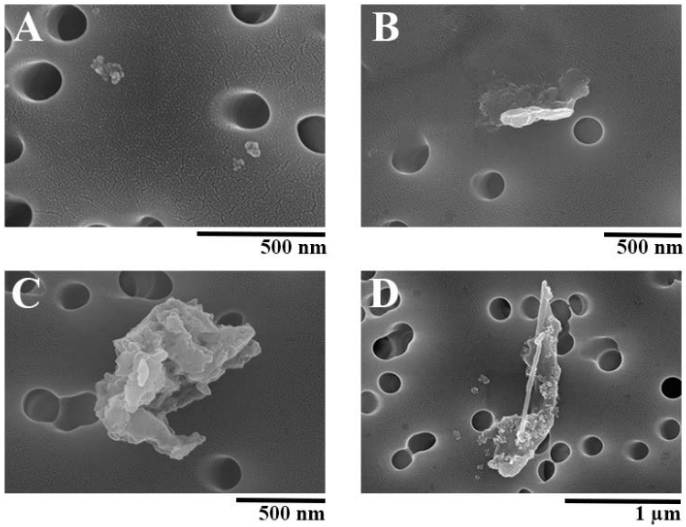
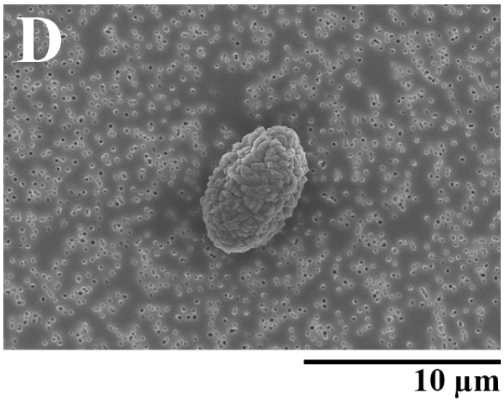
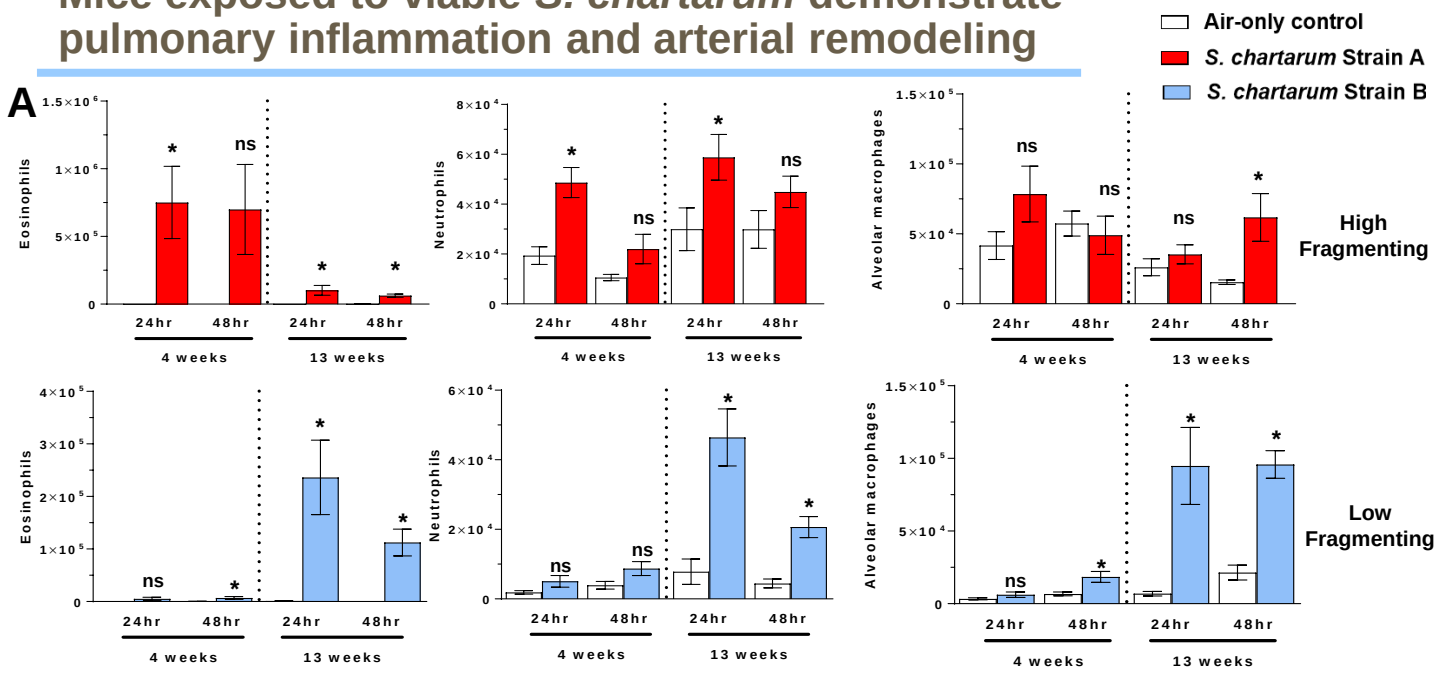
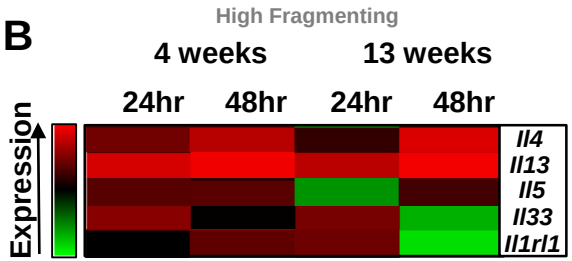


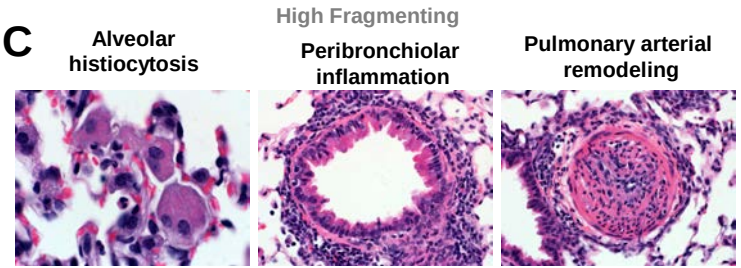
- Strain B
- Lower levels of trichothecene
- Lower amount of fragmentation



Mice exposed to viable *S. chartarum* demonstrate pulmonary inflammation and arterial remodeling







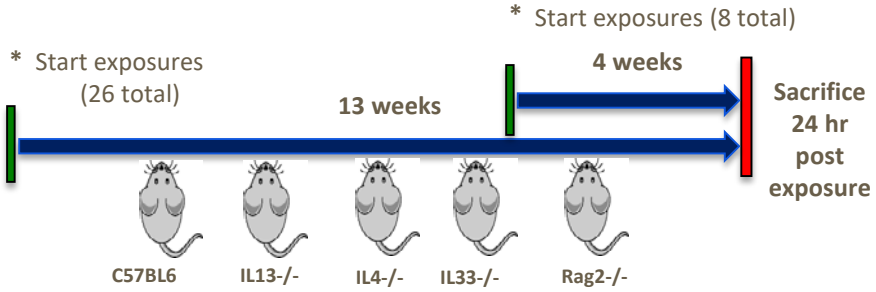
Croston et al 2019, Under Review

Task 2 - Highlights

- Repeated fungal exposures results in varied pulmonary immunological responses depending on the species.
  - *A. fumigatus* drives a Th2-biased immune response.
  - *S. chartarum* drives a mixed Th1 and Th2 response.
- Varied responses due to the biology of the fungal species.
  - spore viability vs fragmentation.
- Pulmonary arterial remodeling observed in all tested species.
  - Ingenuity pathway analysis identified potential cardiac involvement.
  - Mechanisms of arterial remodeling the focus of future studies.

Pulmonary Arterial Remodeling – Future Studies

- Utilize knockout mouse strains.
  - Identify the role of IL4 and IL13 in fungal spore-induced lung and cardiac pathology.
  - Elucidate the cellular mechanisms of ILC2s that drive host responses to spores.



- Exposure groups
  - Air-only control
  - *S. chartarum* (1 x 10^4 spores)
  - *A. versicolor* (5 x 10^4 spores)

Summary

- Leveraging an interagency collaboration between the NIEHS and NIOSH we have;
  - Developed contemporary methods to identify fungal exposures.
  - Utilized a NIOSH inhalation exposure system that simulates occupational fungal exposures and/or mimics the conditions found in damp or water-damaged buildings.

- Contemporary molecular methods have resolved a richer assemblage of fungal species in indoor, outdoor and occupational environments.

- Mice repeatedly exposed to fungal bioaerosols demonstrate pulmonary inflammation, a mixed immunological response, and pulmonary arterial remodeling.
  - **Responses vary between species**: spore viability vs fragmentation

Future Directions

- *Aspergillus versicolor* (Vuillemin) Tiraboschi ATCC 9577/NRRL 238.
  - Pulmonary Immunology studies completed in 2019.
  - NTP Toxicology Study #C15017 scheduled mid FY20.

- Neuroinflammation collaboration - Indiana University.
  - Characterization of neuroinflammation mechanisms.
  - Cognitive dysfunction/behavioral studies.

- Evaluate remaining NTP nominated organisms.
  - *Alternaria alternata.*
  - Overlooked species identified using ITS region sequencing.
  - **Mixed fungal exposures.**

Questions for the NIOSH BSC

1. *Are these datasets reaching the correct stakeholder audience? How can we better communicate the results derived from Task 1 and 2 studies to NIOSH stakeholders and the broader occupational health community?*

2. *Are there additional fungal species, other than those already nominated to the NTP, identified by NIOSH stakeholders that we could include in future studies?*

3. *How can the results derived from fungal exposure and animal model studies be translated into policies that protect workers?*

4. *How could the assays developed in Task 1 be implemented by the broader occupational health and industrial hygiene community to identify microbial exposures?*

5. *How could NIOSH work towards developing an occupational exposure limit for fungi? Would it have to be species based?*

Acknowledgements

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