Mold and Moisture Assessment Based on the Environmental Relative Moldiness Index^{sm}

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NOTICE

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AGENDA

1. **Explain current mold analysis limitations.**

2. **Describe development of the Environmental Relative Moldiness Index (ERMI).** **(More details in session on Wednesday at 9 am).**

3. **Assessment Studies: Cleveland, Cincinnati, Chapel Hill and Detroit.**

Current Methods of Mold Quantification

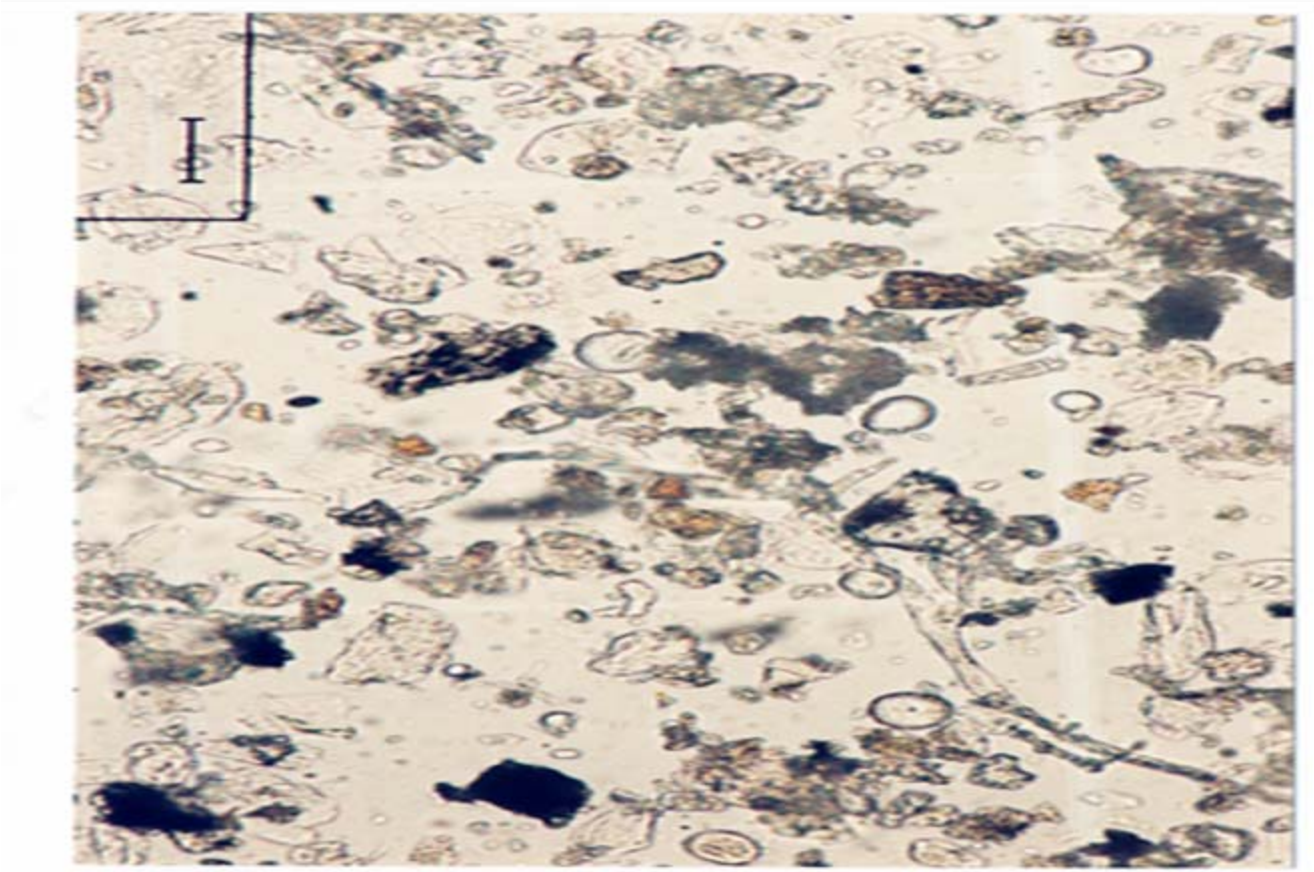
1. **Counting collected spores**

2. **Culturing spores on various media**

Counting Limitations

1. **Limited to short sampling times- 3 to 5 minutes.**
2. **No standard sampling protocol.**
3. **Molds can not be identified, for most species, just by spore.**
4. **No standardized interpretation of results.**

Real Sample



Culture Limitations

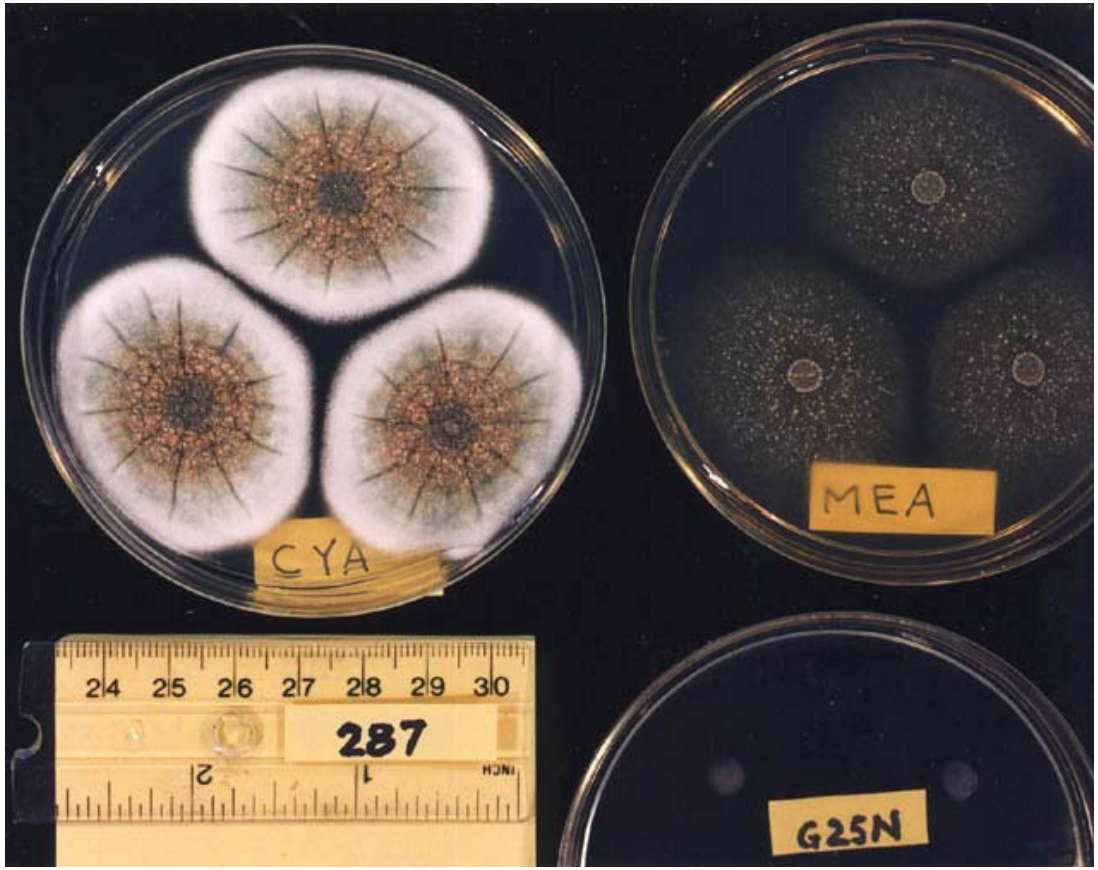
1. **Limited to short sampling times.**

2. **Different species grow on various media and at different rates.**

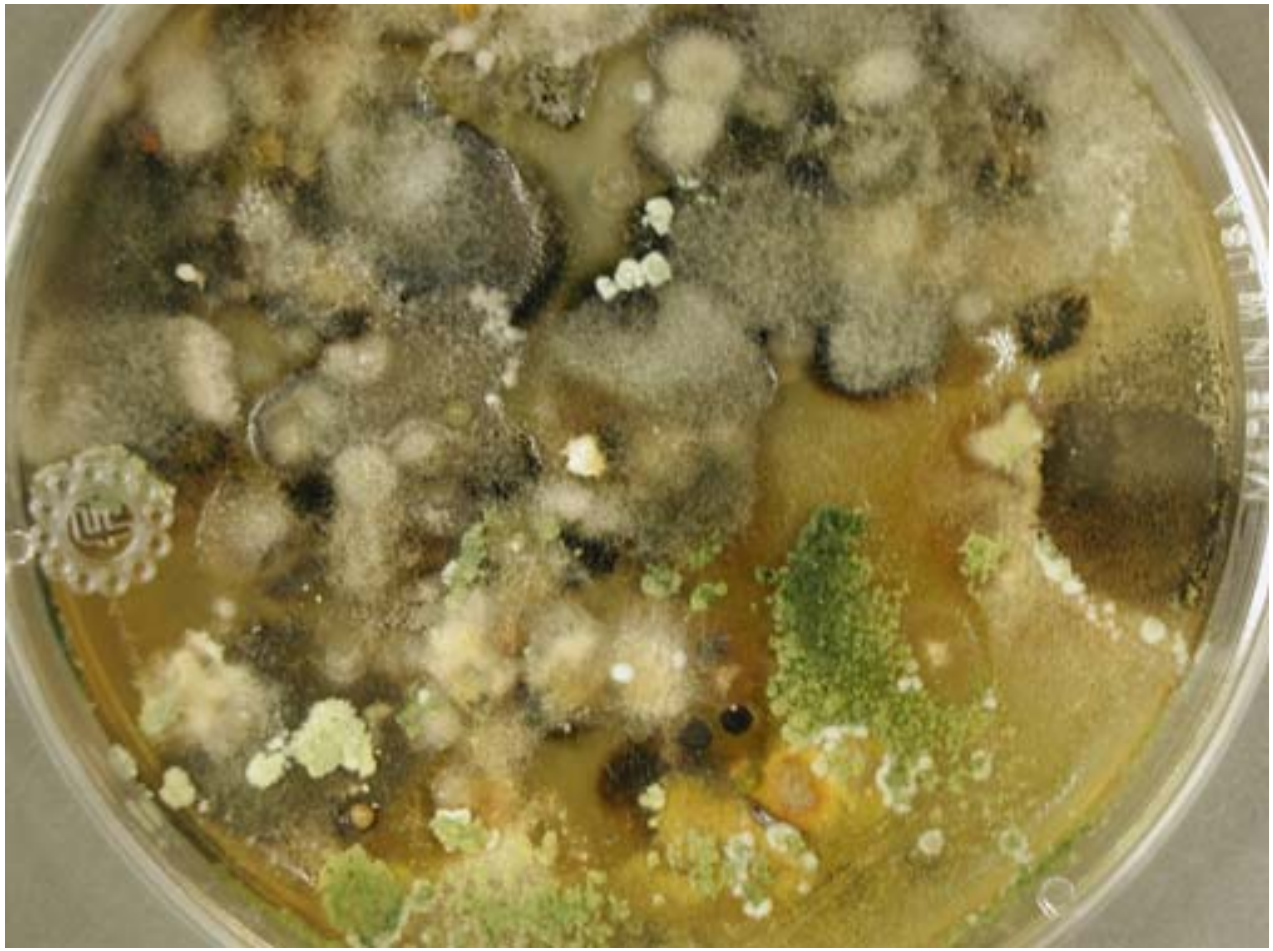
3. **Can take days to weeks to grow to a point of identification which then depends on analyst.**

4. **No standardized interpretation of results.**

Same mold on three media



Over crowding



Goal for Mold Analysis

- **Our Goal is not to measure all molds but indicator mold species.**
- **Dust used to represent the long-term exposure in the home.**

ASSESSMENT METHOD DEVELOPED

- **EPA developed a DNA-based method of mold identification and quantification (Patent 6,387,652).**
- **Creation of Mold Groups from 36 common species in homes**

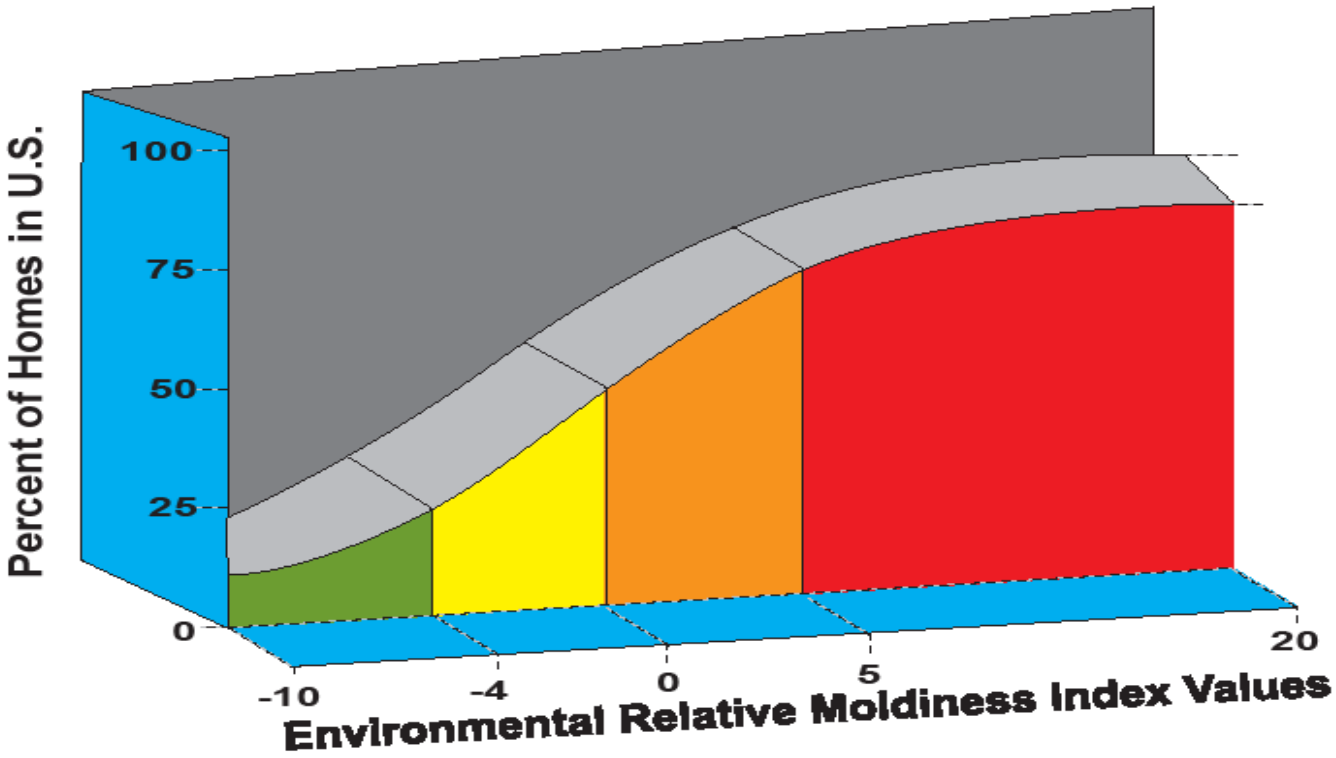
1. **Group 1 Molds, 26 species, are associated with water-damaged environments.**
2. **Group 2 Molds, 10 species, are universal.**

(Vesper SJ et al. Journal of Occupational and Environmental Medicine. 2004:46;596-601. and Meklin T et al. Journal of Environmental Monitoring. 2004:6;615-620.)

The Environmental Relative Moldiness Index-ERMI

- **National HUD American Healthy Home Survey, 1096 homes-standard sampling and DNA-based analysis.** **(More details in session on Wednesday at 9 am).**
- **Assemble ERMI values from lowest to highest and divide into quartiles to create the "Environmental Relative Moldiness Index (ERMI) scale (Published: Vesper et al. Journal of Occupational and Environmental Medicine 2007; 49:829-833.)**
- **The higher the ERMI value the greater is the likelihood of a moisture and mold problem.**

ERMI Scale


Percent of Homes in U.S.
100
75
50
25
0
-10
-4
0
5
20
Environmental Relative Moldiness Index Values

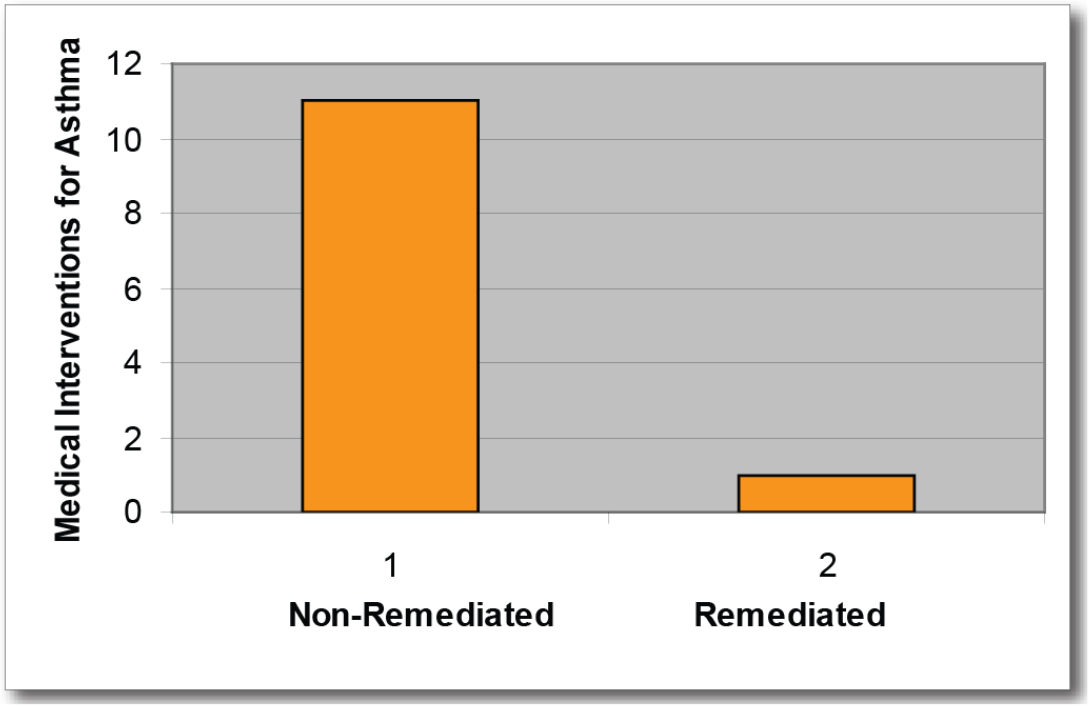
Assessment Studies

Cleveland (OH)-

- **Significantly higher ERMI values in asthmatic's homes.** **Vesper et al. Journal of Occupational and Environmental Medicine. 2006:48;852-858.**
- **Remediation of mold and moisture = less asthma.** **Kercsmar et al. Environmental Health Perspectives. 2006:114;1574-1580.**

Health outcomes of non-remediated versus remediated

Benefit: 10-Fold Reduction in Medical Intervention



Other Studies

Cincinnati (OH)-

- **Prospective study of infants; higher ERMI values predictive of development of wheeze.**

(Vesper et al. Journal of Exposure Science and Environmental Epidemiology. 2007:17;88-94.)

Other Studies

Chapel Hill (NC)-

- **Higher ERMI values in homes of children with asthma.** **(Vesper et al. Journal of Environmental Monitoring. 2007:9:826-830.)**

Detroit (MI)-

- **Significantly higher ERMI values in homes of severely asthmatic children.** **(Vesper et al. Science of the Total Environment. 2008:394:192-196.)**

Conclusions

1. **The ERMI is based on a standardized sampling and DNA analysis protocol. The human element is removed.**
2. **ERMI is not an instantaneous measurement but a "long term" measure of exposure.**
3. **ERMI is a mold index not a health index. Many more epidemiological studies needed.**

Future Studies in Conjunction with HUD

- In Progress- Boston, Kansas City, San Diego

Biological Contaminants Sampled from Surfaces in Flooded Homes of New Orleans

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Background

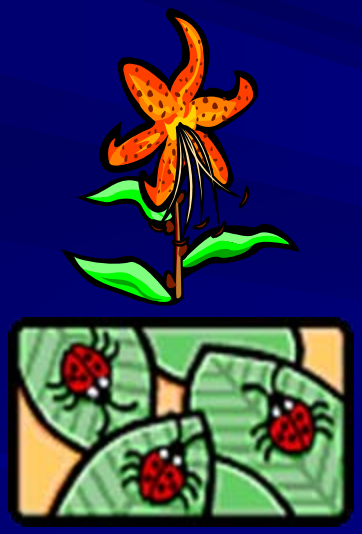
- The unprecedented disasters caused by Hurricanes Katrina and Rita in 2005 disrupted the infrastructures in New Orleans and created many environmental health challenges.
- There are significant public health concerns about exposure to airborne biocontaminants, and the associated respiratory health effects in the population re-occupying homes in New Orleans.
- The process of aerosolization of biocontaminants, such as airborne fungi, (1→3)-β-D-glucan, endotoxin, and dust mite allergens, from affected surfaces in homes is not sufficiently understood. This creates uncertainties in assessing exposure and establishing the exposure-health relationships.

A flood-affected home in New Orleans with fungal growth

Exposure Paradigm and Exposure Assessment

Aerosol Transport

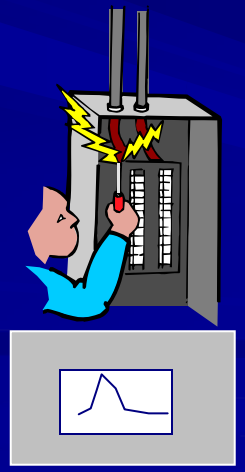
Exposure Assessment



Source

Surface Sampling (Scraping)

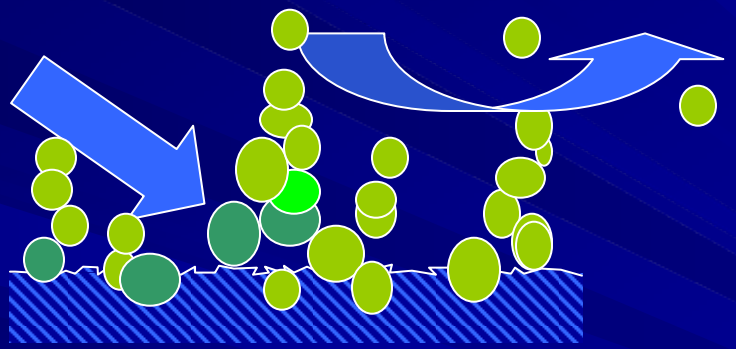
Aerosol Sampling



Respiratory health effects are caused by aerosol particles, e.g., by fungal spores aerosolized from moldy surfaces

Energy Input:
- Air flow
- Vibration

Airflow to entrain particles

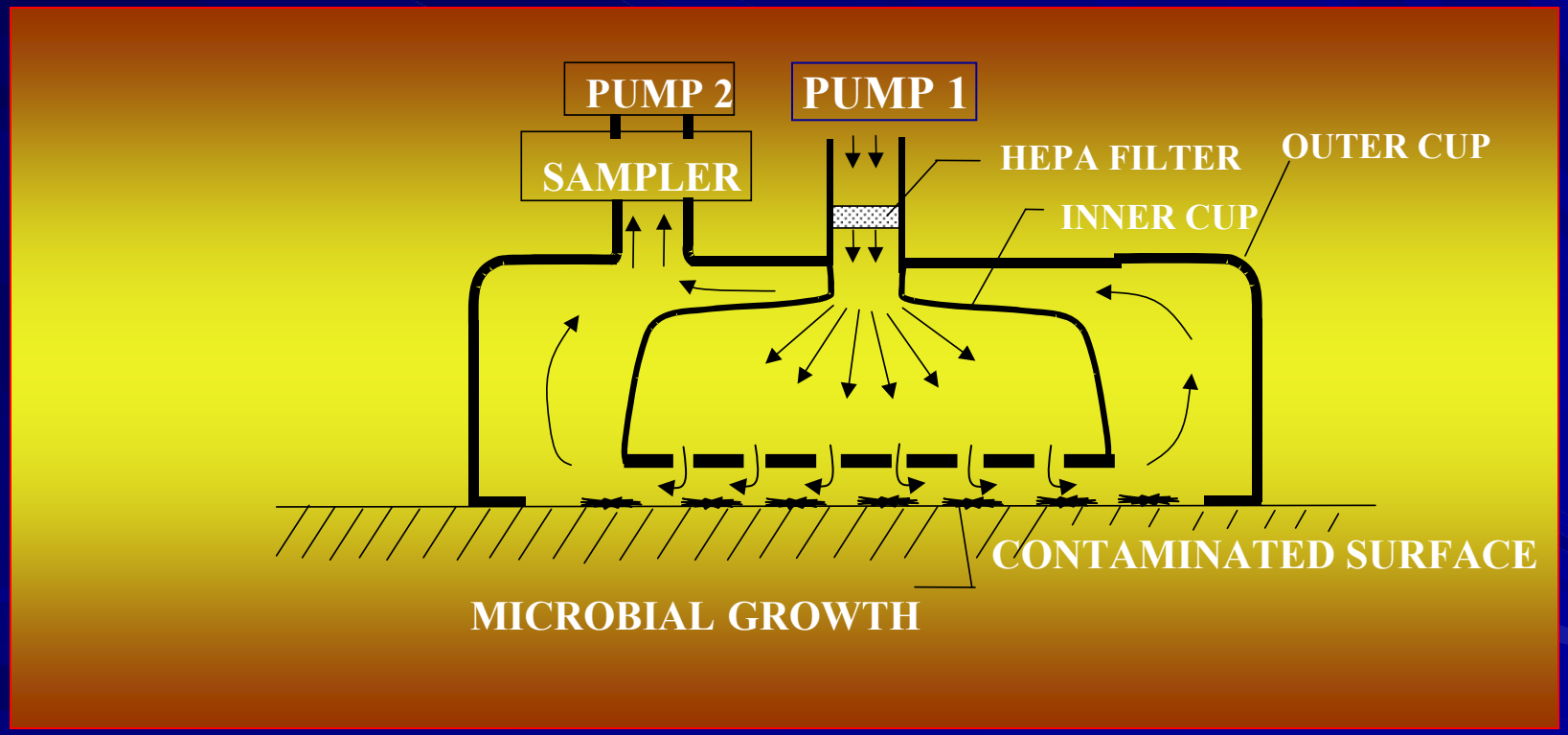


Overcome adhesion between spore and mycelium

Factors affecting aerosolization

- Air velocity
- Turbulence intensity
- Vibration
- Microbial culture
- Growth material, age
- Agglomeration
- Growth conditions (RH, T)

Fungal Spore Source Strength Tester (FSSST)



Fungi

- Heterotrophic single-celled, multinucleated, or multicellular organisms
- Absorb food in solution directly through their cell walls
- Mostly reproduce through spores
- Fungi are associated with allergic respiratory disease

(1→3)-β-D Glucan

- Major polysaccharide component of fungal cell wall
- Contain glucose as structural components
- (1→3)-β-D Glucans are chains of D-glucose molecules
- Glucans are associated with dry cough, phlegmy cough, hoarseness, atopy

Endotoxin

- A component of the outer membrane of Gram-negative bacteria
- Composed of lipopolysaccharides as a main constituent
- Inhaled endotoxin can induce airway inflammation and dysfunction

Dust mite allergens

- One of the most ubiquitous indoor allergens known
- Sensitization to dust mite allergens is a very strong risk factor for asthma

Laboratory Study

Specific Aims

1. To determine aerosolization of culturable and total fungi, (1→3)-β-D glucan, endotoxin, and dust mite allergens (Der f1 and Der p1) from eight different flood-affected materials.

2. To investigate aerosolization of (1→3)-β-D glucan and endotoxin using a particle size selective sampling method.

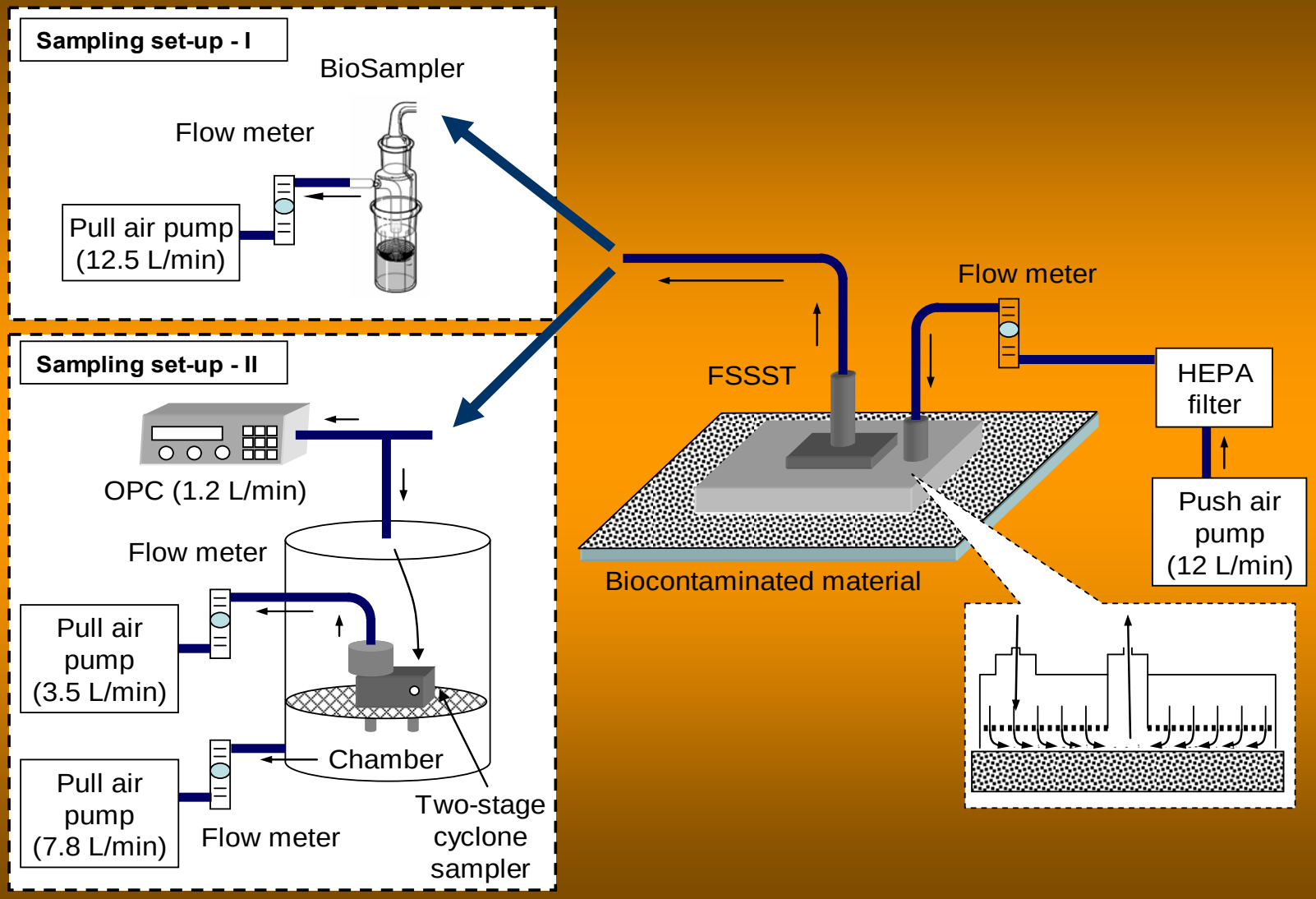
3. To compare the levels of biocontaminants measured with the FSSST and using a vacuum cleaner, respectively.

4. To estimate the respiratory deposition of biocontaminants aerosolized from surfaces (conservative estimate).

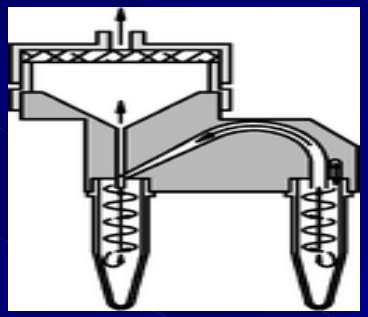
Methods

- Six floor materials: linoleum, small rug, water-affected small rug, area rug, thick carpet, and thin carpet
- Two bedding materials: pillow and mattress
- Six flood-affected homes in New Orleans
- Sampling time for aerosolization tests = 5, 15, and 35 min (cumulative)
- Test instruments for aerosolization test:
  - FSSST + BioSampler
  - FSSST + NIOSH 2-stage cyclone (glucan and endotoxin)
- Dust collection:
  - Collection area on surfaces: >0.25 m^2
  - Vacuum cleaner; collection time = 5 min
  - Standardized HUD dust sampling protocol (HUD, 2004)
- Respiratory deposition analysis: LUDEP ICRP lung deposition model

Sampling Set-up



Size-selective Sampling with the NIOSH 2-stage Cyclone Sampler



- Collection stages:
  - 37-mm filter holder with 0.8 µm polycarbonate filter
  - Two screw-top 1.5 mL micro-centrifuge tubes
- Air flow rate = 3.5 L/min
- Particle size fractions:
  - <1.0 (on filter)
  - 1.0 – 1.8 (in tubes)
  - >1.8 µm (in tubes)
- Sample analysis: for (1→3)-β-D glucan and endotoxin (5 materials)

Analysis of Biocontaminants

Fungi

- Colonies of culturable fungi were identified to genus, based on gross morphology and spore-forming structures
- For total fungi, samples were prepared by filtering extract through mixed cellulose ester filter, clearing by acetone vapor; spores were identified using a phase contrast light microscope

(1→3)-β-D Glucan

- Kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay

Endotoxin

- Endotoxin-specific kinetic chromogenic LAL-assay

Dust mite allergens

- Two-site monoclonal antibody sandwich ELISAs for Der p1 and Der f1

Biocontaminants aerosolized from floor and bedding materials tested with FSSST (cumulative levels over 35 min; Mean ± SD)

Material	Culturable fungi; $\times 10^3$ CFU/m^2	Total fungi*, $\times 10^3$ spores/m^2	β-(1→3)-D glucan, $\times 10^3$ ng/m^2	Endotoxin, $\times 10^3$ EU/m^2
Linoleum	<LOD	475	18.25 ± 23.57	59.15 ± 70.45
Small rug	<LOD	259	28.54 ± 32.94	93.09 ± 141.52
Small rug (moist)	8 ± 8	615	21.56 ± 26.45	9.86 ± 4.16
Area rug	25 ± 22	1,145	12.18 ± 7.40	37.37 ± 30.28
Thick carpet	8 ± 8	207	3.43 ± 1.90	0.70 ± 0.58
Thin carpet	11 ± 19	324	2.00 ± 2.26	0.81 ± 0.31
Pillow	<LOD	710	13.12 ± 8.90	8.39 ± 4.91
Mattress	259 ± 353	1,598	2.01 ± 0.64	11.52 ± 4.81

* n = 1 for total fungi; n = 3 for other contaminants; LODs: Culturable fungi: 8,264 CFU/m^2; Total fungi: 5,587 spores/m^2; (1→3)-β-D-glucan: 8.39 ng/m^2; Endotoxin: 86.76 EU/m^2

Aerosolized Culturable Fungi

- <LOD (~10^4 CFU/m^2) - ~ 2.59×10^5 CFU/m^2
- Prevalent genera: *Aspergillus, Penicillium*, and *Cladosporium*, and non-sporulating colonies
- Highest level: contaminated mattress
- Culturability range: 3% (area rug) - 41% (mattress)

Aerosolized Total Fungi

- 2.07×10^5 - 1.6×10^6 spores/m^2

- Prevalent spore types (12 genera/classes):
  - *Aspergillus/Penicillium*
  - *Cladosporium*
  - *Alternaria*
  - *Stachybotrys*
  - *Chaetomium*
  - Ascospores

Strong association with allergic asthma

Strong association with allergic asthma

Increased risk of building-associated pulmonary disease

Aerosolized and Dust-borne (1→3)-β-D glucan

- Aerosolized glucan: (2.0 – 29.0) $\times 10^3$ ng/m^2
- Dust-borne glucan: (2.0 – 2,006) $\times 10^3$ µg/m^2

Glucan level in dusts from 574 Cincinnati homes: 18.4 µg/m^2

Aerosolized and Dust-borne Endotoxin

- Aerosolized endotoxin: (7.0 – 930.0) $\times 10^2$ EU/m^2
- Dust-borne endotoxin: (8.2 – 61.4) $\times 10^5$ EU/m^2

Endotoxin levels in dusts from New York : 3,892 EU/m^2; from Cincinnati : 24 EU/m^2

Significant correlation between aerosolized glucan and endotoxin ($r = 0.425$; $p<0.001$)

Future research:

- Role of immuno-modulators (glucan and endotoxin) in public health
- Role of synergistic interactions between glucan and endotoxin in respiratory disease

Aerosolized and Dust-borne Dust Mite Allergens

- Dust mite allergens:
  below the lowest LOD (0.025 µg/g) in most samples
- Detectable Der f1:
  dust samples from pillow: 0.38 µg/g
  small rug: 0.4 µg/g
- Detectable Der p1:
  dusts from small rug: 1.3 µg/g

Dust mites rarely infested flood-affected materials

Dust mite allergens were altered in some way that affected measurement results

Dust mite allergens >LOD occurred in samples with low CFU; this suggests antagonistic ecological relationship

Choosing the Optimal Sampling Period for Testing Aersolization

- **Total fungi:**
  - **Difference between 5 and 35 min: significant ($P < 0.05$)**
  - **Difference between 15 min and 35 min: not significant ($P > 0.05$) (ANOVA and Scheffe post hoc tests)**
- **Glucan and endotoxin:**
  - **Difference between 5, 15, and 35 min: not significant in most samples ($P > 0.05$ in ANOVA and Kruskal Wallis tests)**

Short sampling periods of 5 min for endotoxin and 15 min for fungi and (1→3)-β-D glucan were found to be sufficient

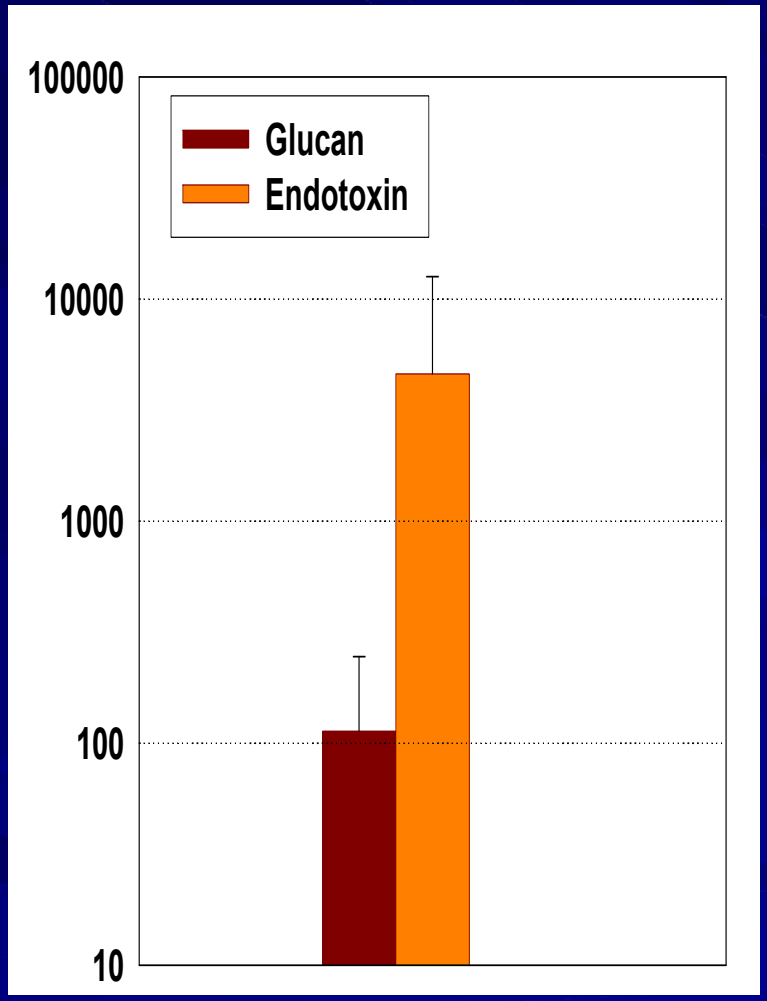
Particle Size Selective Analyses of Glucan and Endotoxin

No statistically significant differences between concentration levels in 3 size fractions (P>0.05; ANOVA, Kruskal Wallis tests, and Bonferroni post-hoc test)

Smaller aerosolized particles (<1.0 µm) have glucan and endotoxin levels comparable to larger (>1.8 µm) particles

Finer particles can be inhaled deeper into airways raising additional exposure concerns

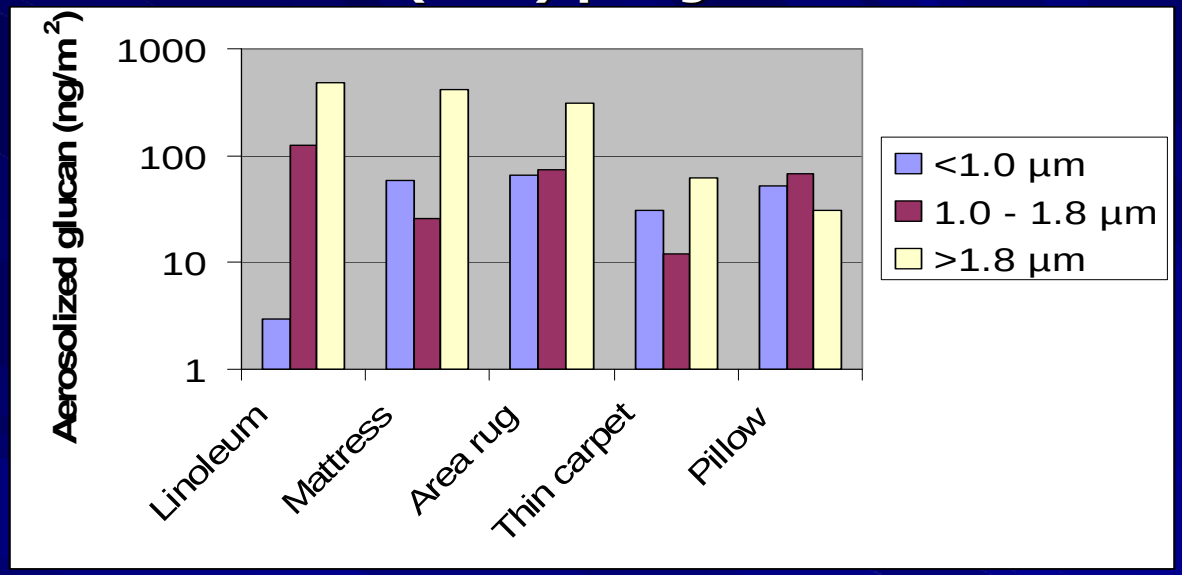
Glucan and Endotoxin Levels: Vacuum Cleaner versus FSSST



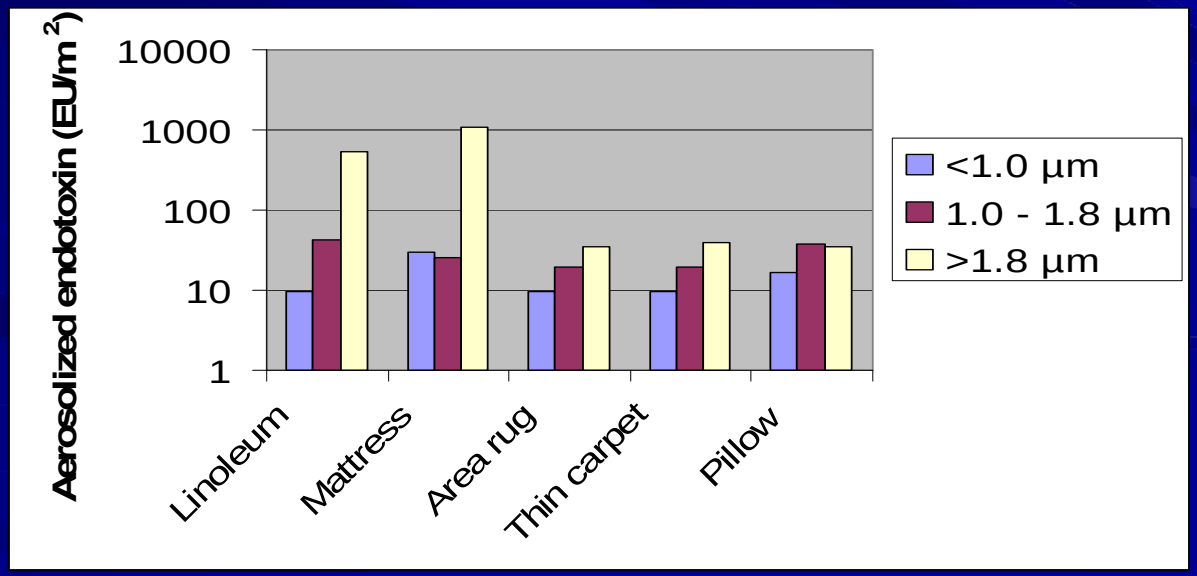
Vacuum samples may considerably overestimate the inhalation exposure risks of aerosolizable surface biocontaminants

Total Respiratory Deposition

(1→3)-β-D glucan



Endotoxin



Field Study

Specific Aims

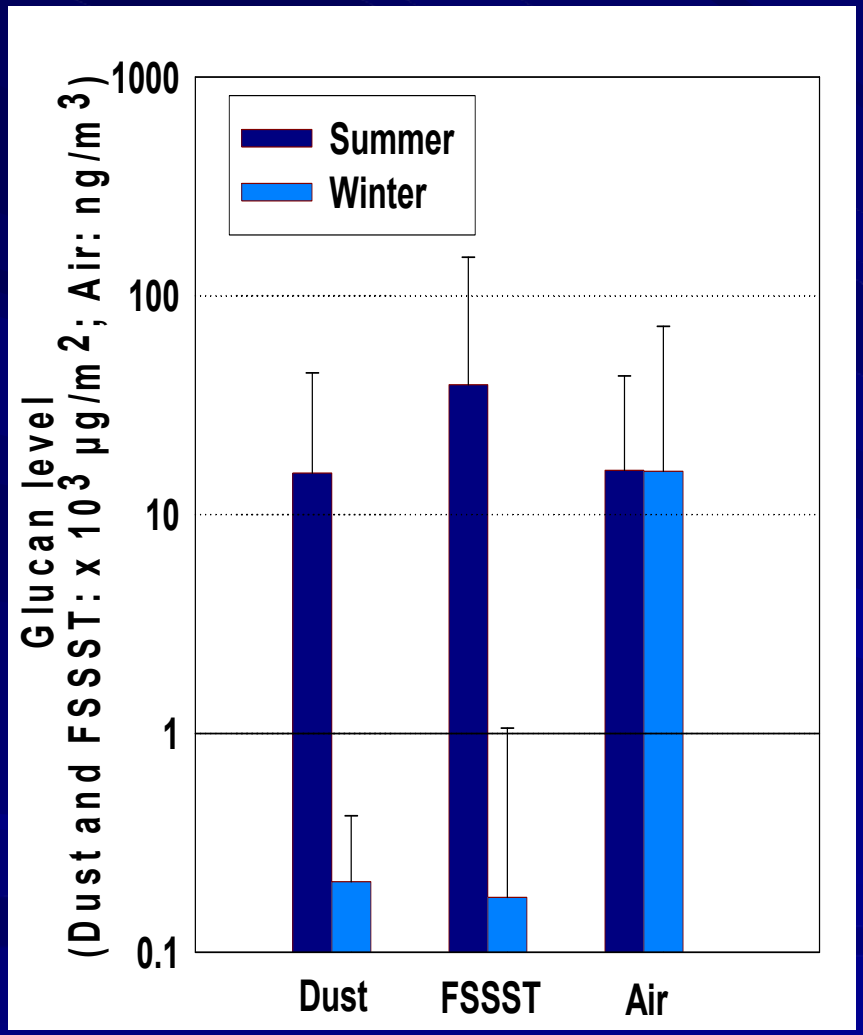
Main aim:

Determine the levels of total fungi, (1→3)-β-D glucan, endotoxin, and dust mite allergens (Der f1 and Der p1) inside 30 selected flood-affected homes

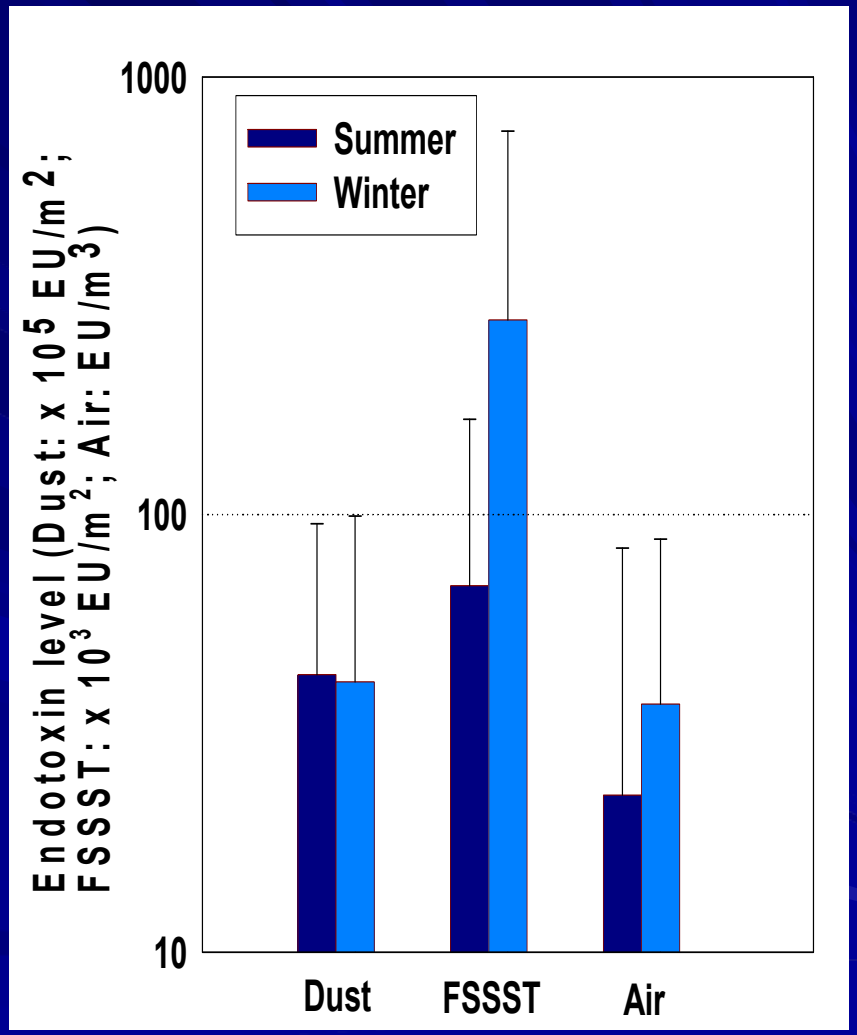
Additional aim:

Implement the community outreach and education program (health effects of molds and moisture damage in homes)

Biocontaminant Levels: Summer vs. Winter



(1→3)-β-D Glucan



Endotoxin

Findings from the Field Study (as of Today)

- **(1→3)-β-D-glucan: significantly decreased from summer to winter (paired t-test: p = 0.004 for dust samples, p = 0.020 for FSSST samples, and p = 0.032 for air samples).**

- **Endotoxin: no statistically significant difference between seasons (paired t-test: P = 0.385); moreover, the endotoxin levels in FSSST and air samples have significantly increased from summer to winter (p = 0.010 and 0.009).**

- **Seasonal data are currently being interpreted.**

Glucan in dusts: ~ 6-fold higher than in Cincinnati homes

Endotoxin: 10^2 to 10^4 times higher than in Cincinnati and New York homes

Community Outreach Activities (Summary)

- Recovery Day Celebration
  - Attendance: ~ 75 people
  - Dr. Faye Grimsley/staff informed attendees about the project
  - Community interaction helped identify homes for the study
- Lower Ninth Ward Family Health Fair
  - Attendance: ~ 200 people
  - Pamphlets and packets on mold cleanup and prevention distributed
- Dillard University's 2008 CDC Housing Fair
  - Attendees: representatives from more than 50 organizations
  - Dr. Faye Grimsley/staff distributed info on mold remediation

Photographs from the Lower Ninth Ward Health Fair Event

Dr. Grimsley distributes materials

Participants

- Sponsor: "Links"
- Featured 20 organizations
- <u>Major attendees</u>:
  - LSU Agricultural Center
  - City of New Orleans Health Department
  - LaCHIP for uninsured children
  - City of New Orleans Office of Healthy Homes & Lead Hazard Control
  - Walgreens

Photographs from the Dillard University's CDC 2008 Housing Fair

- **Sponsor: Dillard University's Dept. of Community Development Corporation**

- **<u>Major attendees</u>:**
  - **Deep South Center for Environmental Justice**
  - **Global Green New Orleans**
  - **Federal Housing Authority (FHA)**
  - **several banks**
  - **many private contractors**

Acknowledgements

Participating families, sponsors and community organizations

$$$

US Department of HUD, Grant No. OHLHH 0155-06

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OPTIMIZING EXPOSURE ASSESSMENT METHODOLOGY FOR INVESTIGATING MOLDY BUILDINGS

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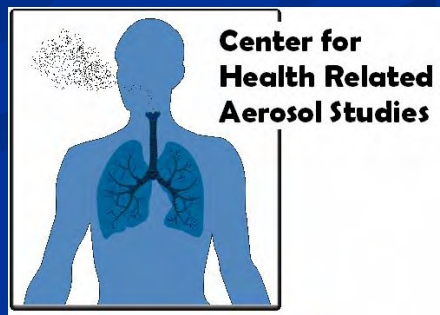
UNIVERSITY OF Cincinnati



OUTLINE

- Background
- Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS)
- Currently ongoing HUD-funded study in CCAAPS sub-cohort

"When the waters subside, the problem is going to be mold"

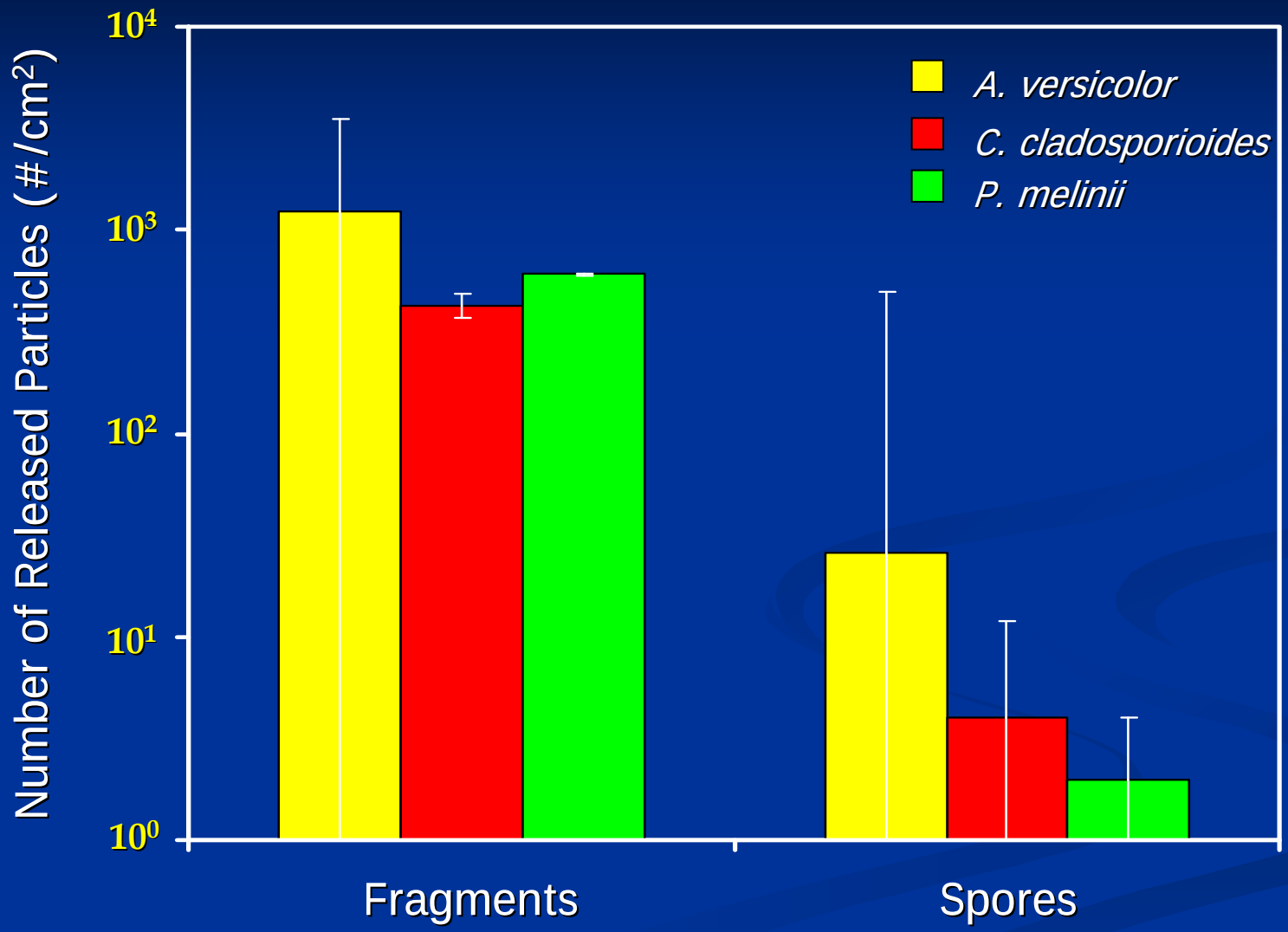
BACKGROUND

- Visible mold and moisture in buildings is associated with occupant respiratory diseases and symptoms.
- Much less in known about association between mold exposure and development of asthma.
- Jaakkola et al. (2005): Parental report of moldy odor in home was associated with doctor-diagnosed asthma in children.
- Pekkanen et al. (2007): Moisture damage and visible mold in main living quarters were associated with increased risk of doctor-diagnosed asthma in children.

BACKGROUND

- Previous studies have not been able to establish clear association between airborne fungal spore concentrations and adverse respiratory effects.
- Interaction with other air pollutants?
- New methods are available to assess mold exposure, for example
  - PCR
  - (1-3)-β-D-glucan
  - Fungal fragments

RELEASE OF FUNGAL PROPAGULES FROM MOLDY SURFACE



Gorny et al., 2002

SIGNIFICANCE OF SMALL PARTICLE SIZE

- Long residence time in the air
- Different behavior in control devices
- Deposition deep into the lung
- Slow clearance rate
- Large surface area →
- Increased bioavailability

PARTICLE SETTLING IN STILL AIR

Time to settle 5 feet (1.5 m) by gravitation

0.5 µm
Bacteria, fragments

41 hours

3 µm
Fungal spores

1.5 hours

5 µm
Fungal spores, Aggregates

33 minutes

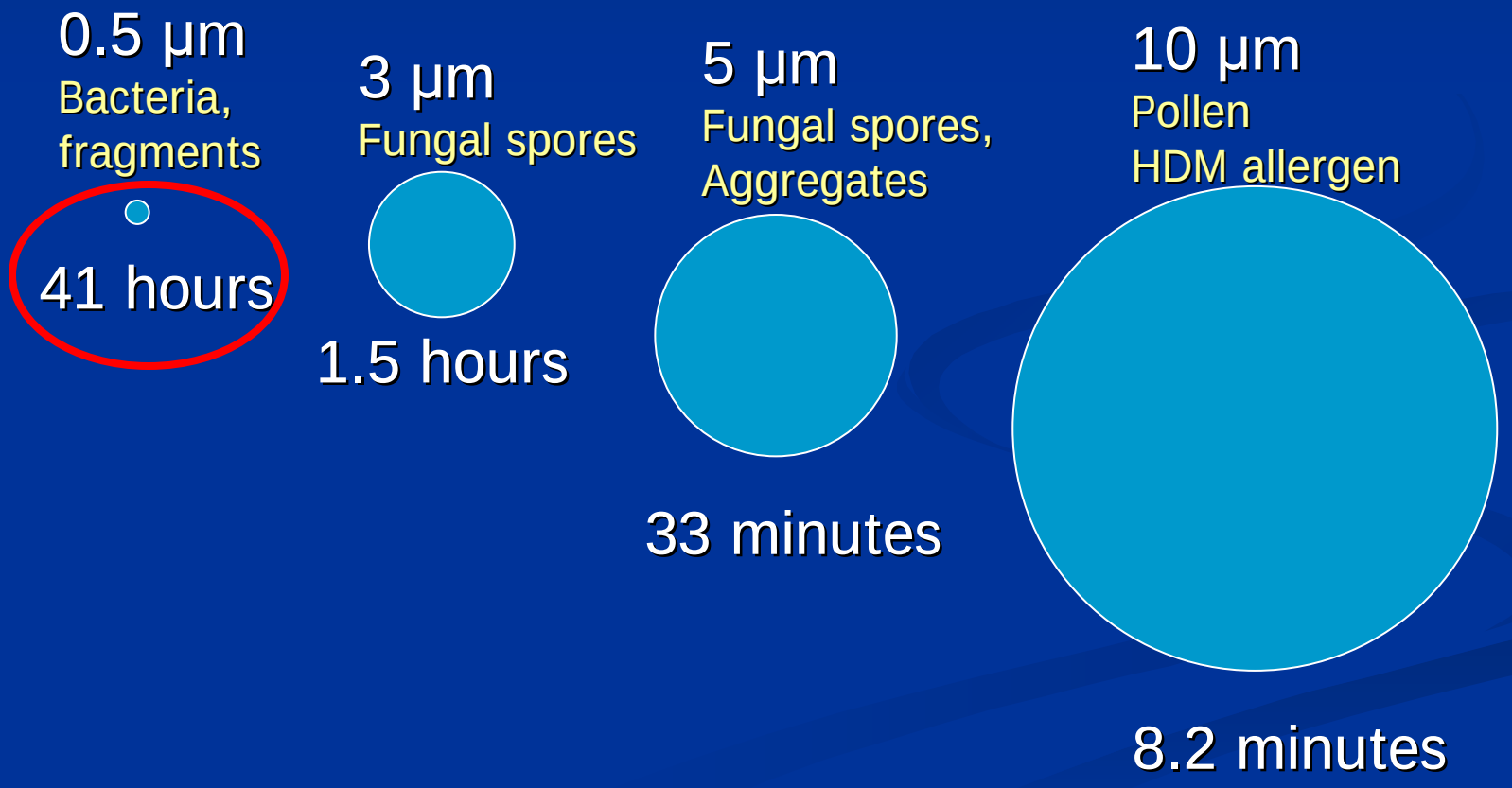
10 µm
Pollen
HDM allergen

8.2 minutes

Courtesy of Dr. Baron, NIOSH

PARTICLE SETTLING IN STILL AIR

Time to settle 5 feet (1.5 m) by gravitation

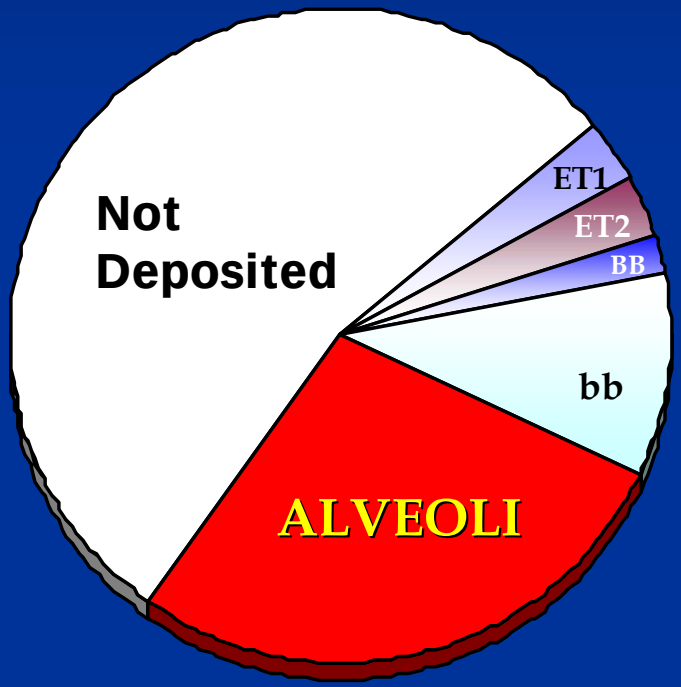


Courtesy of Dr. Baron, NIOSH

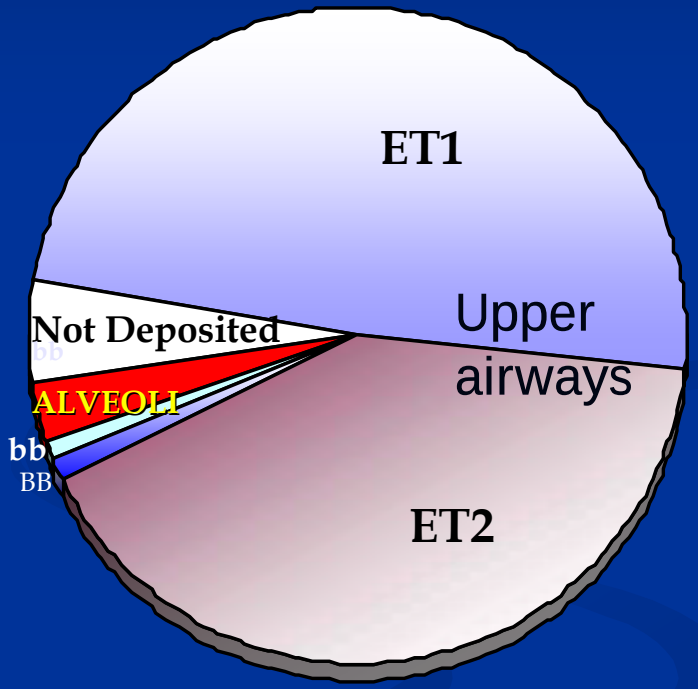
Respiratory Deposition of *S. chartarum*

3-Month-Old Infant

Fragments



Spores



ET1 = Anterior nasal region (ET1)
BB = Bronchial region
AL = Alveolar interstitial region

ET2 = Main extrathoracic region
bb = Bronchiolar region
ND = Not deposited

Cho et al., 2005

WHY ARE CHILDREN PARTICULARLY SUSCEPTIBLE TO AIR POLLUTION?

- Lungs not developed at birth
  - Alveolar and epithelium development
  - Development of respiratory bronchioles
- Immature respiratory defense
- Greater exposure than adults
  - Mouth breathing
  - Larger lung surface area per unit body weight
  - Breathe greater air per unit body weight

BIRTH COHORT
Cincinnati Childhood Allergy and Air Pollution Study - CCAAPS

Purpose

- Determine if children who are exposed to diesel exhaust particles (DEP) are at increased risk for developing allergic diseases and asthma.
- Investigate interaction between DEP and aeroallergens.

CCAAPS - RECRUITMENT

- **7352 families with newborn children were contacted**
- 758 infants were recruited
- Recruitment criteria
  - Location of home (close/far from highway)
  - At least one atopic parent

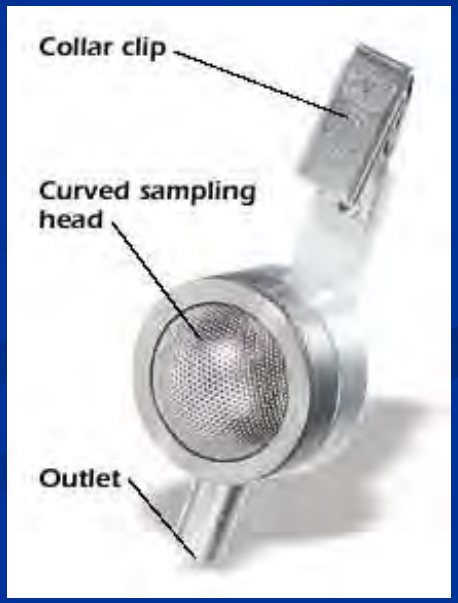
CCAAPS CLINICAL METHODS

Annual study visits ages 1-4

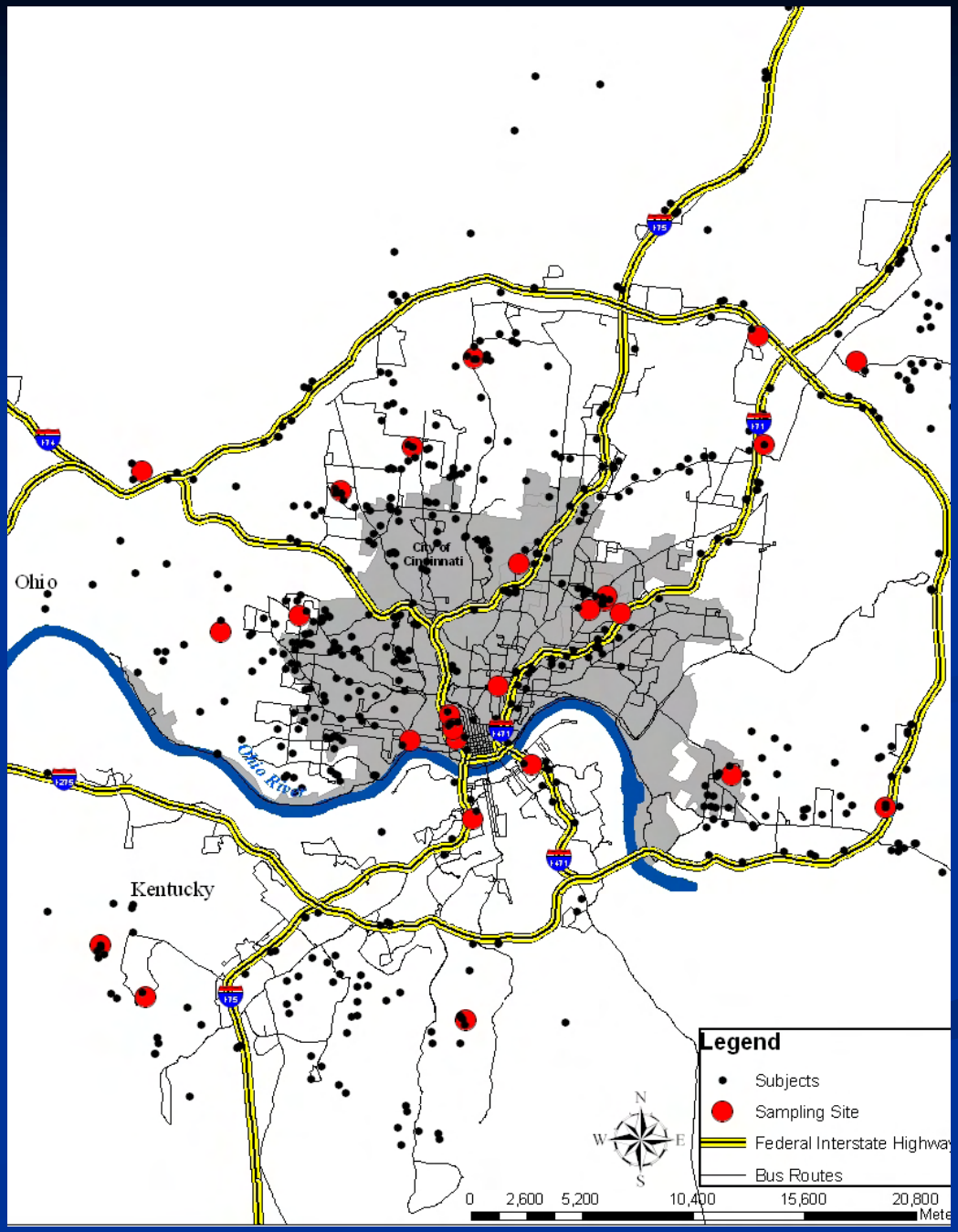
- Skin Prick Test
- Symptom questionnaire
- Physical examination
- Hair sample
- DNA sample

CCAAPS OUTDOOR AIR SAMPLING METHODS

- 24 sampling sites for $PM_{2.5}$
  - Elemental analysis by XRF
  - Elemental / Organic carbon (EC/OC)
  - **ECAT-- <u>E</u>lemental <u>C</u>arbon <u>A</u>ttributable to <u>T</u>raffic**
- 2 sampling sites for pollen and mold
  - Inhalable particles using Button sampler



CCAAPS AMBIENT AIR SAMPLING NETWORK



CCAAPS INDOOR EXPOSURE ASSESSMENT AT AGE 1

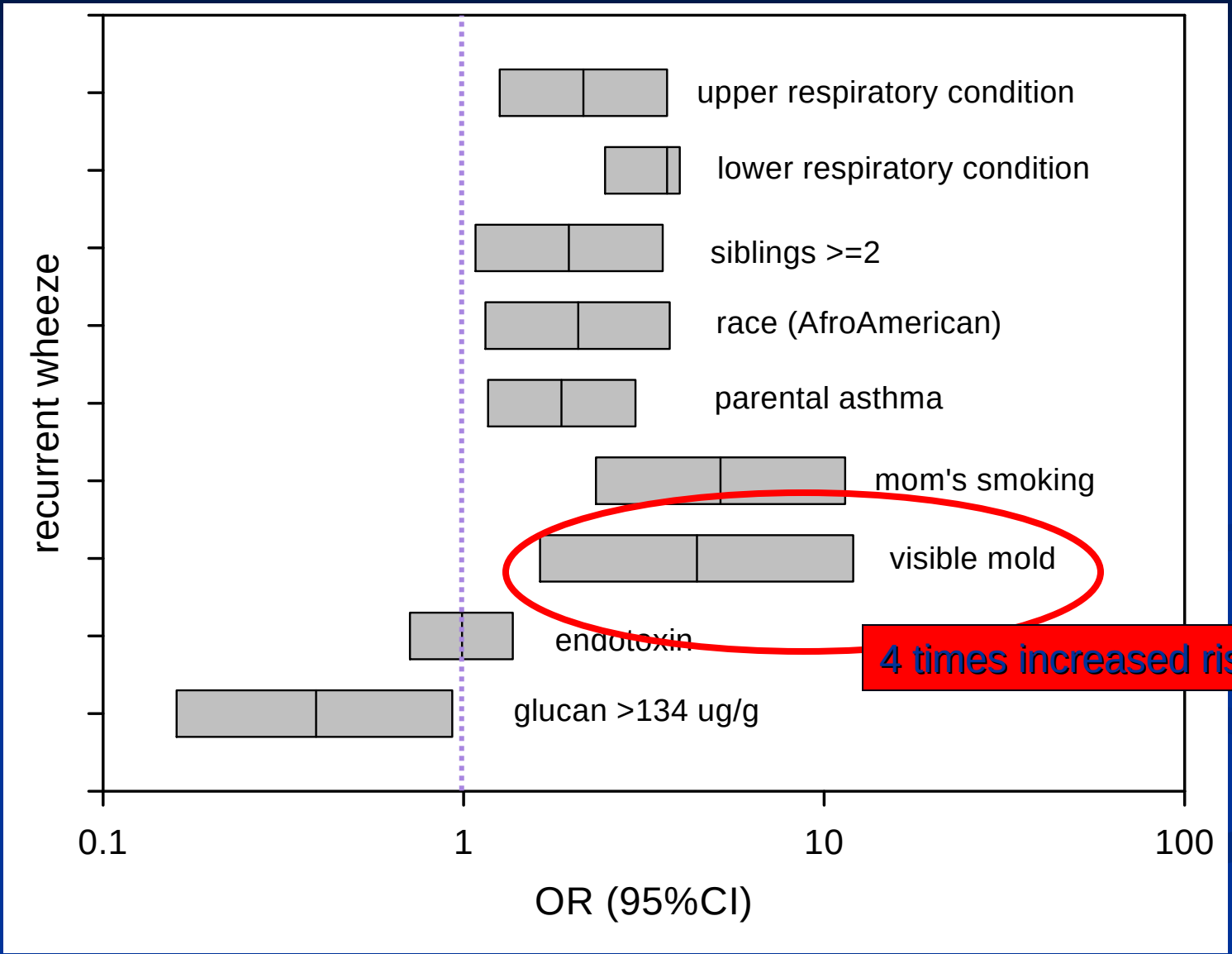
- Questionnaire and walk through for home characteristics
- Measure visible mold and moisture damage
- Collect dust sample from child's primary activity room
  - (1-3)-β-D-glucan
  - endotoxin
  - cat, dog, dust mite, cockroach

MOLD CATEGORIES

- **NO MOLD**:
  - No water damage
  - No visible mold
  - No moldy odor
  - No water/mold damage history
- **HIGH** (must have visible mold):
  - Mold alone in the entire room ≥ 0.2 m^2 **OR**
  - Combined area of visible mold + water damage on the same surface ≥ 0.2 m^2
- **LOW**
  - Between categories None and High

Meklin et al., 2004

RECURRENT WHEEZING AT AGE ONE VS. EXPOSURE



Data based on: Iossifova et al., Allergy 62:504-513, 2007

IS ASTHMA DEVELOPMENT ASSOCIATED WITH MOLD EXPOSURE?

IF YES, WHICH METHOD IS BEST PREDICTOR

HUD-FUNDED STUDY - OBJECTIVE

- Identify optimum method to predict development and severity of allergic diseases associated with mold exposure.
- Two new concepts will be tested:
  - Relative moldiness index (RMI)
  - Fungal fragment sampling in combination with (1-3)-β-D-glucan assay and immunochemical assay.
- Study will utilize existing CCAAPS birth-cohort
- Association between early vs. later mold exposure with asthma development

CCAAPS CLINICAL VISIT AT AGE 6-7

(FUNDED BY NIEHS)

- Identical assessments as 1-4
  - SPT, symptom questionnaire, clinical exam
- Pulmonary function testing
- Bronchodilator
  - Methacholine challenge on subset
- Exhaled Nitric Oxide (eNO)
- Serum sample

INDOOR EXPOSURE ASSESSMENT

Age 6-7 (HUD-funded study)

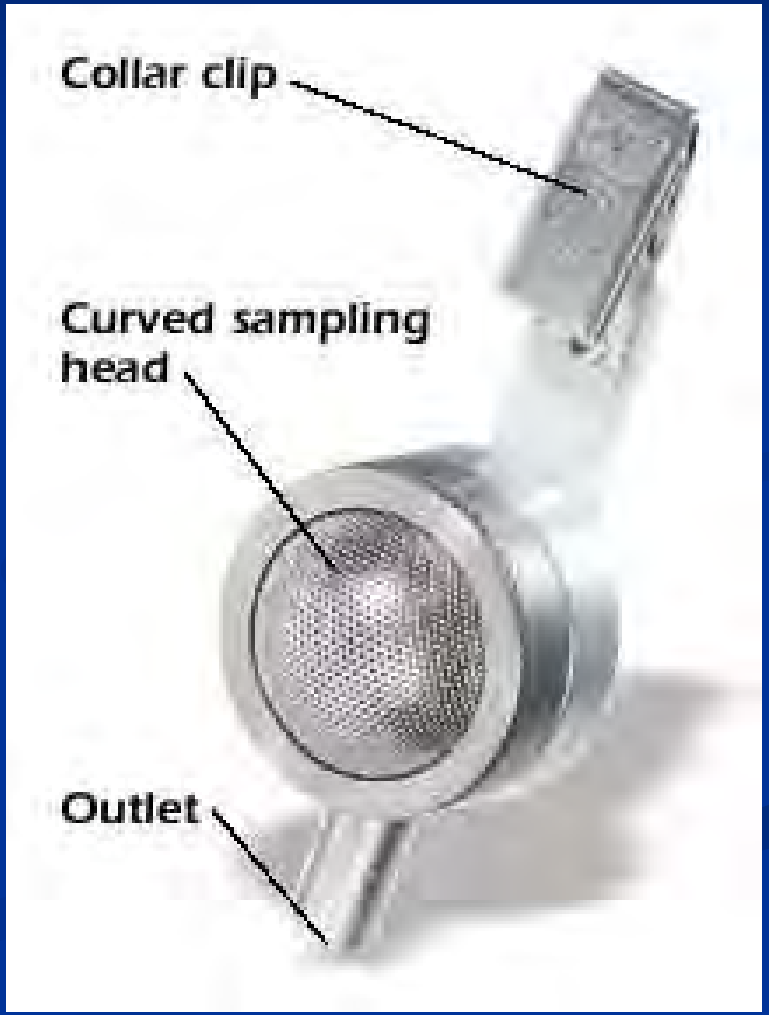
- Follow-up visit on 200 homes with high/no mold exposure
- Questionnaire and walk through for home characteristics
- Measure visible mold and water damage
- Collect dust sample
- Collect 24-hour air sample

DUST SAMPLE ANALYSIS

- (1-3)-β-D-glucan
- Endotoxin
- Cat, dog, dust mite, cockroach, mouse (Roda)
- PCR for 30 mold species, relative moldiness index (Dr. Vesper)

NON-SIZE-SELECTIVE AIR SAMPLING

- Button Sampler
- Microscopic counting of fungal spores
- (1-3)-β-D-glucan assay



SIZE-SELECTIVE AIR SAMPLING

- NIOSH 2-stage sampler for fungal fragments
- (1-3)-β-D-glucan assay
- *Pen/Asp* fungal immuno-assay (Dr. Schmechel)

Lindsley et al., JEM 8:1136, 2006

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