# Creating a Healthy Indoor Microbiome:
# From Mold Control to Virus Surveillance

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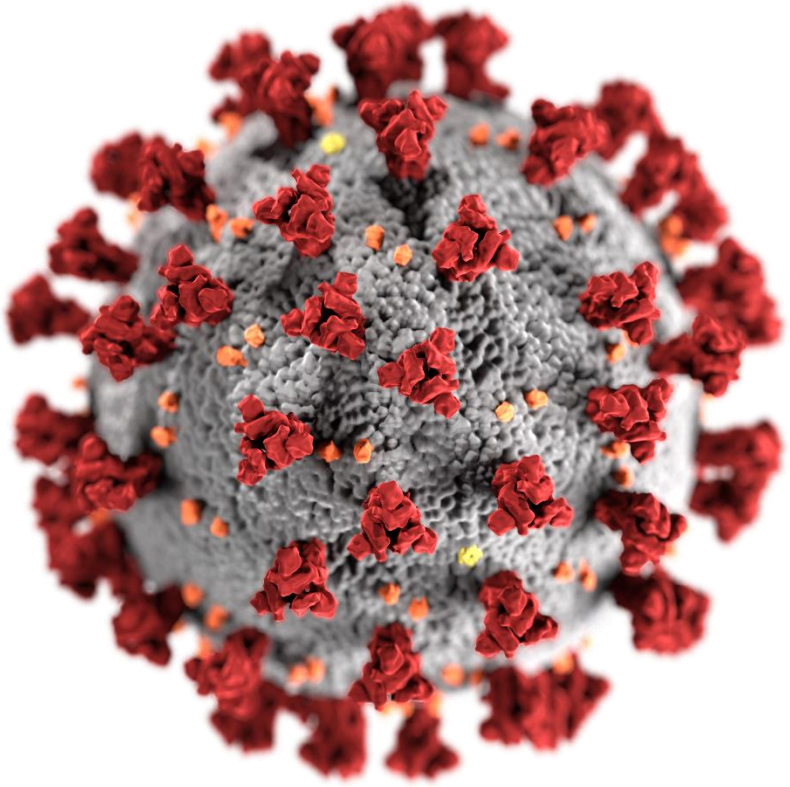
@KarenCDannemill



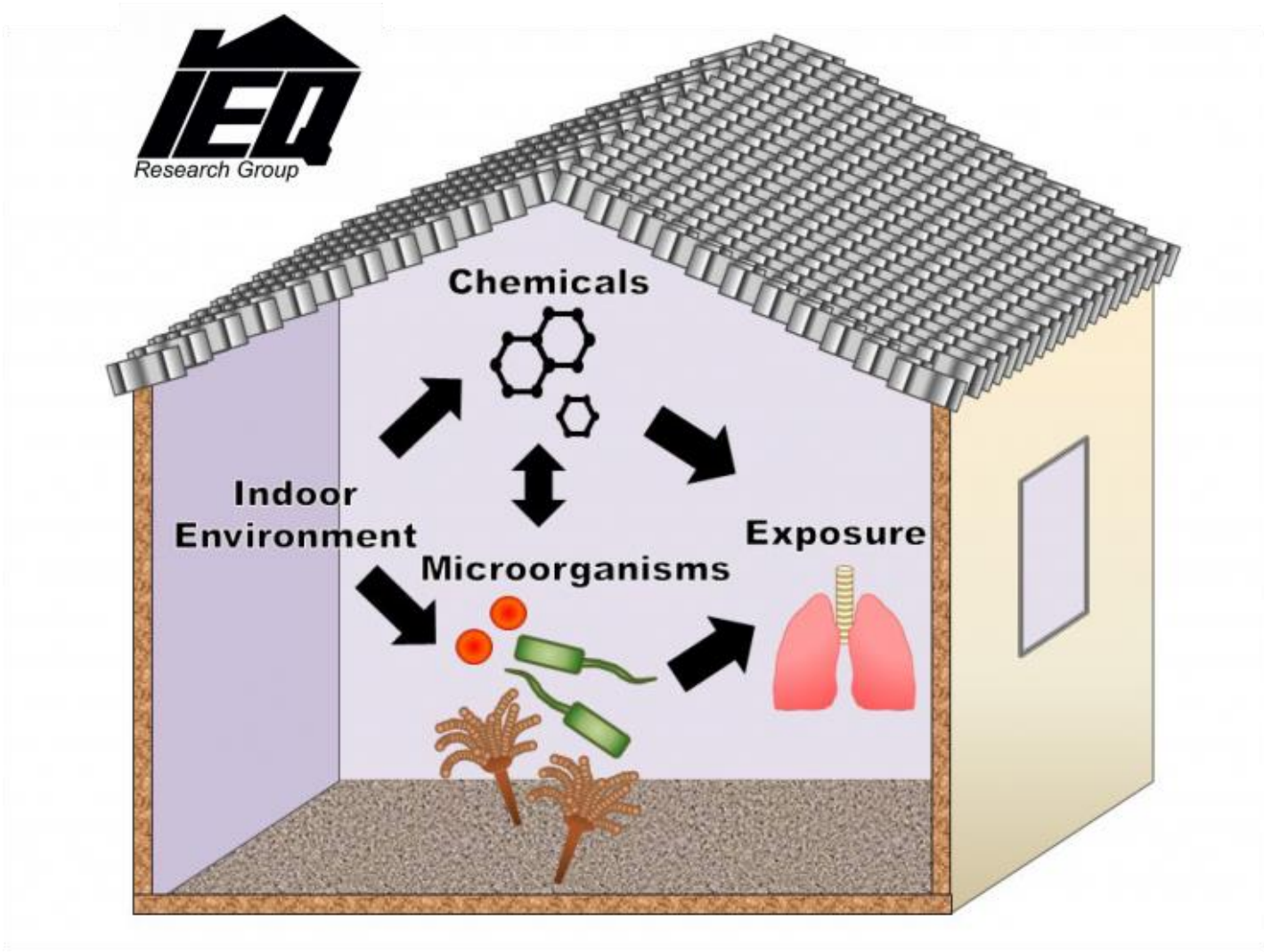
AEE – Northern Ohio Chapter

October 29, 2021

THE OHIO STATE UNIVERSITY

Image: CDC PHIL

# Indoor Environmental Quality



# Today, exposure to mold in homes costs $22.4 billion per year

Artwork by Daniele Del Nero

16% of cost associated with:

- Allergic Rhinitis
- Acute Bronchitis
- Asthma



Mudarri, D., J Environ Public Health. 2016; 2016: 2386596.

# Understanding the indoor environment can lead to new monitoring techniques

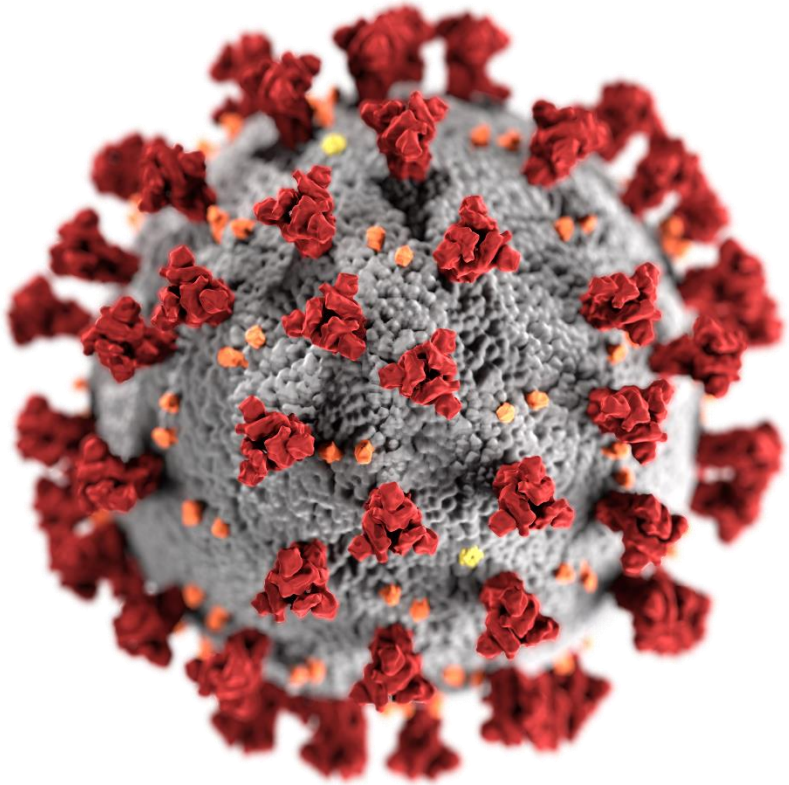
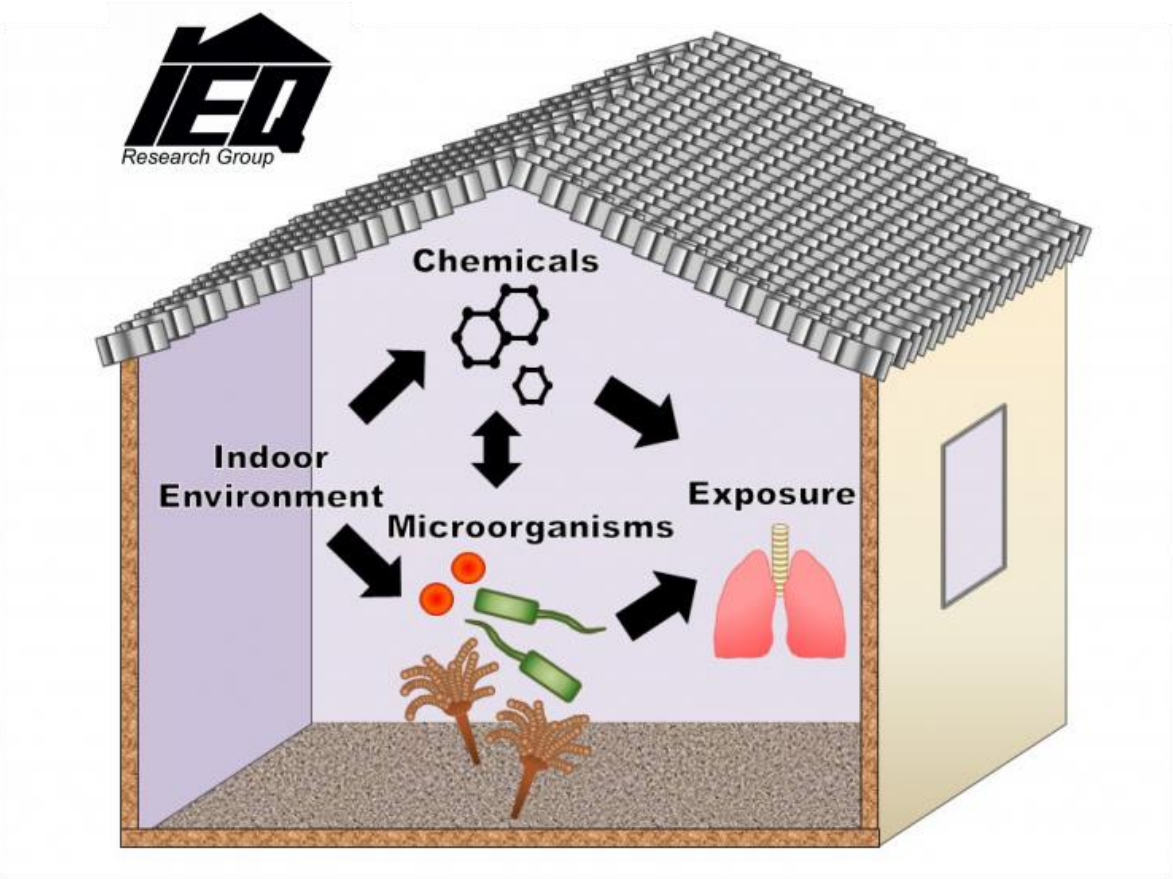


Image: CDC PHIL

# Talk Outline
# *Healthy Indoor Microbiome*




**Part 1:** Moisture and carpet

**Part 2:** Chemical emissions

**Part 3:** Infectious disease (COVID-19)

# Part 1: Building materials and moisture associated with indoor microbiome

**We know about growth on drywall:**

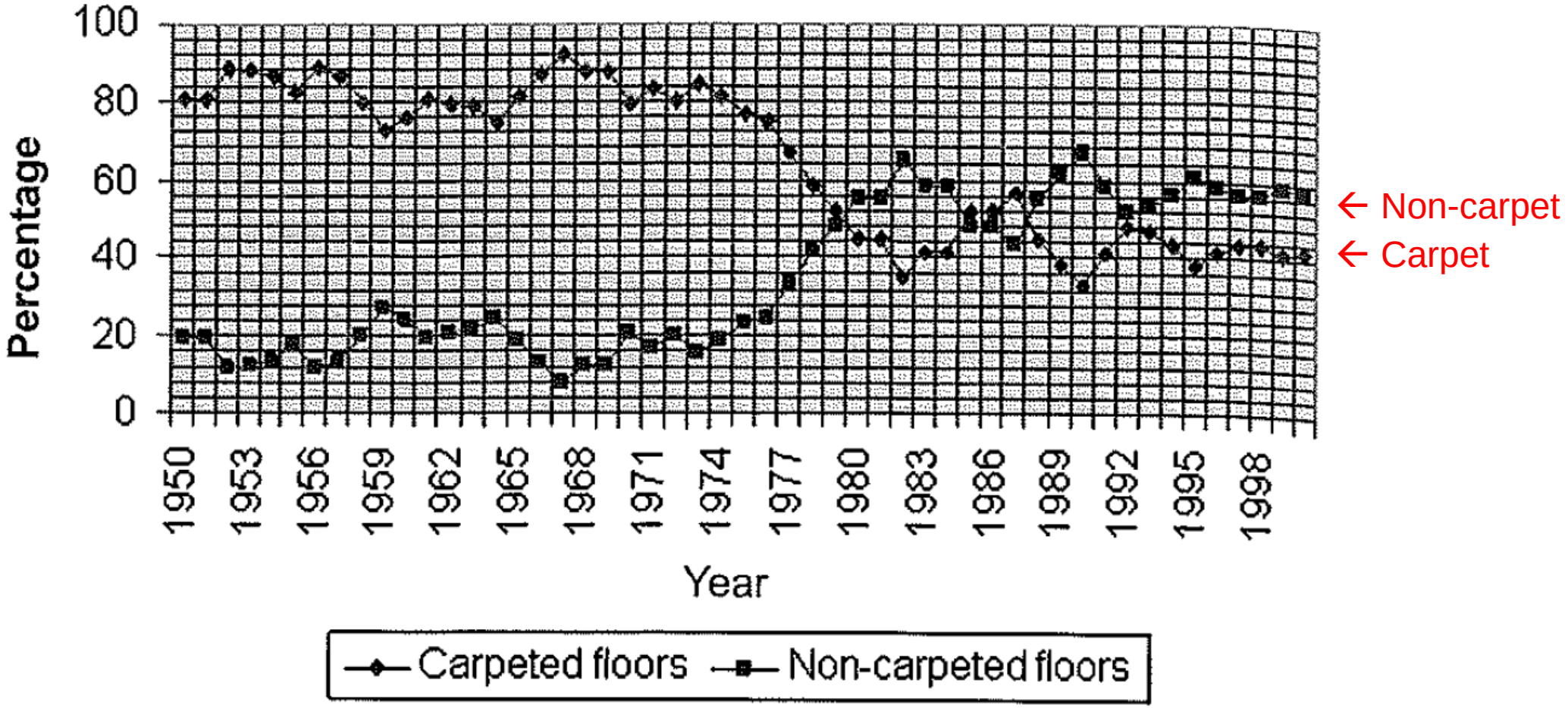
REVIEW Open Access

Moisture parameters and fungal communities associated with gypsum drywall in buildings

Sandra Dedesko[1] and Jeffrey A. Siegel[1,2*]

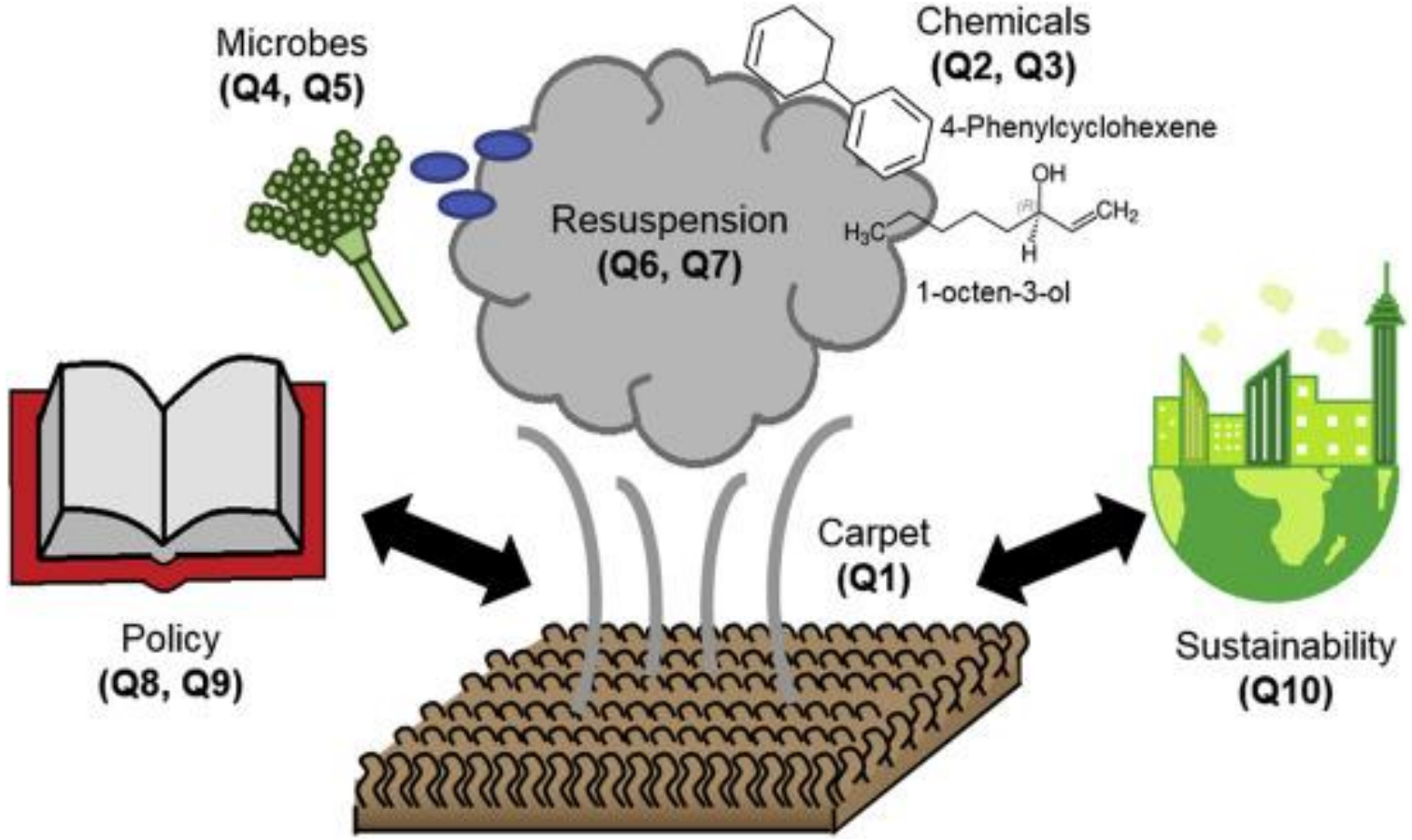
**What about in carpet?**

# Carpet is prevalent in homes post-WW II




From: Ulrich and Lee, TJTI 2008, 99, 67-75

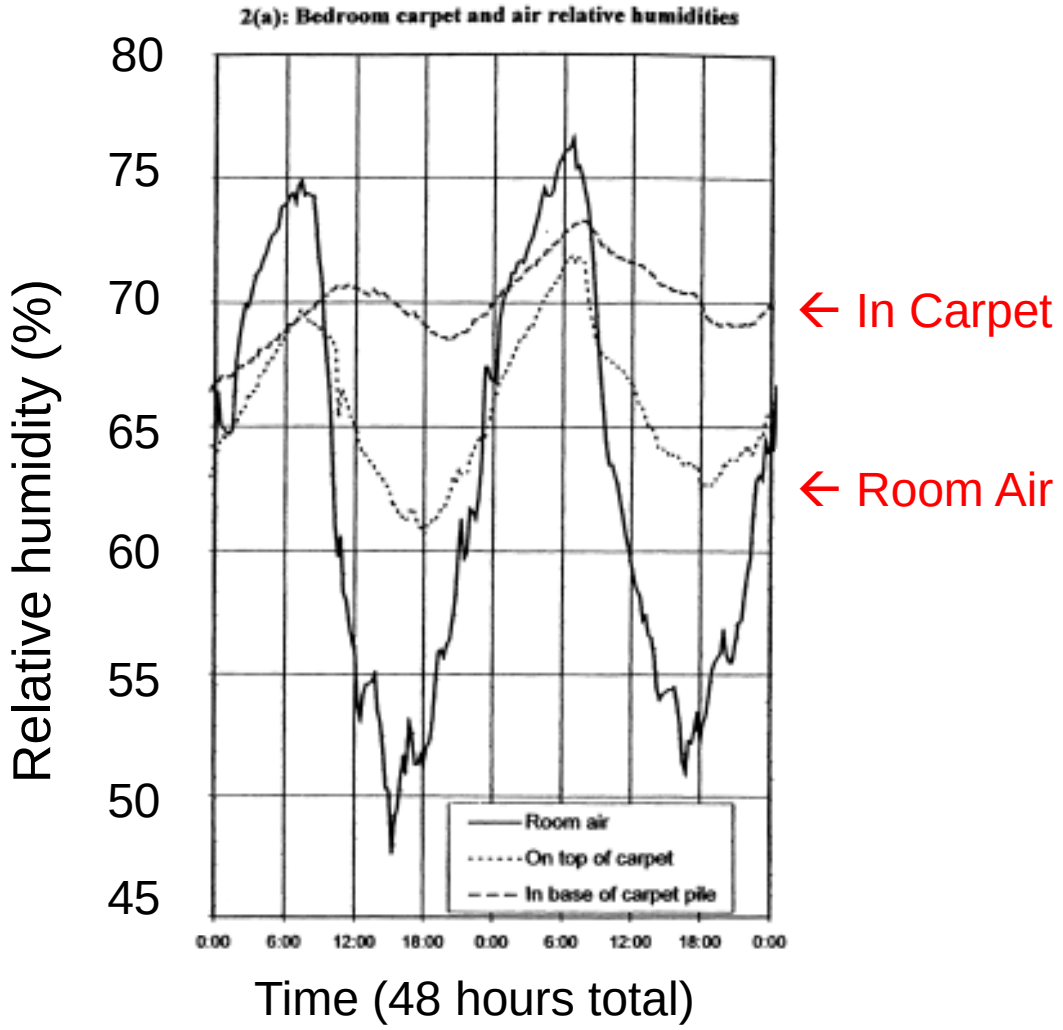
# Carpet is an important reservoir for human exposure to dust




Haines et al, 2020, Building and Environment, https://doi.org/10.1016/j.buildenv.2019.106589

# Relative humidity in carpet can be elevated above room air

- Dust mites
- Indoor chemistry
- Bacteria
- Fungi (← focus here)



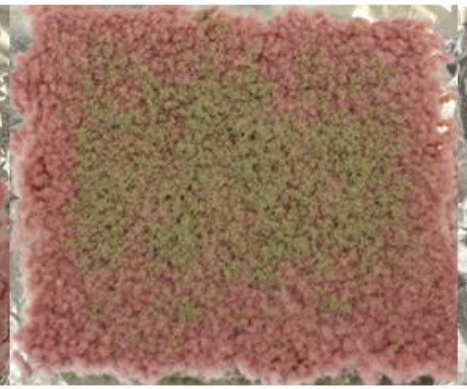

Cunningham, 1998 Clin Exp Allergy

# Is RH in the air sufficient to support Microbial Growth in Carpet?

- Collect carpet/dust from home
- Expose to relative humidity conditions
- Determine microbial growth
  - Rate
  - Function
  - Phthalate degradation


# Dust is embedded in carpet

10 cm x 10 cm carpet coupon



Apply dust

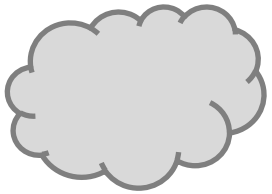
Embed dust with modified ASTM F608−13 method

Dust is embedded

Place carpet coupon in temperature- & relative humidity-controlled chamber

# DNA/RNA-based measurement of microbial exposures in homes: Improved measurement

Dust



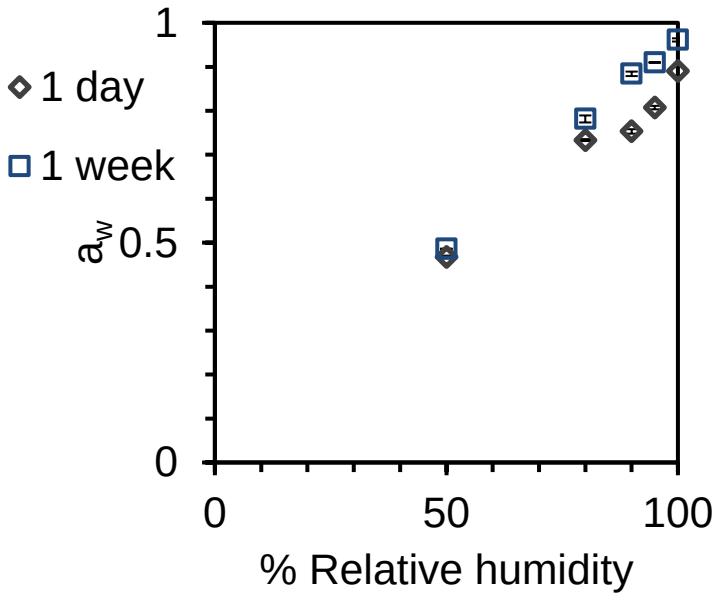
DNA

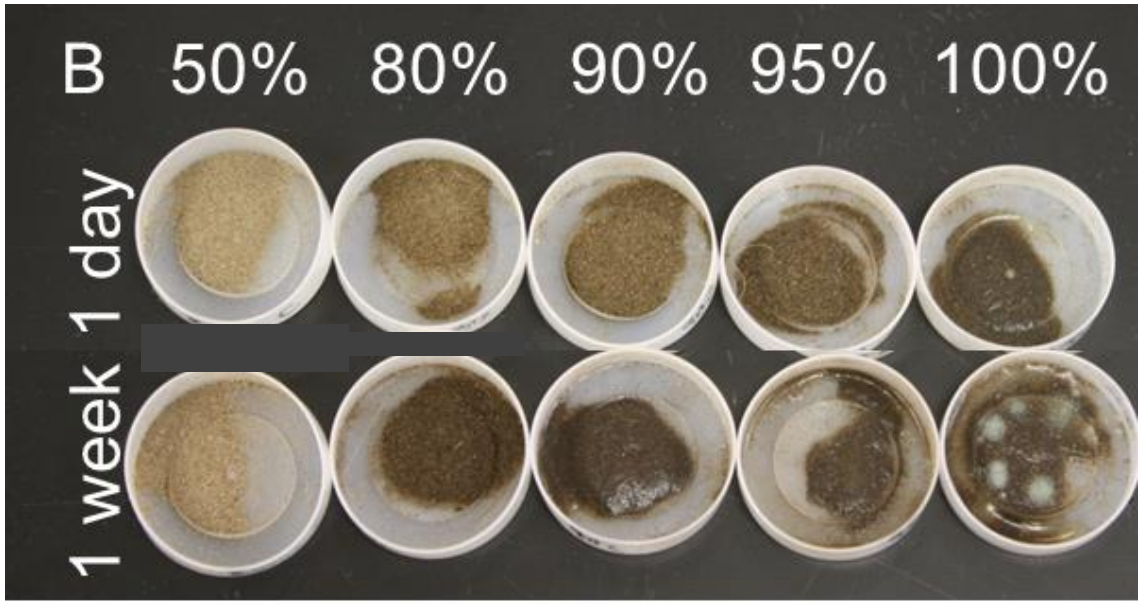
DNA Sequencing

Bioinformatics Tools

- ~Hundreds of thousands of DNA reads per sample
- Taxon identifications
- Quantification
- Diversity

# Water activity ($a_w$) of dust equilibrates quickly with RH

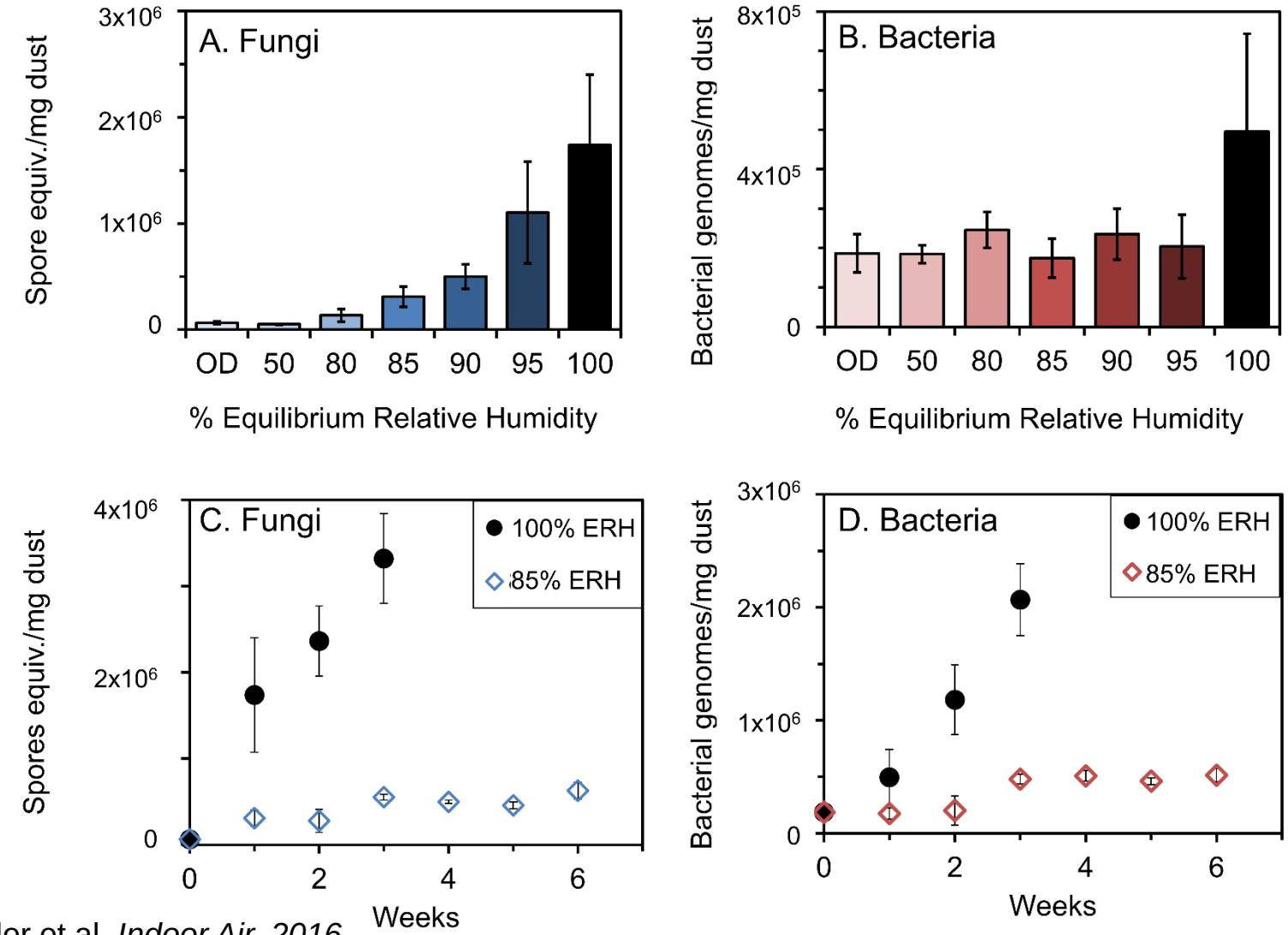



$$a_w = \frac{p_{dust}}{p_{water}}$$

$$Equilibrium\ RH = a_w \times 100\%$$

# Microbial growth occurs above 80% relative humidity

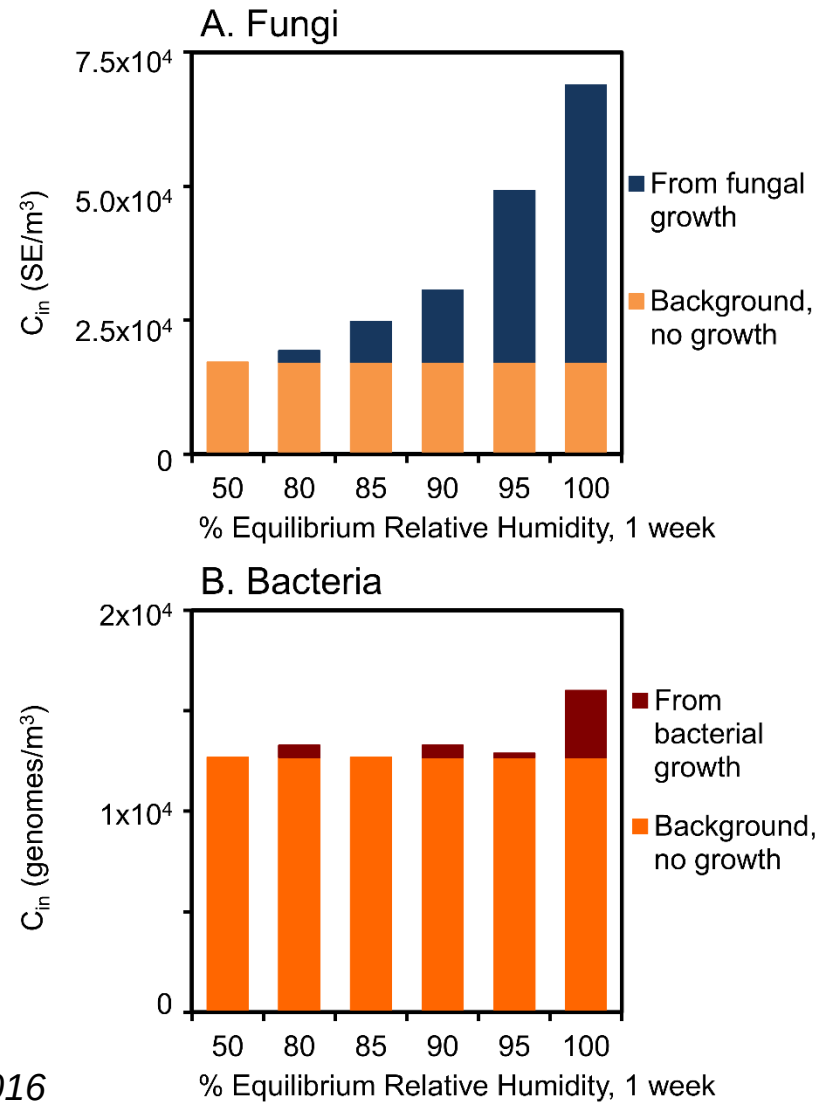



Dannemiller et al. *Indoor Air, 2016*

# Moisture is the limiting factor for growth

| Nutrient/ Salt | Dissolvable amount in dust (mg/kg dust) | Estimated amount needed to support growth (mg/kg dust) |
|---|---|---|
| C | 35000 | 7.2 |
| N | 5.7 | 1.3 |
| P | 7.9 | 0.22 |
| S | 9.1 | 0.058 |
| Na | 6300 | - |
| K | 2100 | - |
| Ca | 1600 | - |
| Mg | 220 | - |
| $NH_4$ | 160 | - |
| Cl | 2400 | - |

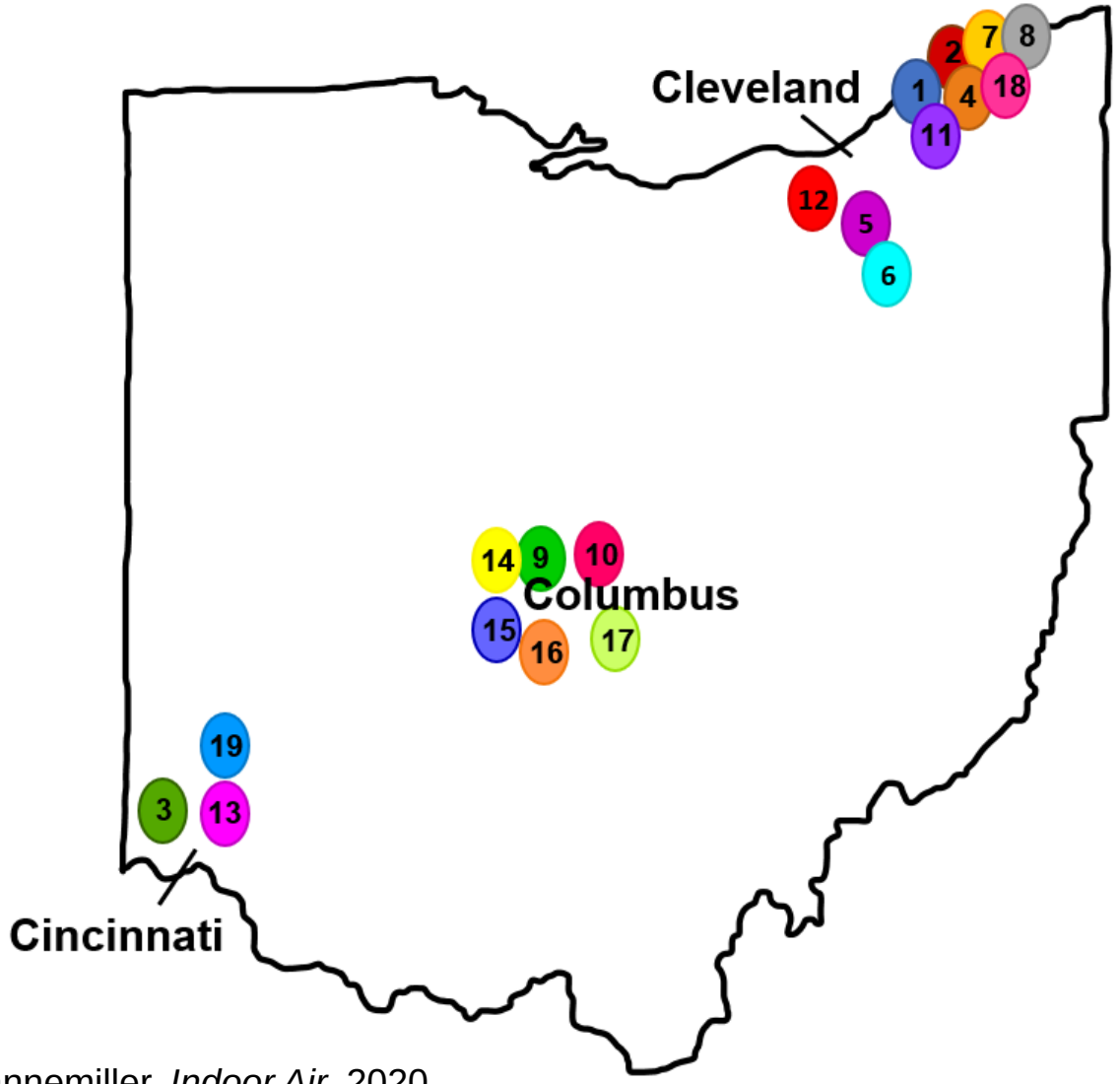
# Growth in dust contributes to human aerosol exposure




Dannemiller et al. *Indoor Air, 2016*

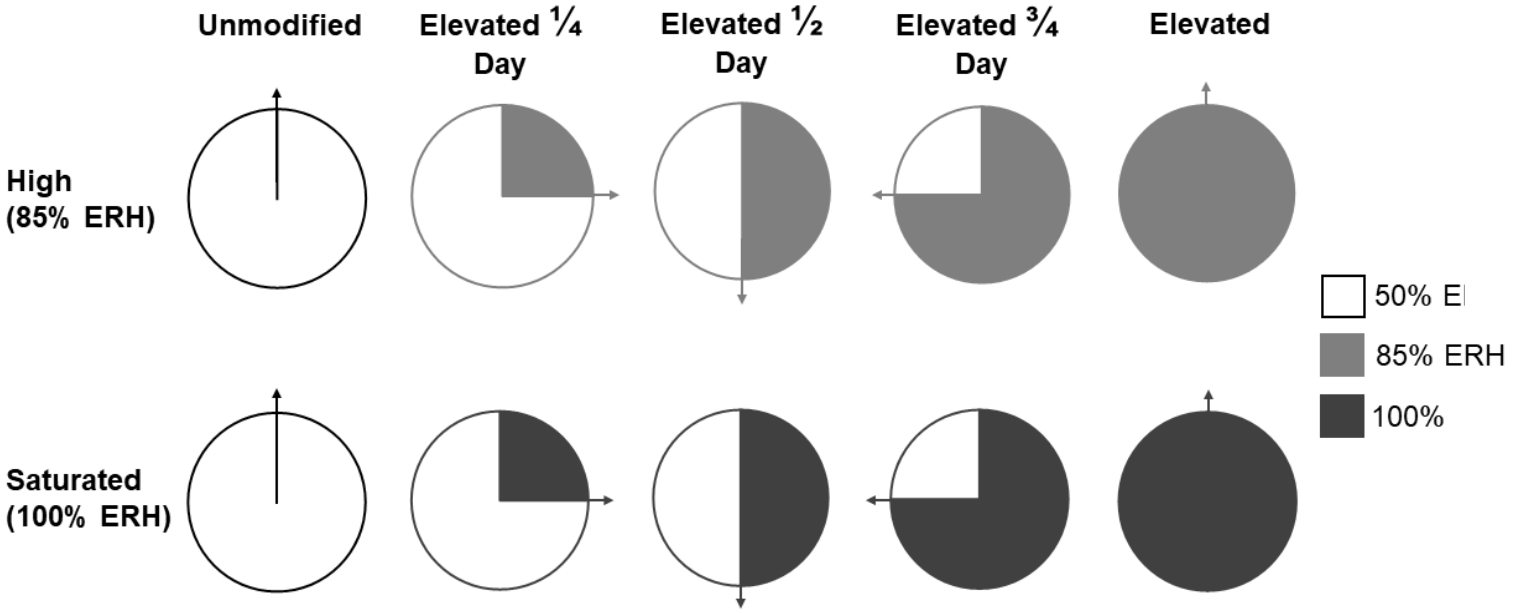
# **Next Goal:** Determine how variations in RH affect fungal growth in carpet

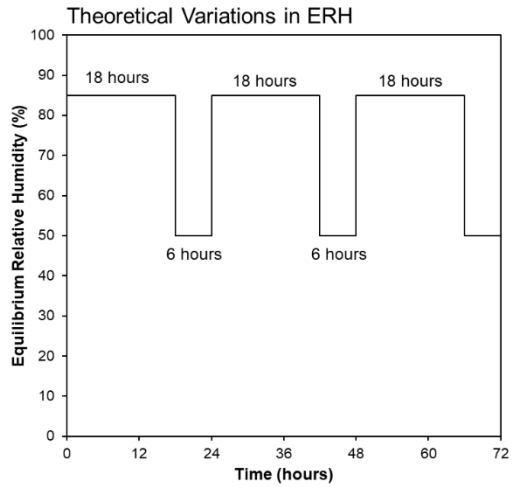
Sarah Haines

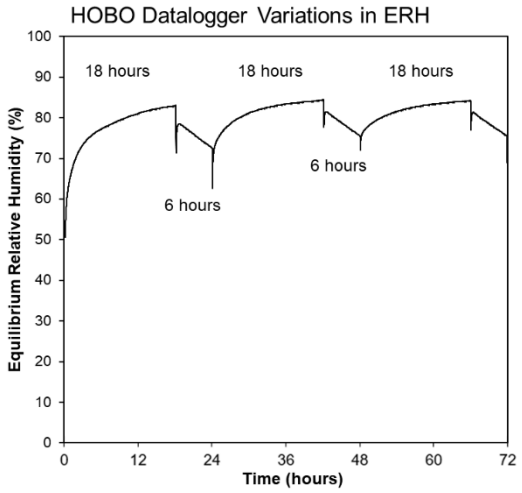



Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

# **Goal:** Determine how variations in RH affect fungal growth in carpet

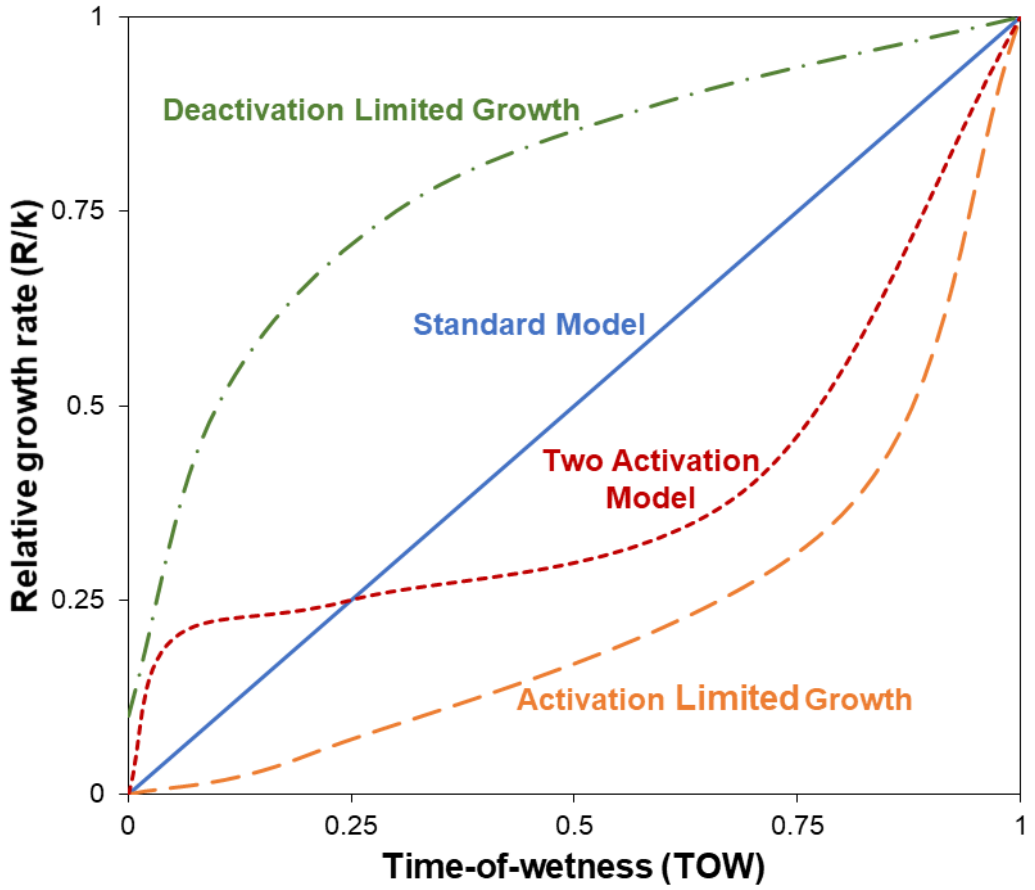



Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

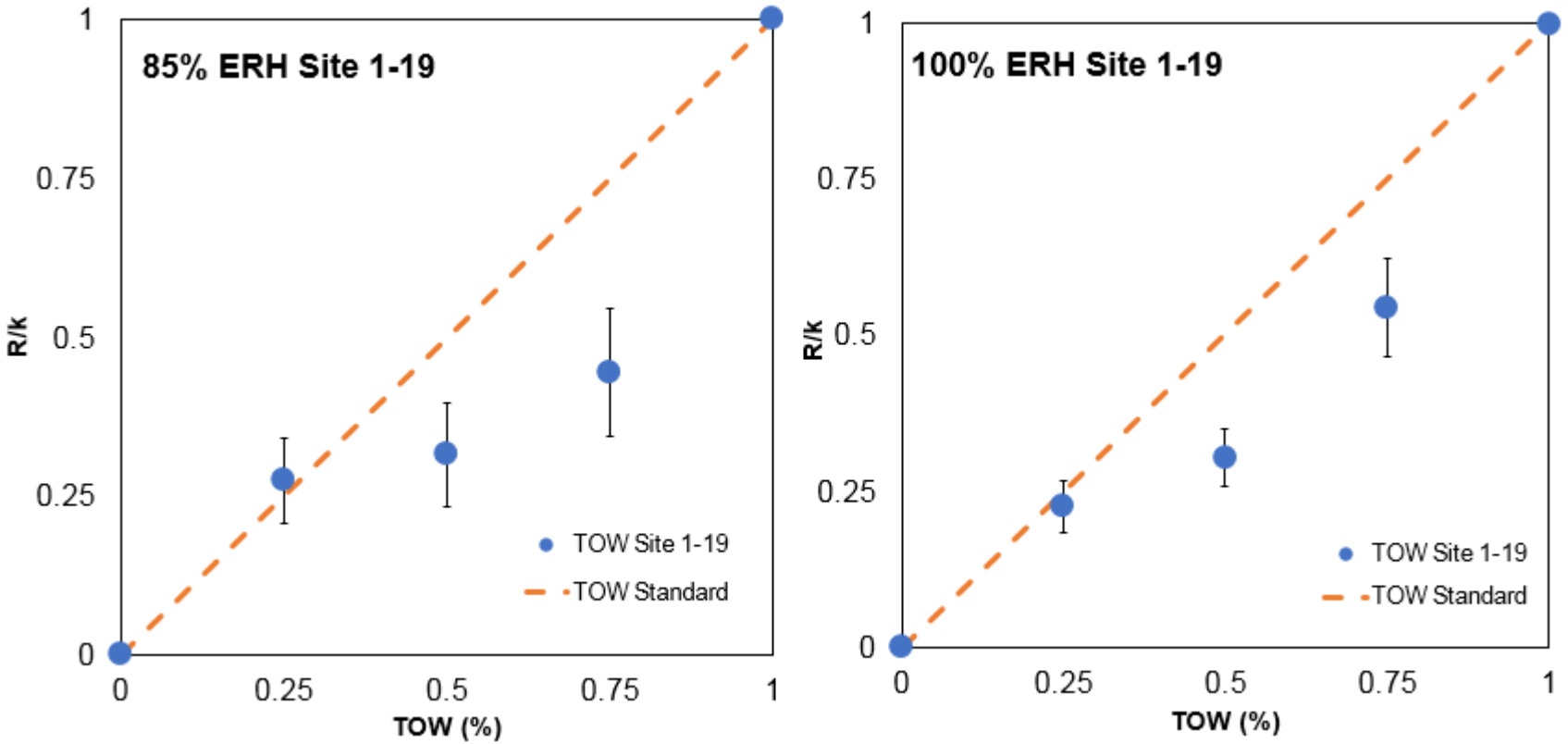
# Apply Time of Wetness model




Adan, O., Huinink, H. "Fungal Growth & Humidity Fluctuations: A Toy Model. *Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living*. 2011

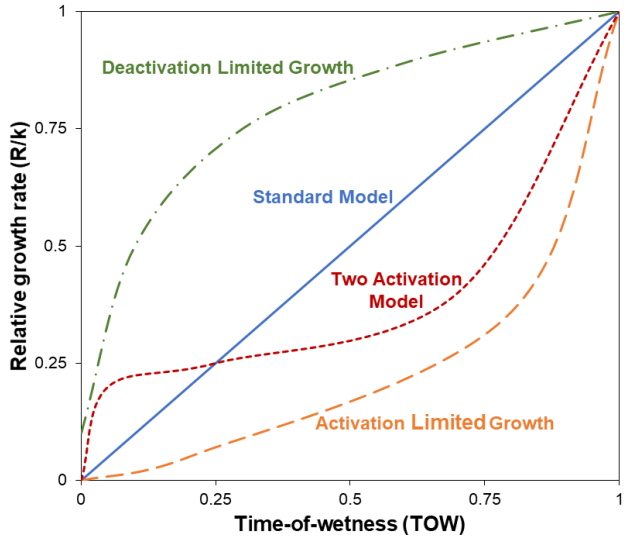
# Fungal growth in carpet dust follows the activation limited growth model

## Carpet TOW data



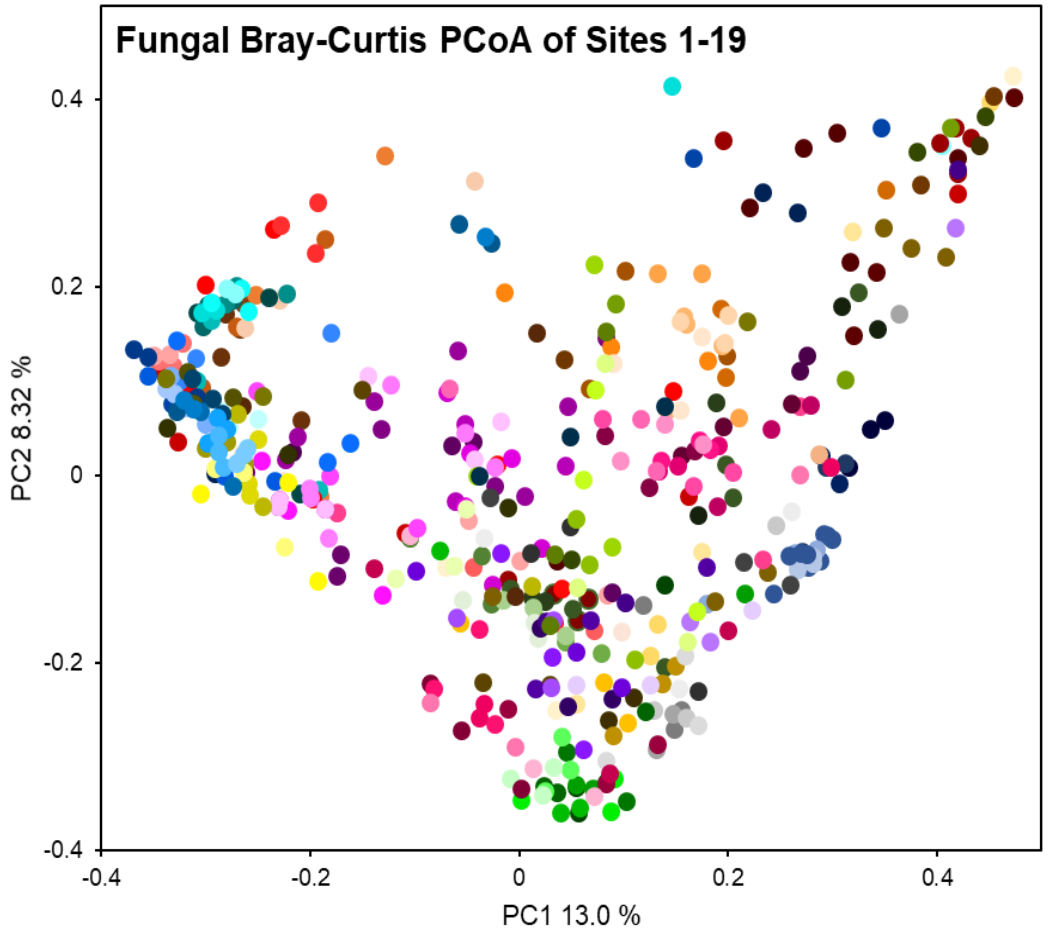

Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

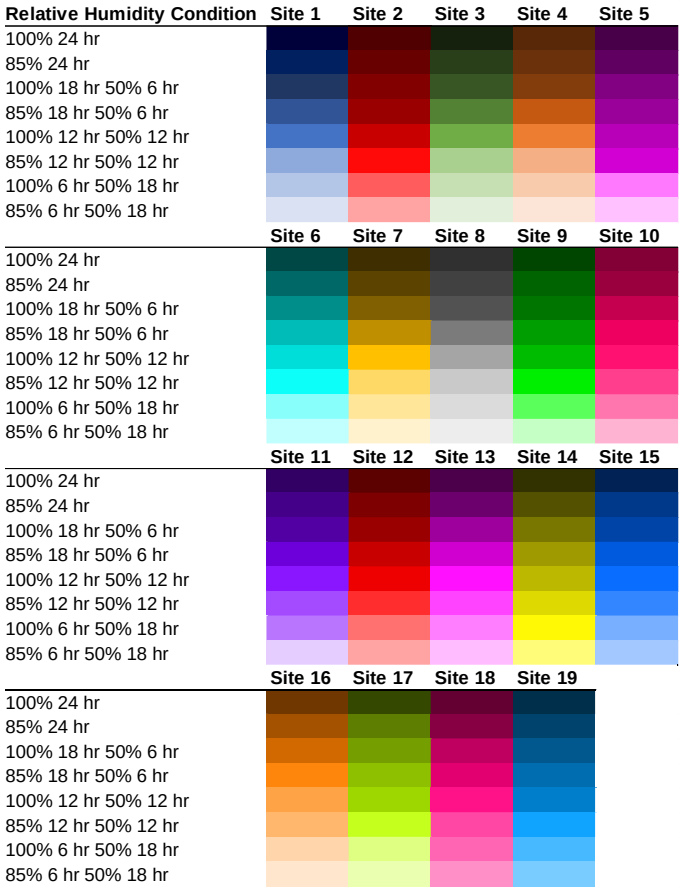
## Standard TOW Model




Adan, Olaf C. G., and Robert A. Samson. (2011) "Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living", *Wageningen: Wageningen Academic*

# Site-specific effects dominate moisture signature

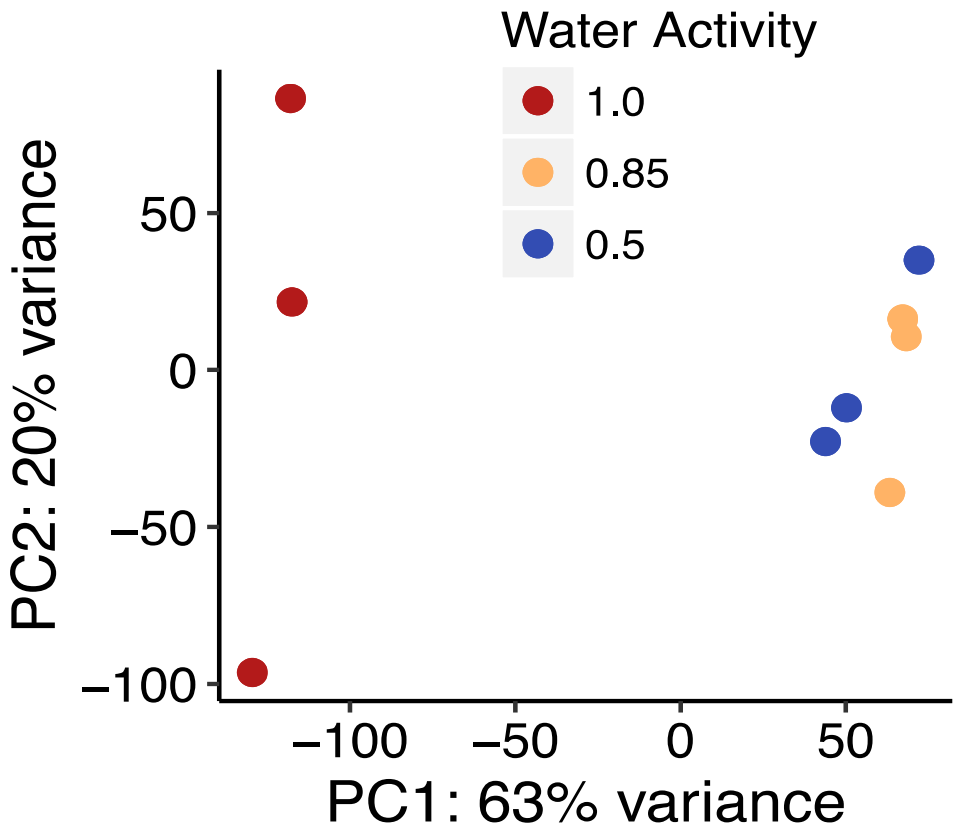



Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

# More moisture creates more “metabolic diversity”

Bridget Hegarty

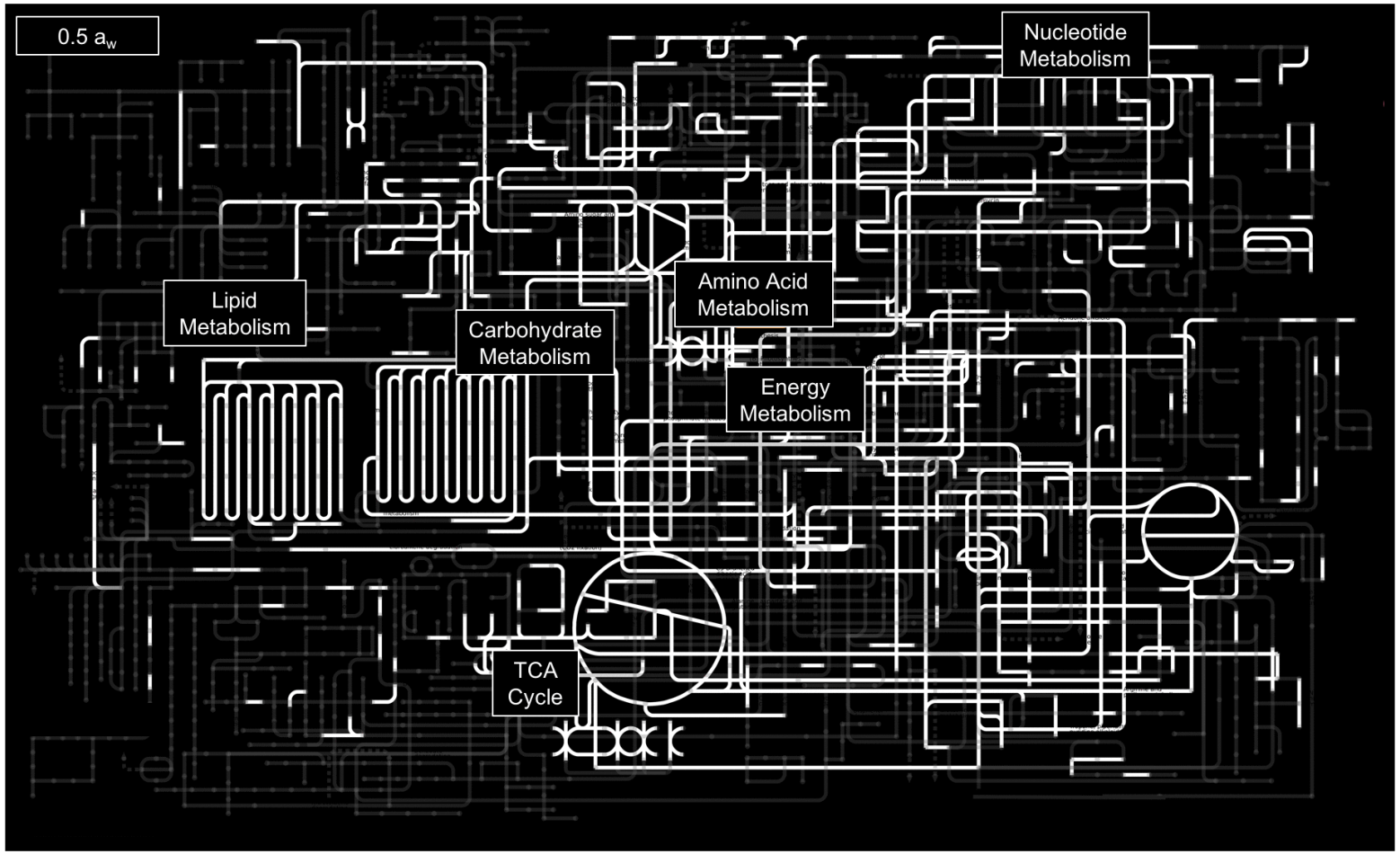



DNA → RNA → Protein

Gene expression

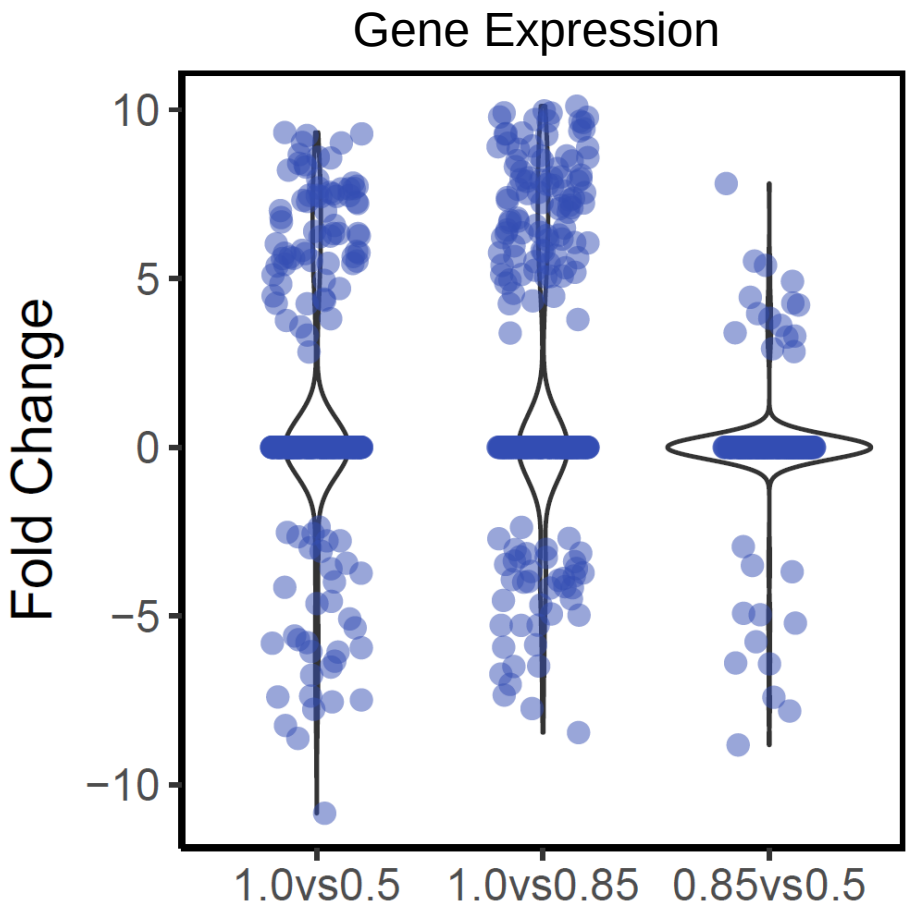
Hegarty, Dannemiller, & Peccia. *Indoor Air*

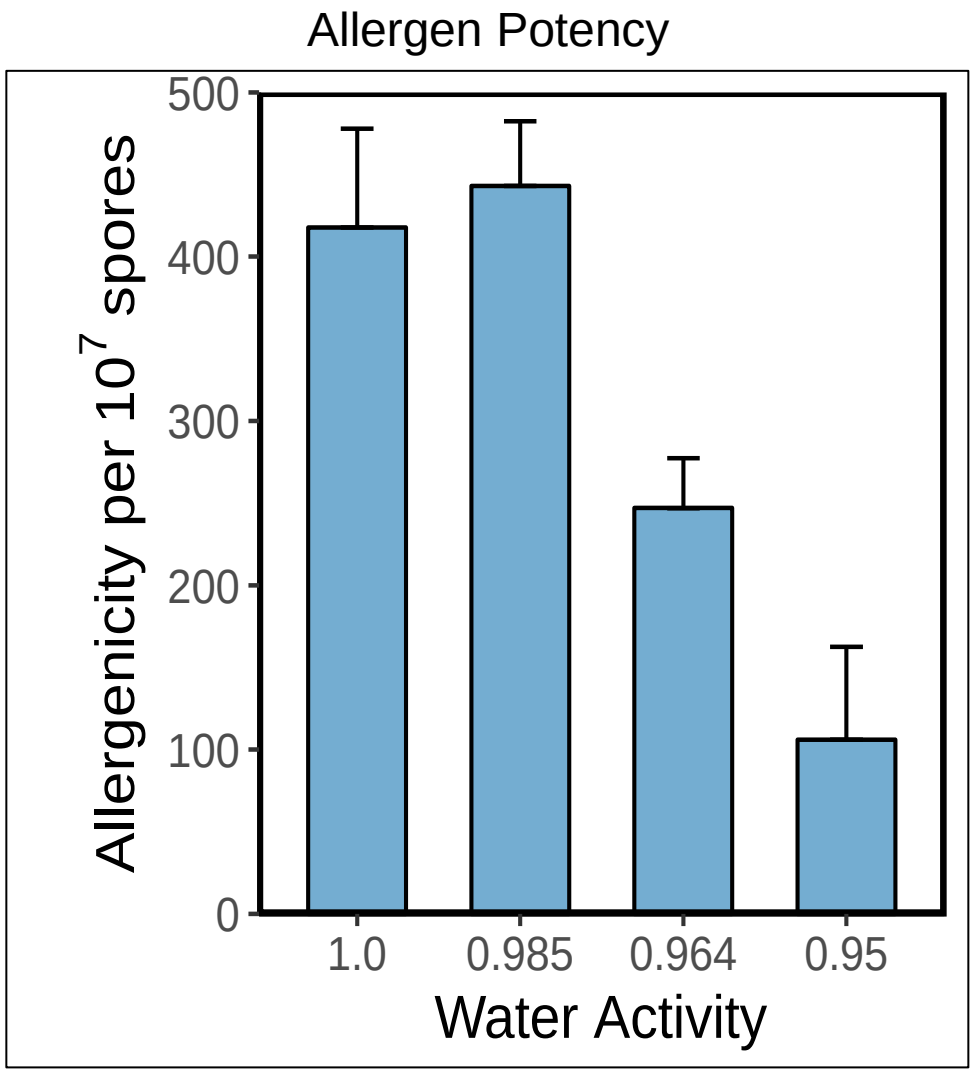
# What happens in fungi as moisture availability increases?




Hegarty, Dannemiller, & Peccia. *Indoor Air*

# Growth at increased water activity increases allergen potency



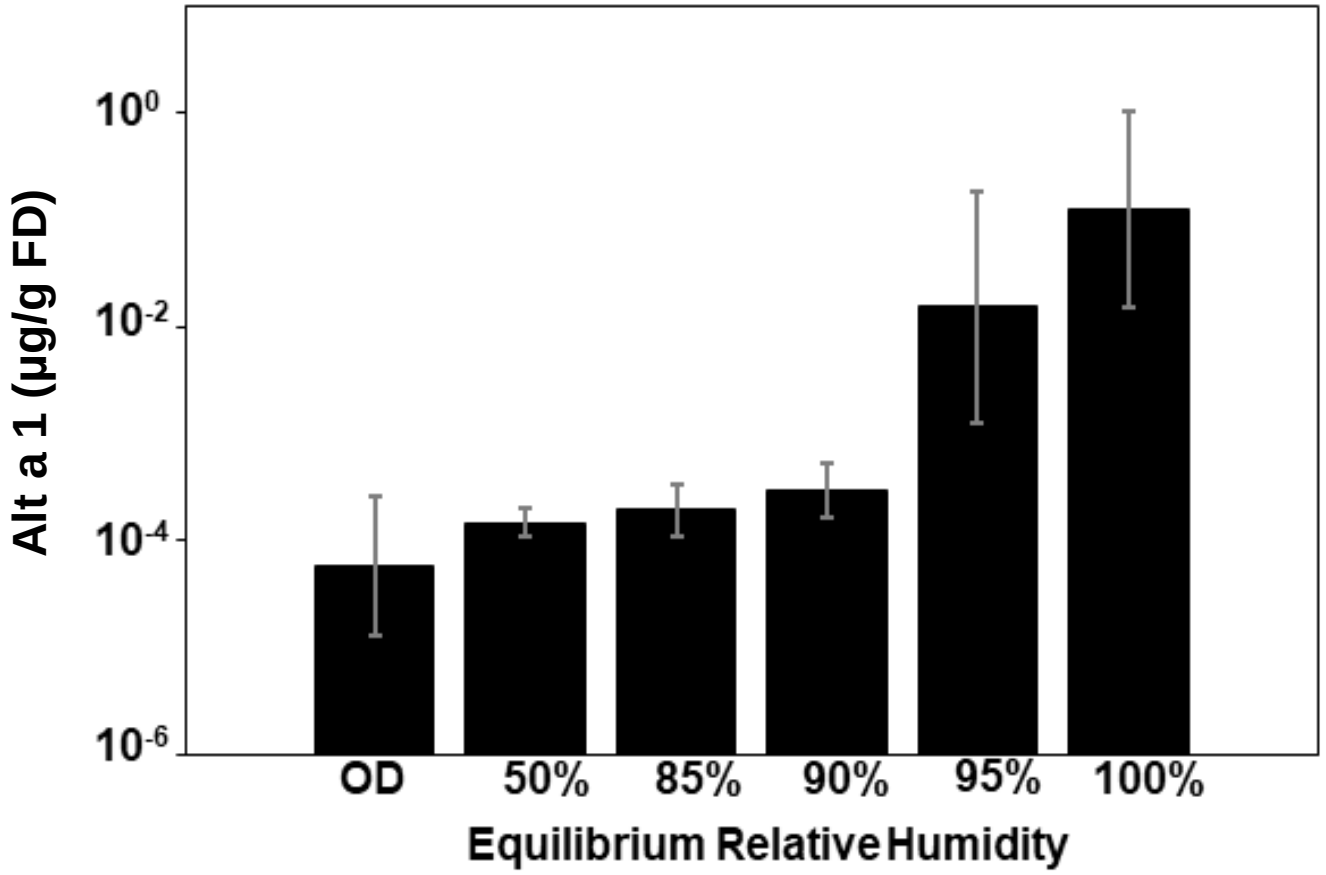




Hegarty, Dannemiller, & Peccia. *Indoor Air*

# Relative humidity associated with increased allergen production

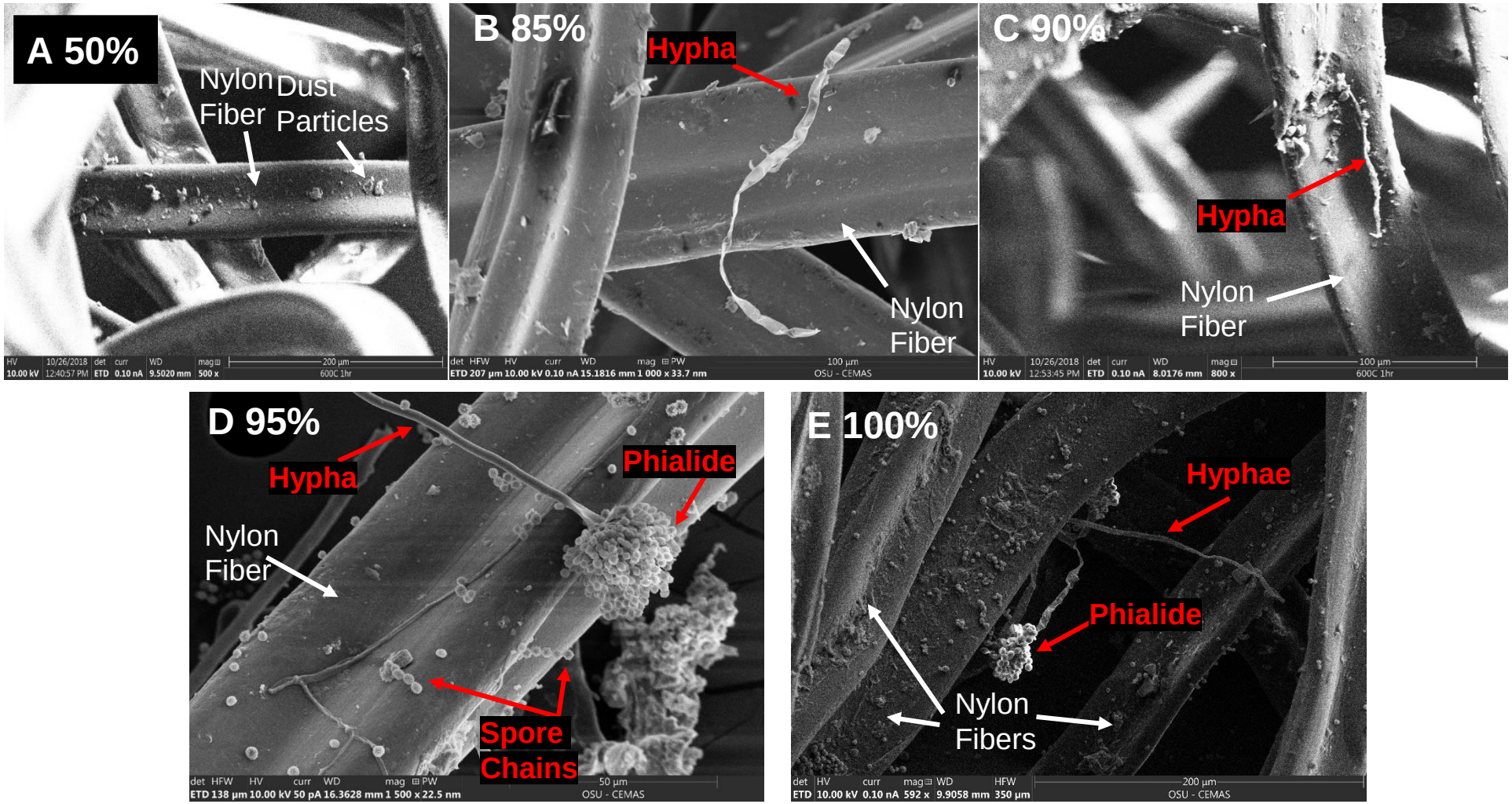
Nick Nastasi

## Moisture > Dust > Fiber Type



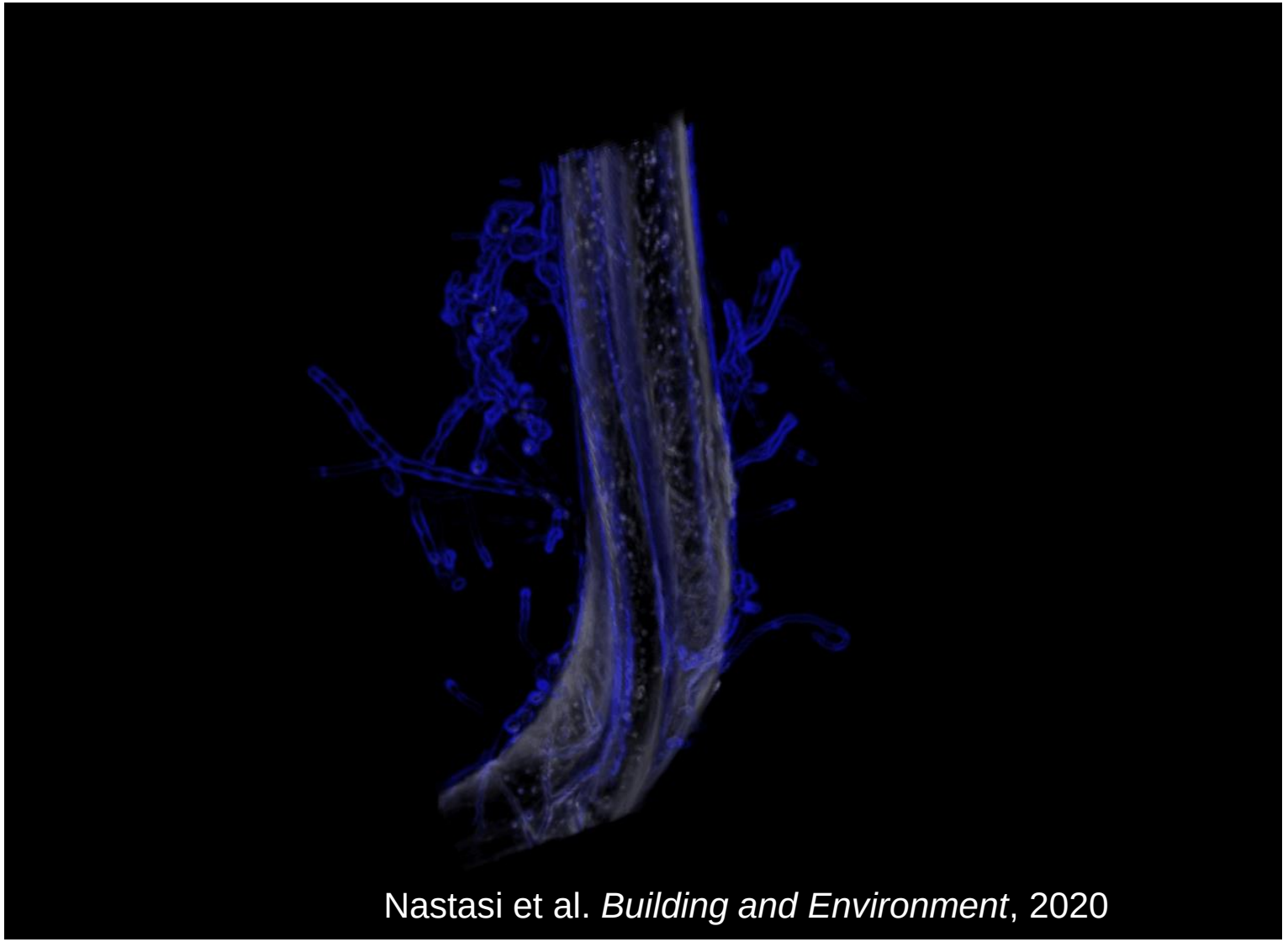

Nastasi et al. *Building and Environment*, 2020

# But what does it look like?
# Low → High RH



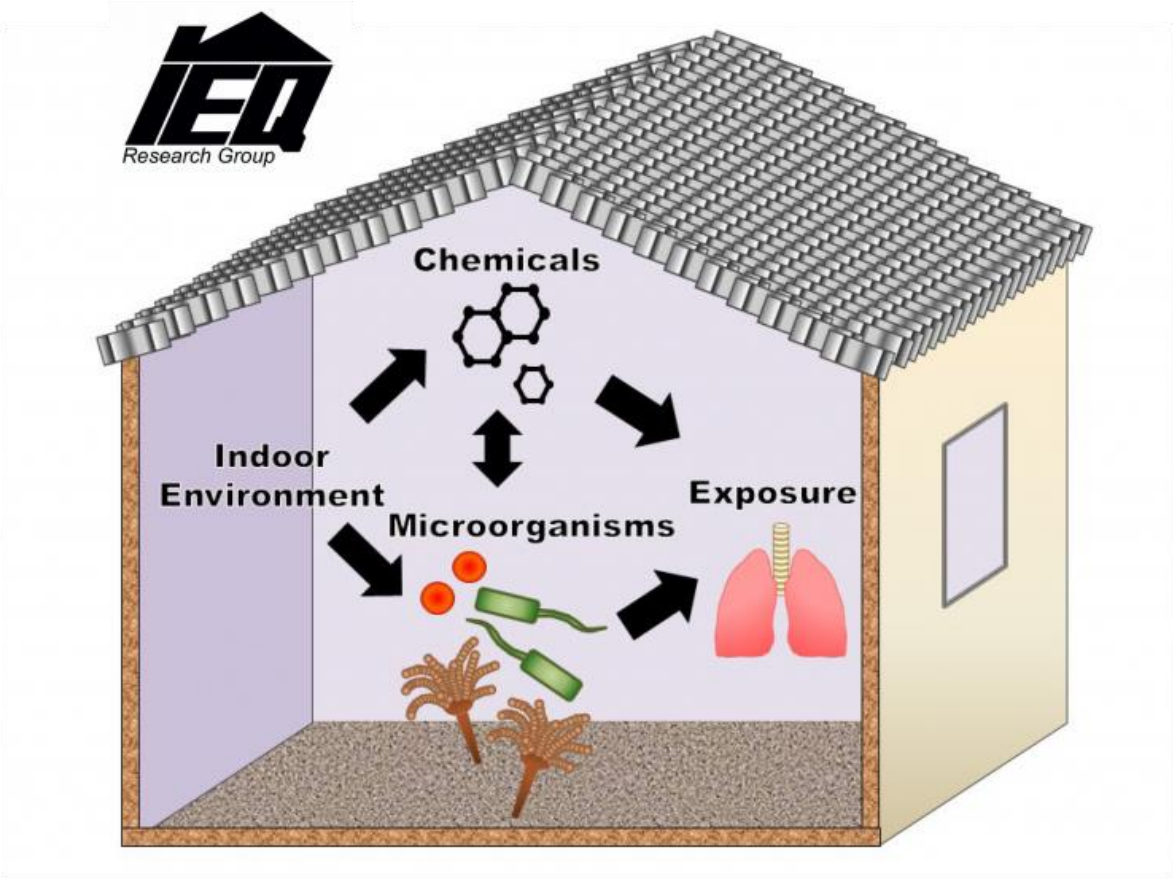

Nastasi et al. *Building and Environment*, 2020

# Fungal growth on carpet



Nastasi et al. *Building and Environment*, 2020

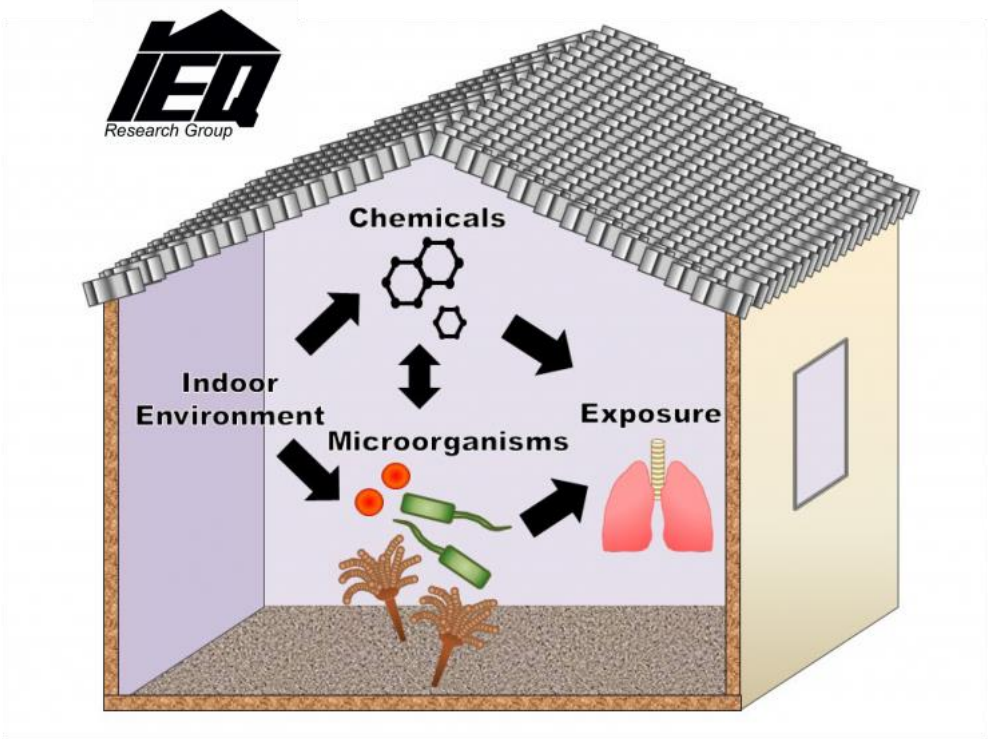
# Part 1 Summary




- Elevated moisture is sufficient to support microbial growth and function in dust
- This has important implications for health and building design

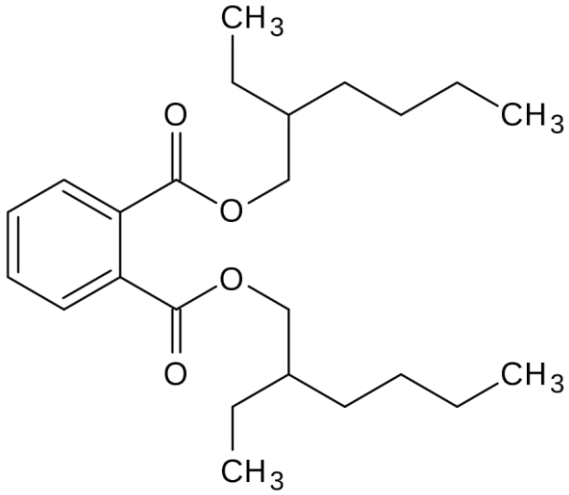
# Part 2: Moisture associations with indoor chemistry

- Degradation of compounds in dust
- Release of volatile organic compounds (VOCs) and microbial VOCs (mVOCs)



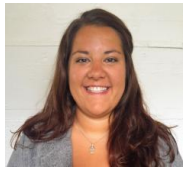
# Can microbial function interact with chemicals like phthalates?

- Plasticizers
- Endocrine disruptors
- Found in high levels in dust (up to 1 mg/g)
- Degraded by microbes in wastewater/soil




**DEHP**

# We incubated dust at elevated RH and measured phthalates



Ashleigh Bope

Sarah Haines

Bridget Hegarty

## Natural Experiment

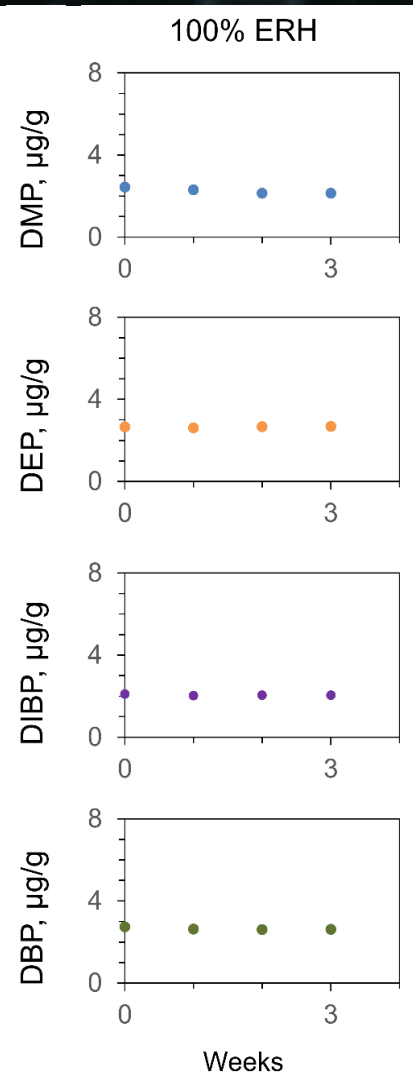
- Dimethyl phthalate (DMP)
- Diethyl phthalate (DEP)
- Diisobutyl phthalate (DiBP)
- Di(n-butyl) phthalate(DnBP)
- Di(2-ethylhexyl) phthalate (DEHP)
- Butyl benzyl phthalate (BBzP)
- Di-n-octyl phthalate (DnOP)
- Diisononyl phthalate (DiNP)
- Diisodecyl phthalate (DiDP

## Spiked Experiment

- Deuterated DEHP

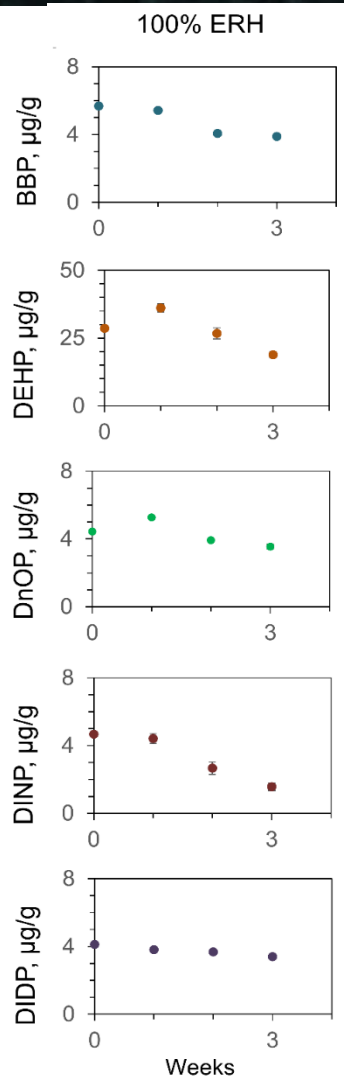

Bope, Haines, et al. *Environmental Science: Processes & Impacts, 2019*

# Preferential degradation of high MW



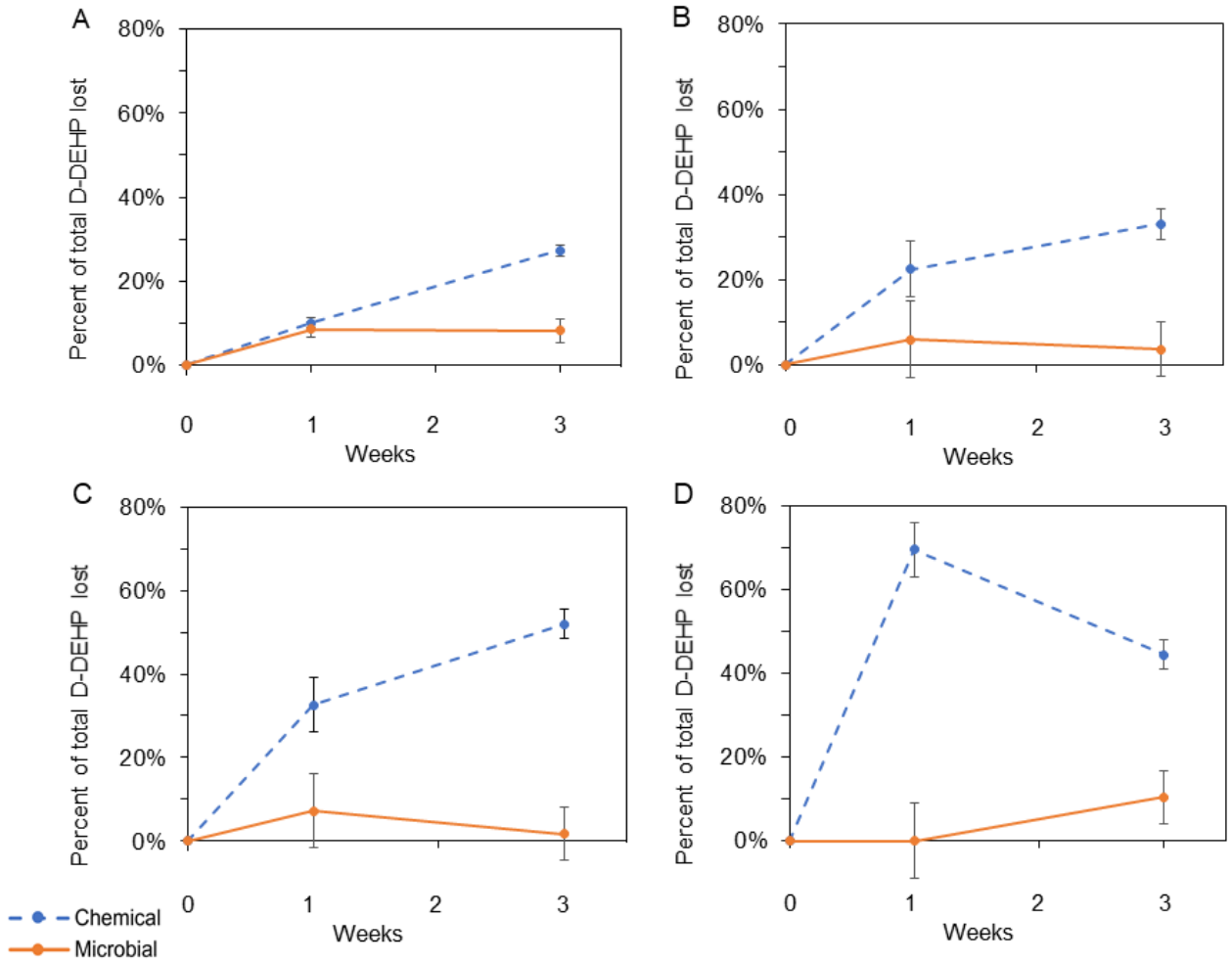

←Low MW

High MW →




Bope, Haines, et al. *Environmental Science: Processes & Impacts, 2019*

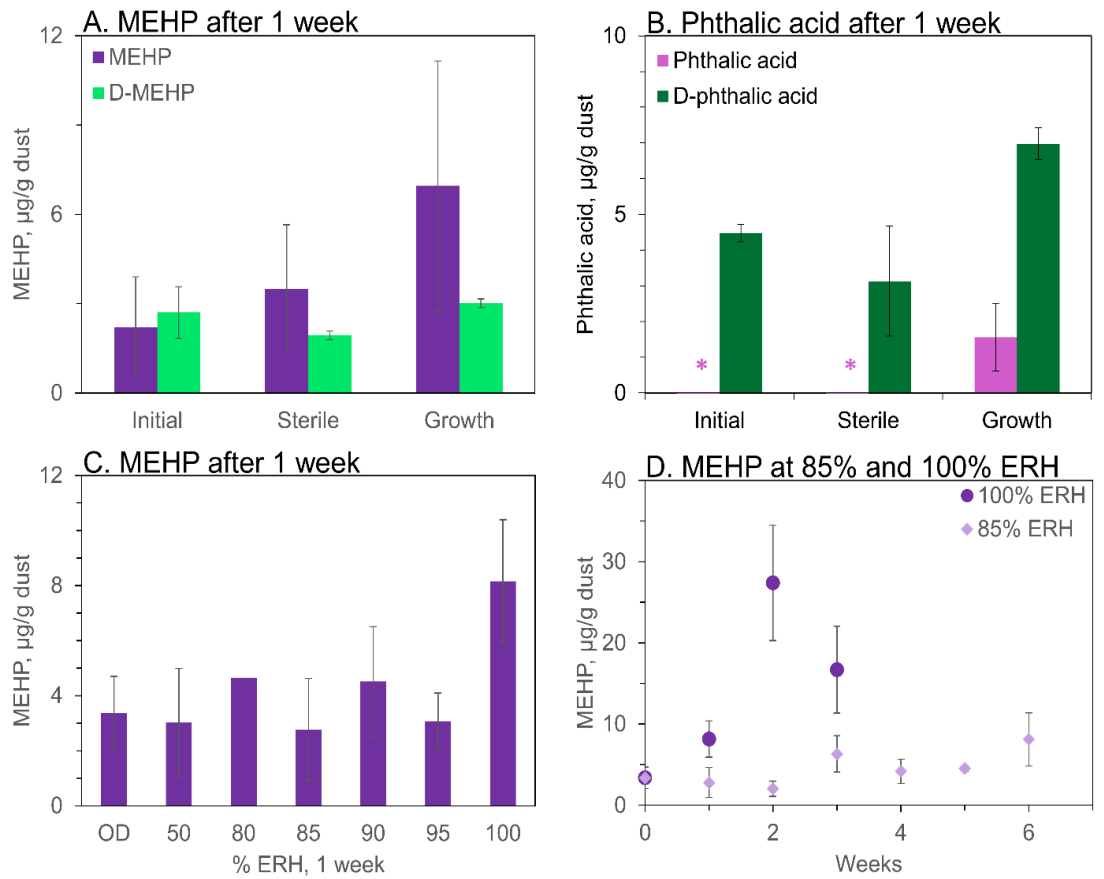
# Degradation due to both microbial and abiotic processes




Bope, Haines, et al. *Environmental Science: Processes & Impacts, 2019*

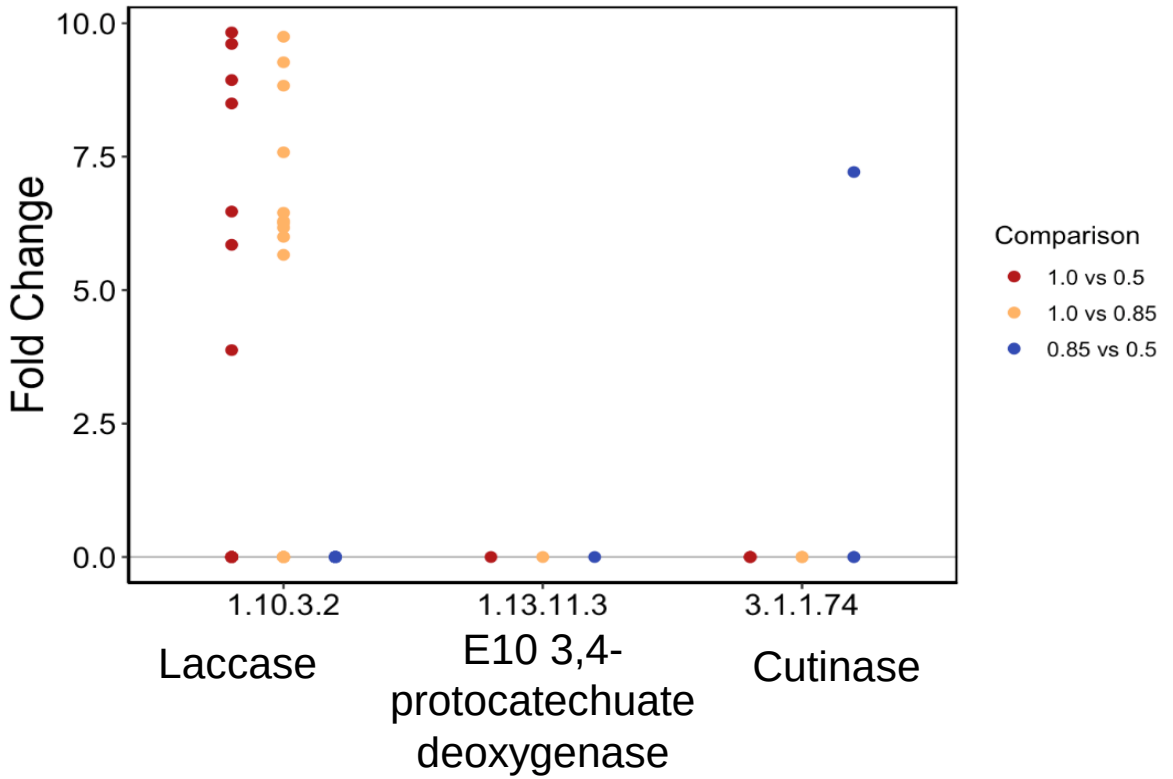
# MEHP and phthalic acid were produced

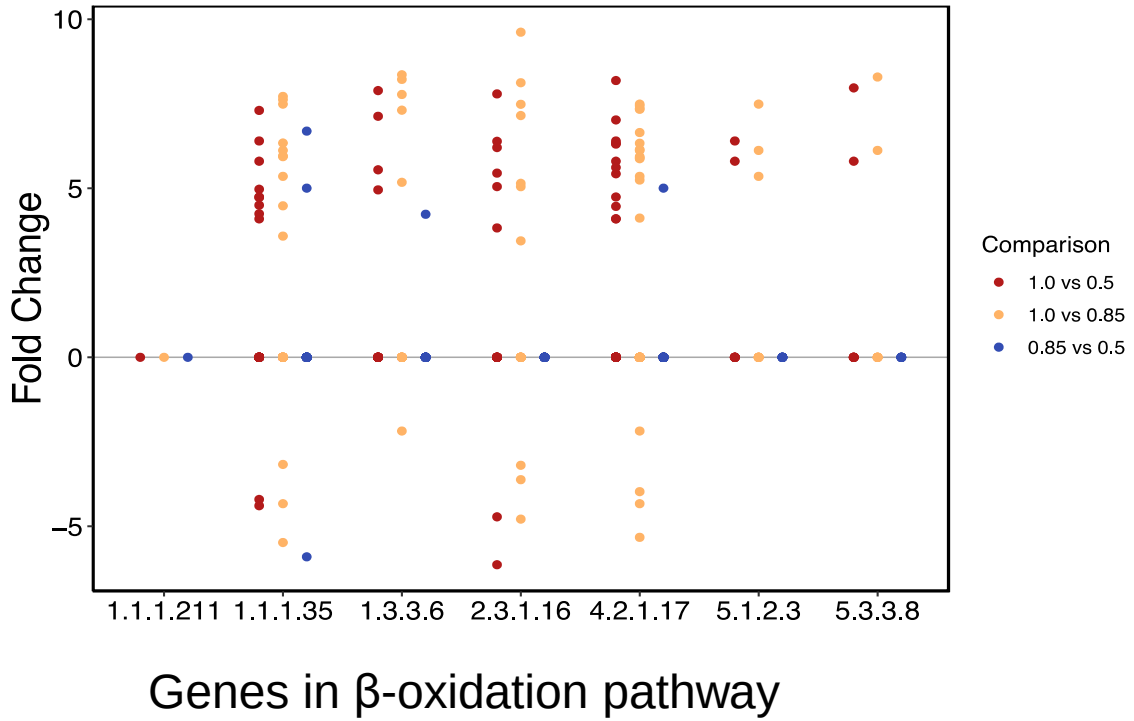
DEHP → MEHP → Phthalic acid




Bope, Haines, et al. *Environmental Science: Processes & Impacts, 2019*

# Genes associated with phthalate degradation upregulated at high RH; associated with high MW

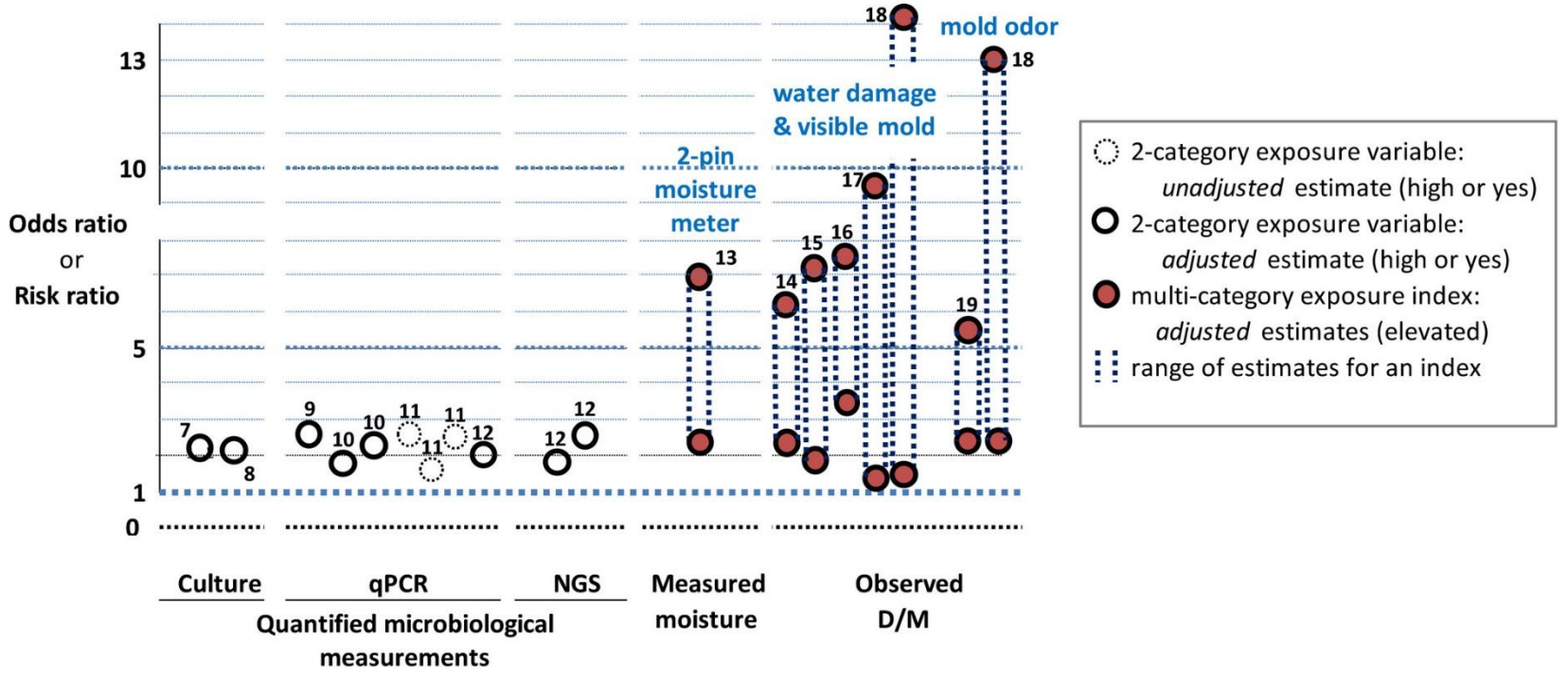



Bope, Haines, et al. *Environmental Science: Processes & Impacts, 2019*

# What about volatile emissions?

- Moldy odor is consistently and highly associated with health outcomes




Mendell, Adams, *Indoor Air*, 2019

# Release of chemicals from microbes

Rachel Adams

Pawel Misztal

Sarah Haines

Emma Hall

**<u>Goal</u>**

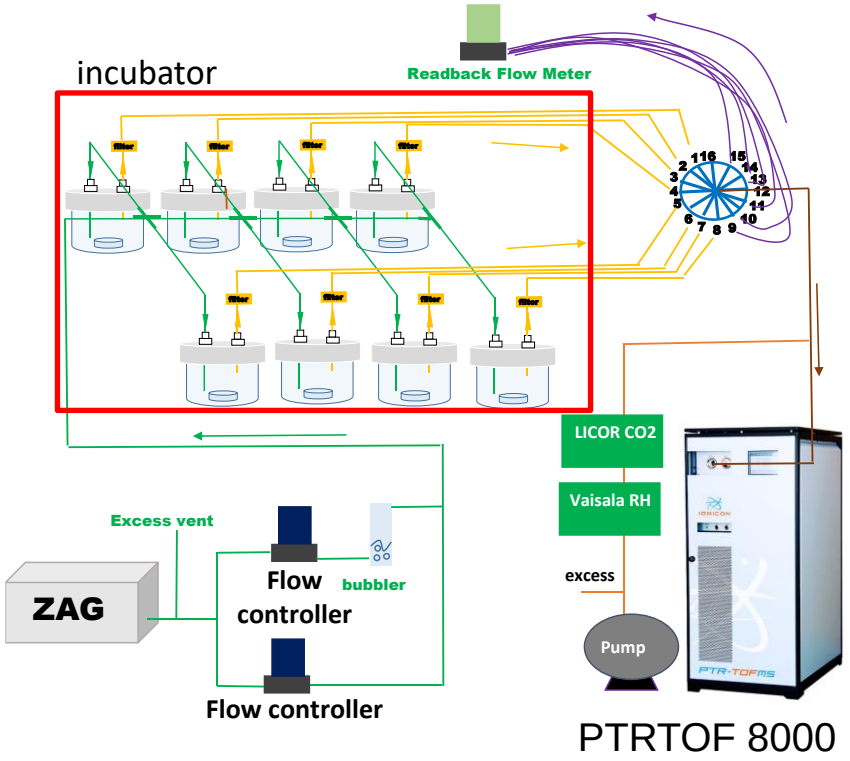
Understand how:

- moisture availability
- geographic location
- material type

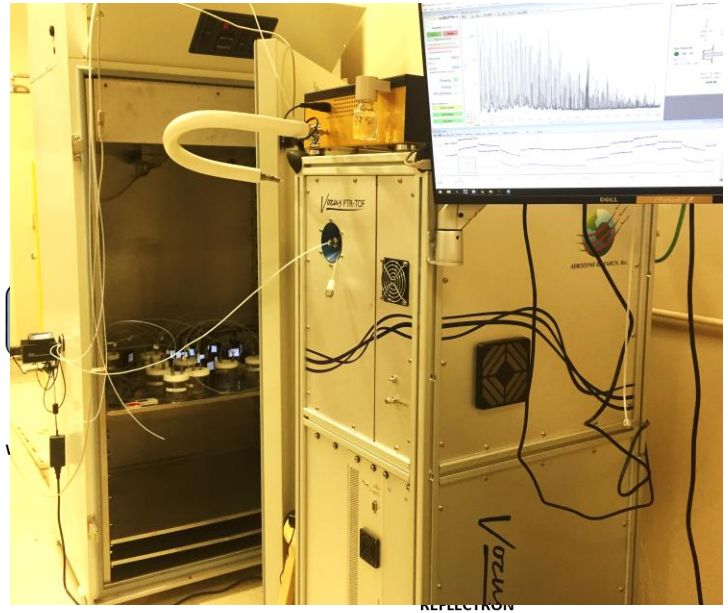
Impact:

- microbial growth
- mVOC production

# PTR-ToF-MS detects >1000 VOCs




PTRTOF 8000



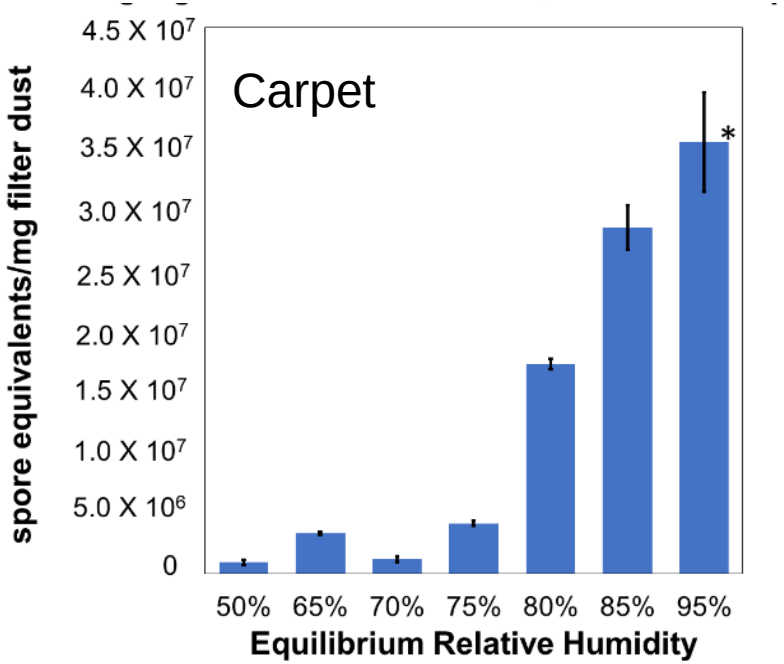
**Vocus advantages:**

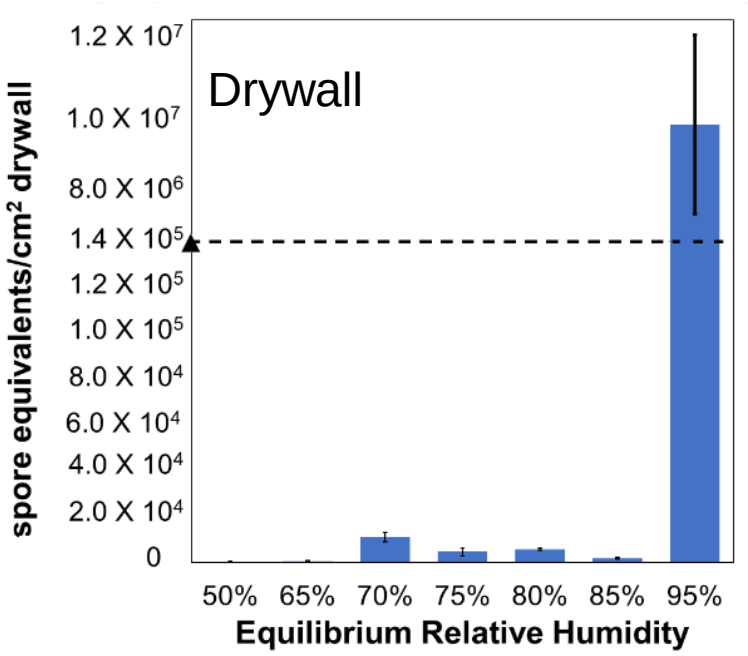
- 10-100 x higher sensitivity than previous generation (sub-ppt detection limit)
- Improved reaction chamber for SVOC
- Higher mass resolution 15,000 m/dm

Misztal et al., ES&T, 2018

Adams et al., Microbiome, 2018

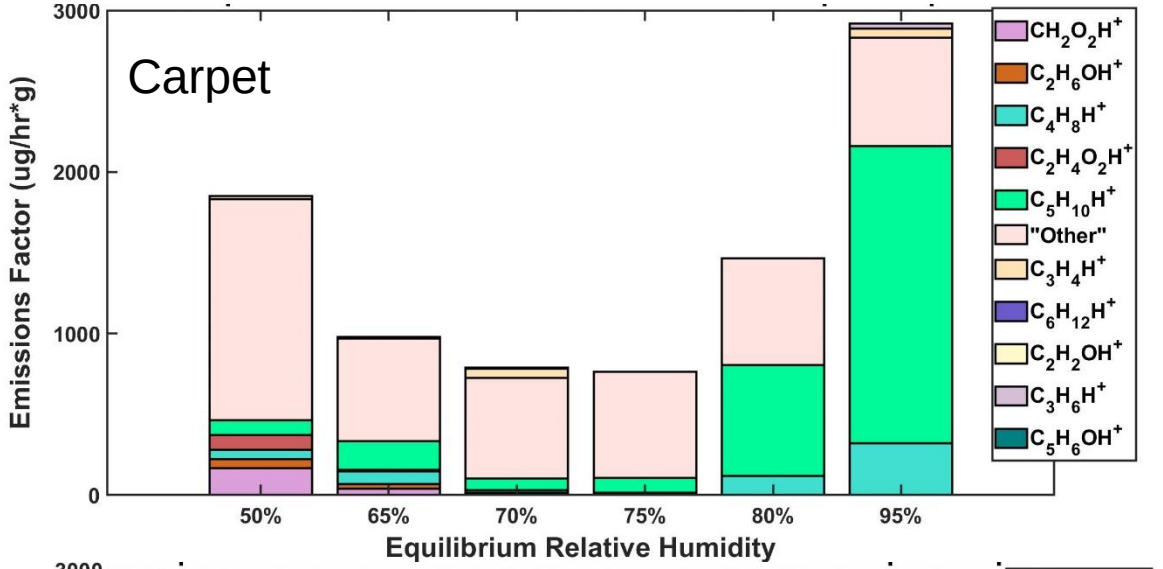
# Growth occurs at lower RH in dust: More microbial chemistry there

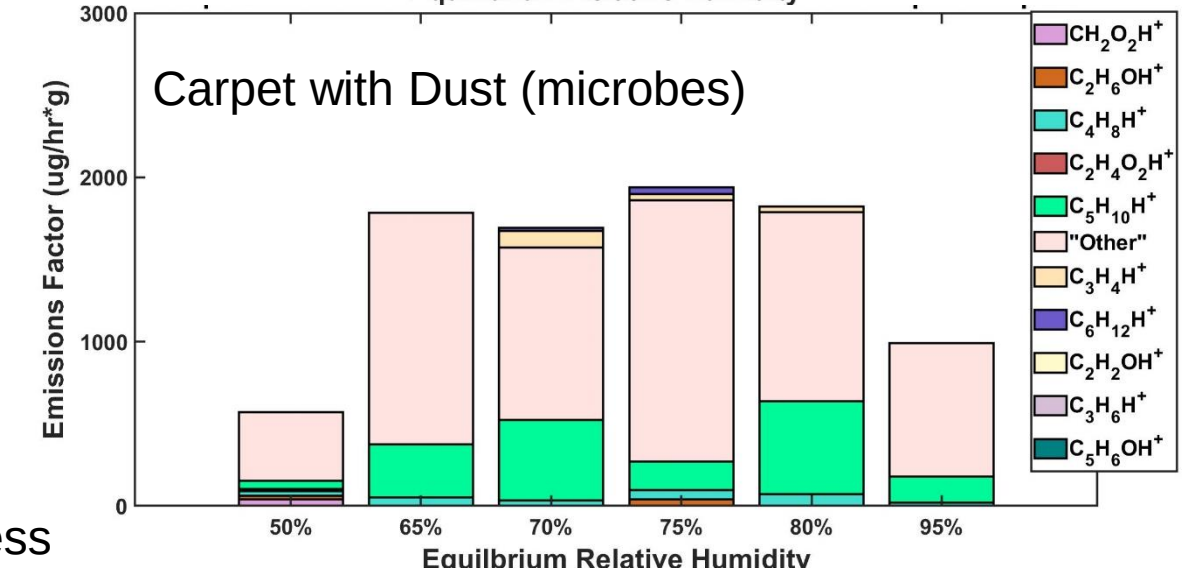



Haines et al, Microbiome, In press

# Most VOCs from building material directly, but some come from microbes




Haines et al, Microbiome, In press

# Most VOCs from building material directly, but some come from microbes

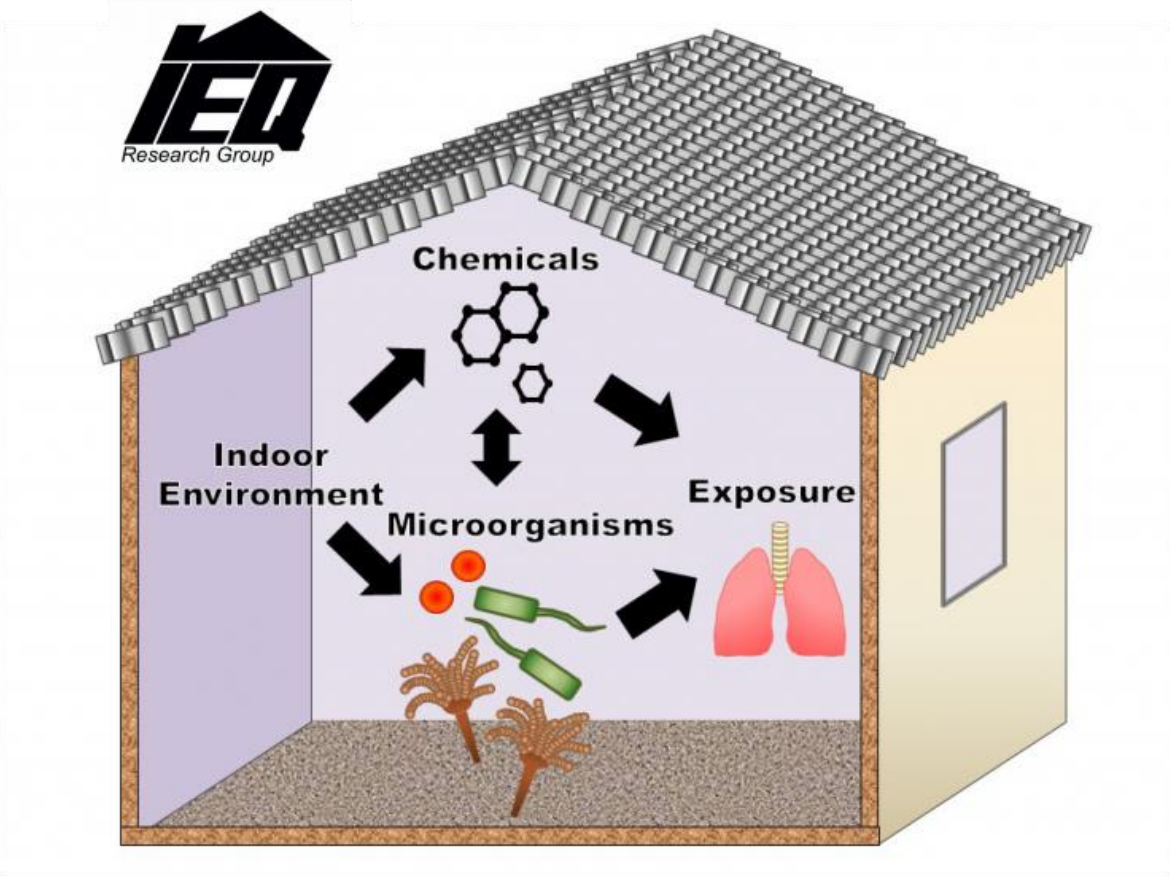
- Possible mVOCs:
  - Monoterpenes
  - Camphor
  - Dimethyl sulfide
- Many from microbes are odorous
- Some microbial terpenoids may react with ozone for secondary chemistry

Haines et al, Microbiome, In press

# Part 2 Summary:

- Microbes can degrade phthalate esters in house dust with sufficient moisture
- Moisture impacts indoor chemistry differently based on building material
- Moisture has both direct and indirect influence on indoor chemistry
- **Future work:** VOC signatures that indicate moisture

# Part 3: COVID-19 Surveillance



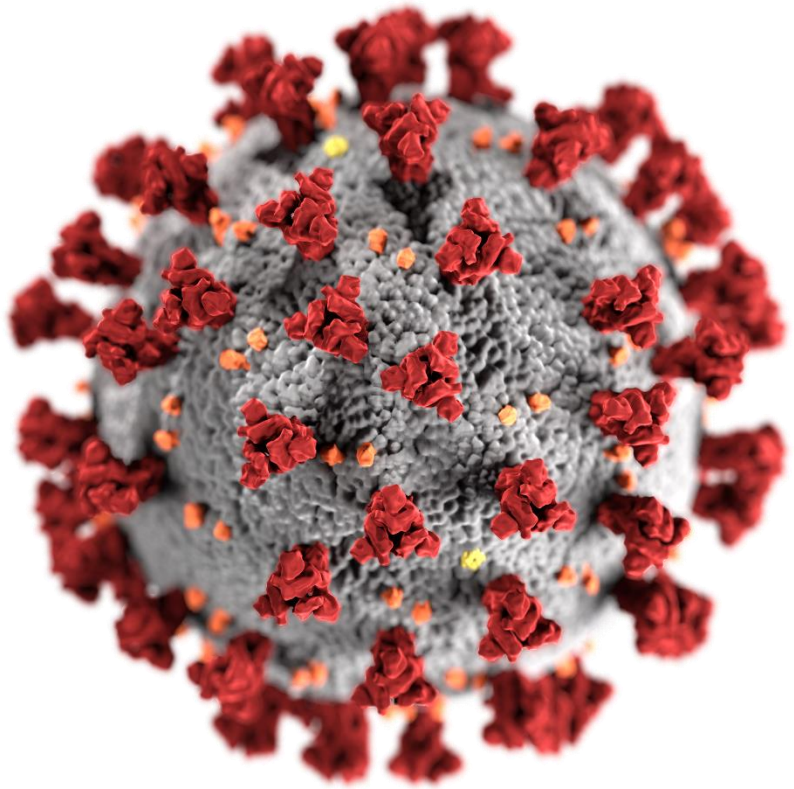
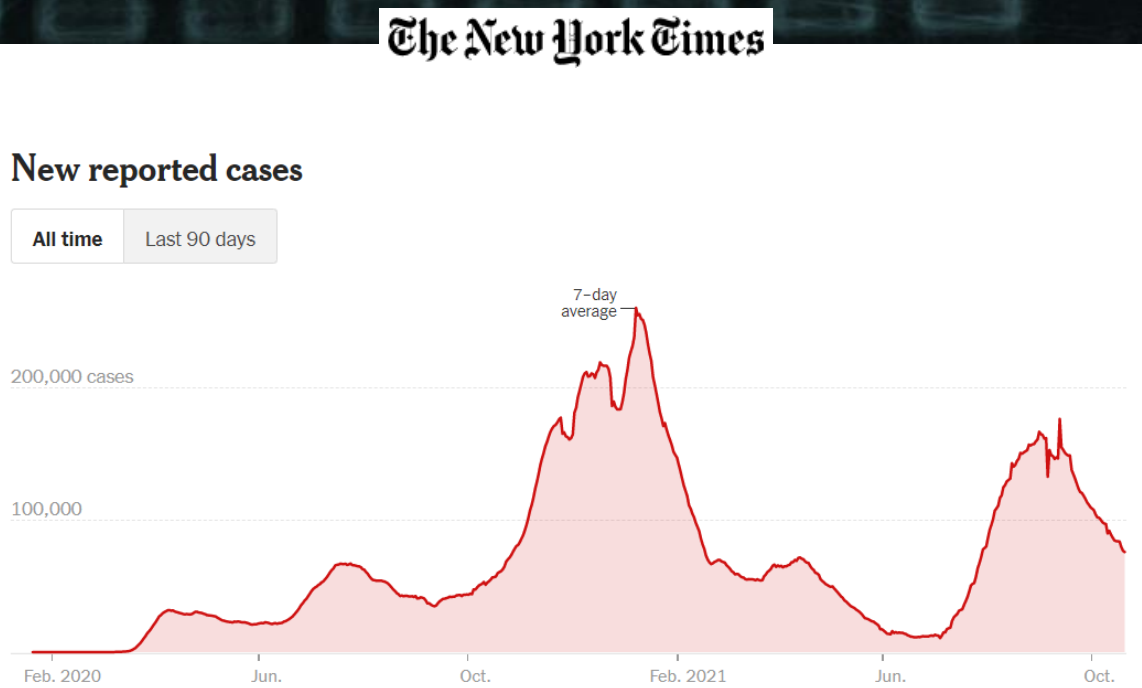



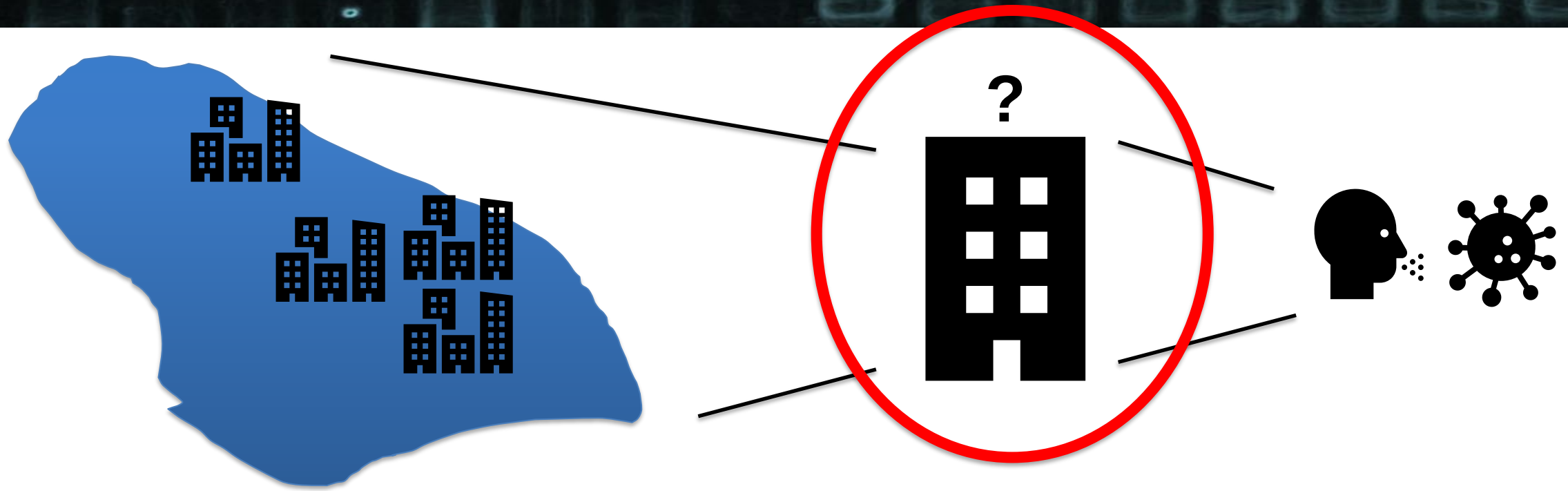
Image: CDC PHIL

# In future years: Long-term monitoring needs

- SARS-CoV-2 may continue to cause future outbreaks
- Vaccination reduces need for individual testing
- Long-term monitoring in high-risk areas needed
  - Variants that evade immunity
  - Individuals without immunity

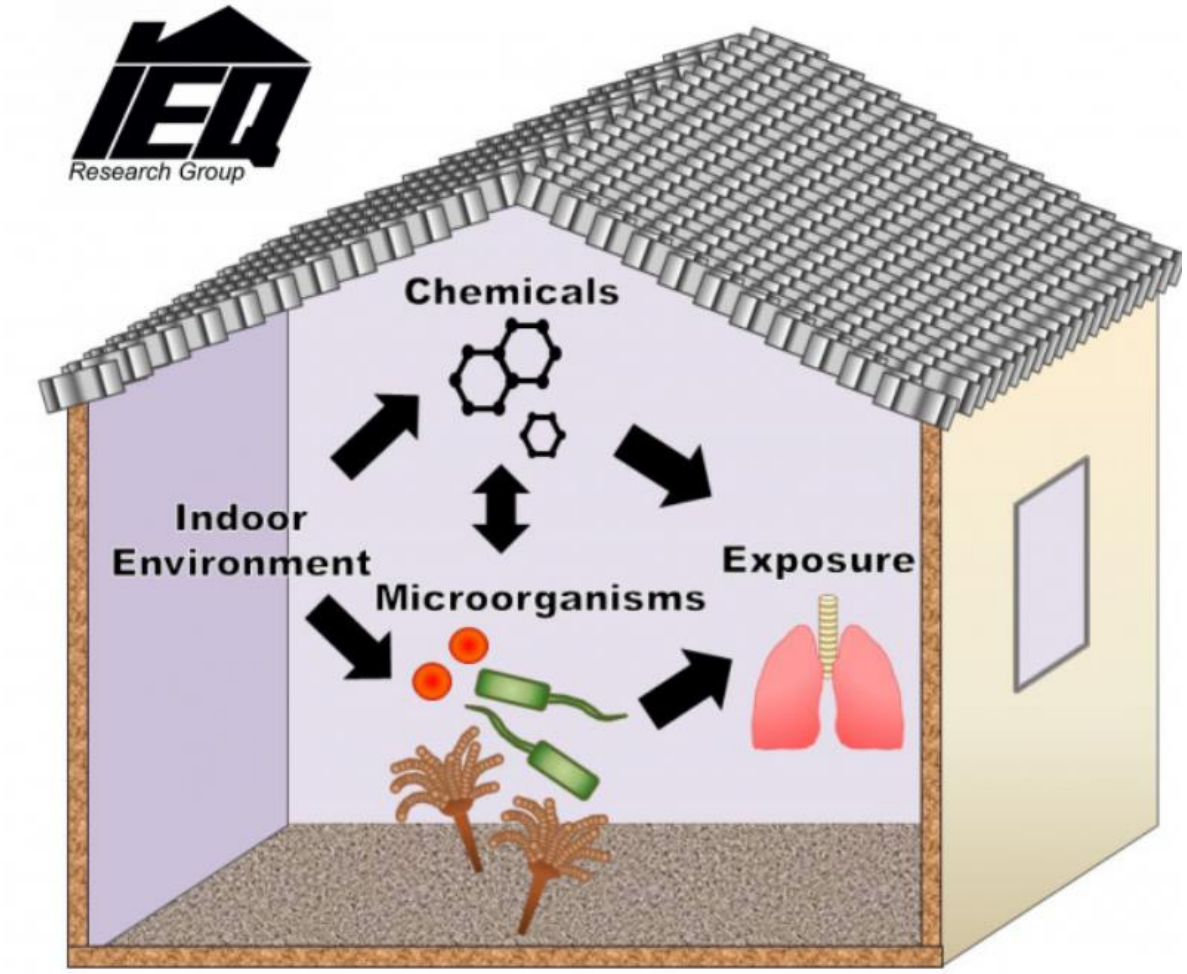


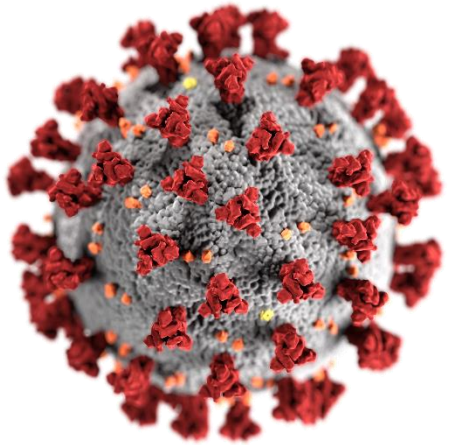
# We need a new long-term surveillance solution




**Solutions:** Wastewater Monitoring | Building Monitoring???? | Individual Testing

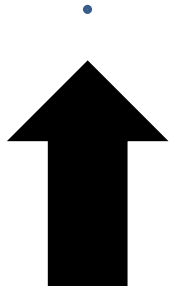
# Indoor Environmental Quality



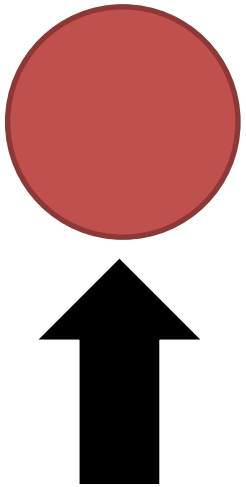



# Most spread occurs indoors: Where does the virus go?

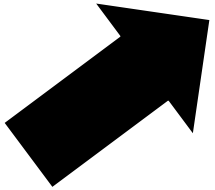
# Viruses are in a wide range of particle sizes



Volatile Organic Compound (VOC) (things that you smell)



Virus, 0.12 micrometer (not usually floating around alone)



Droplet from breathing/coughing/ sneezing/talking that can contain virus (0.1-100 micrometer)

0.1 micrometer

# Particle settling time depends on size

Settles faster

Settles slower

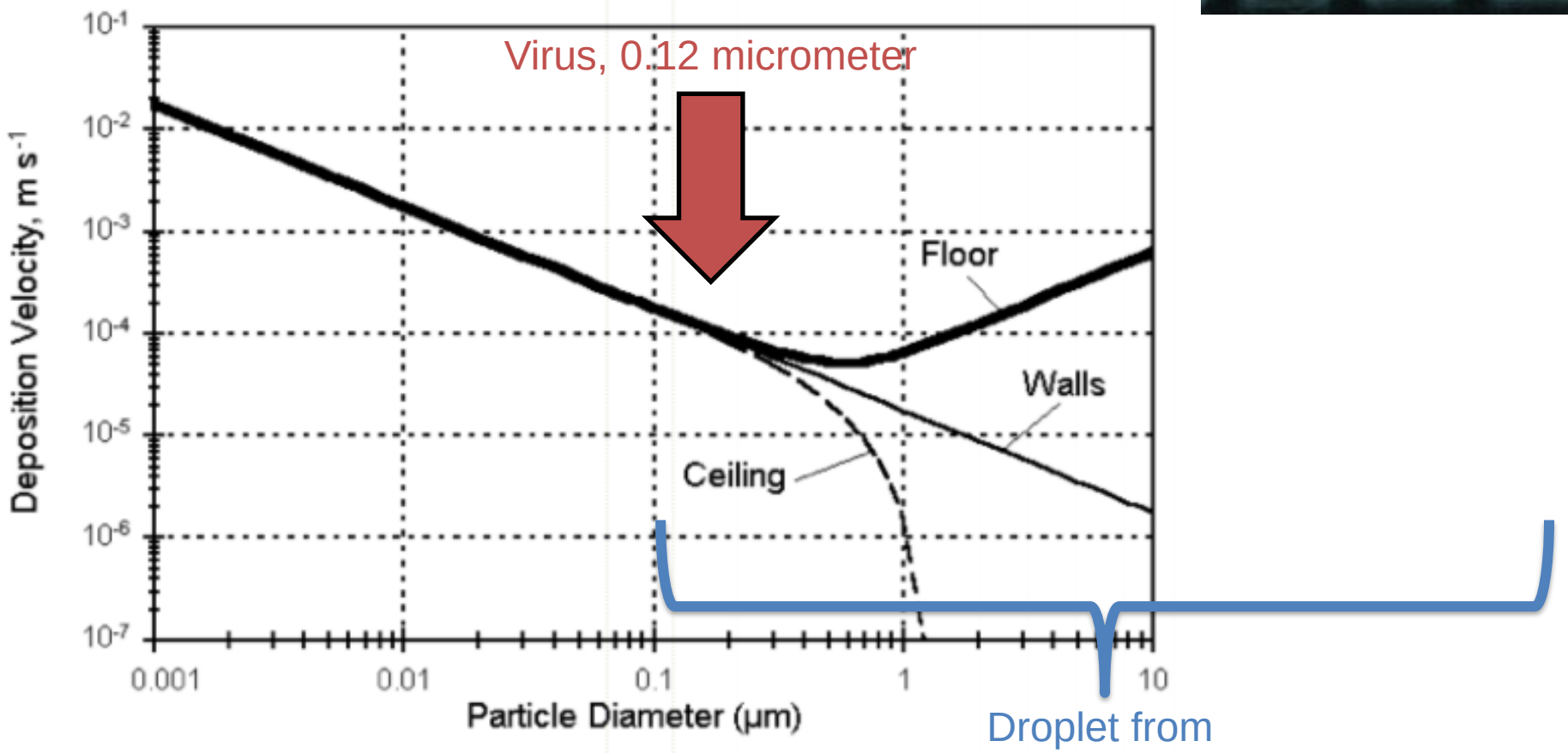



**Figure 19-3. Idealized Patterns of Particle Deposition Indoors.**

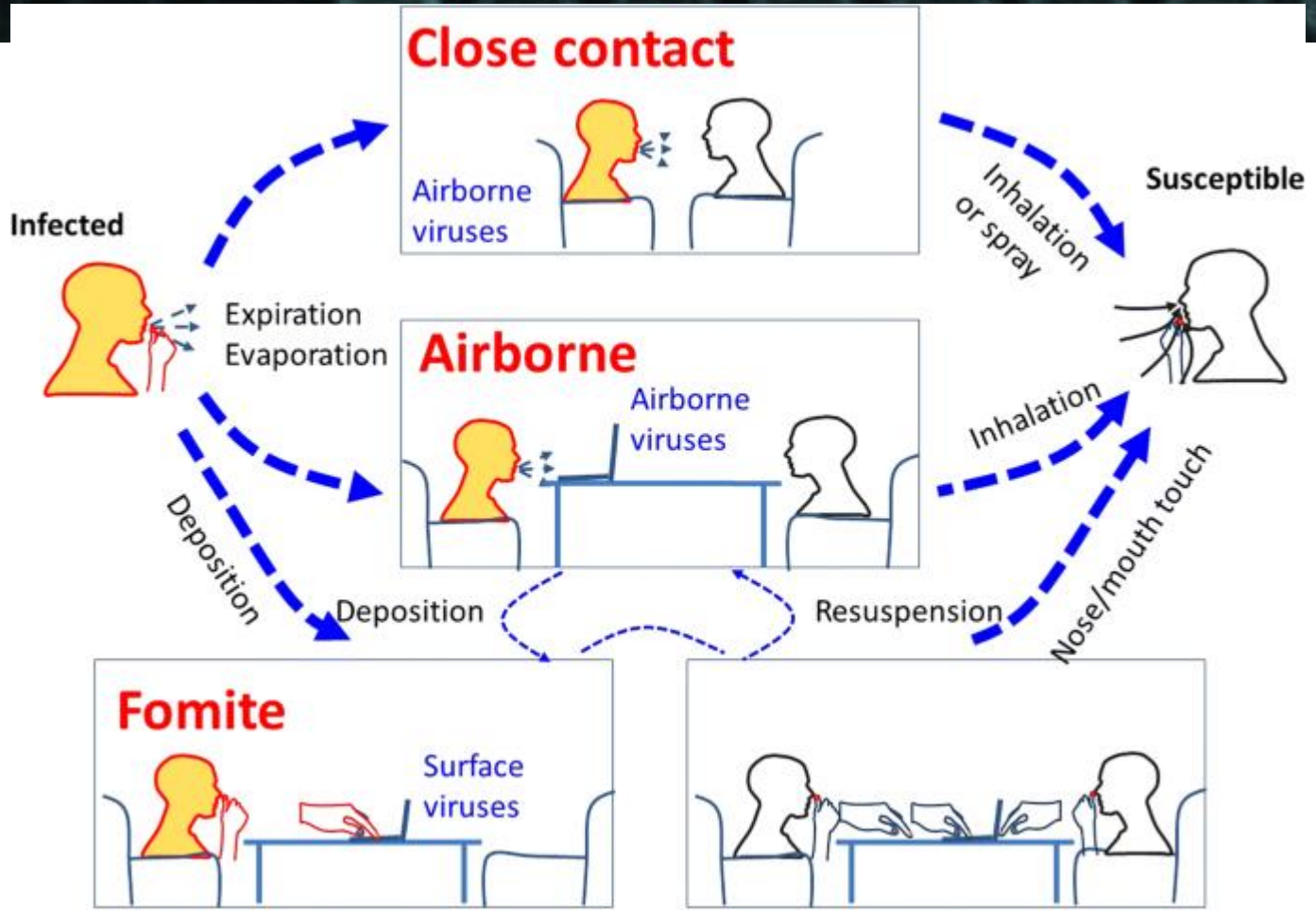
Source: Adapted from Nazaroff and Cass (1989b).

Droplet from breathing/coughing/ sneezing/talking that can contain virus (0.1-100 micrometer)

EPA Exposure Factors Handbook, 2011

# Viral transmission occurs via multiple routes

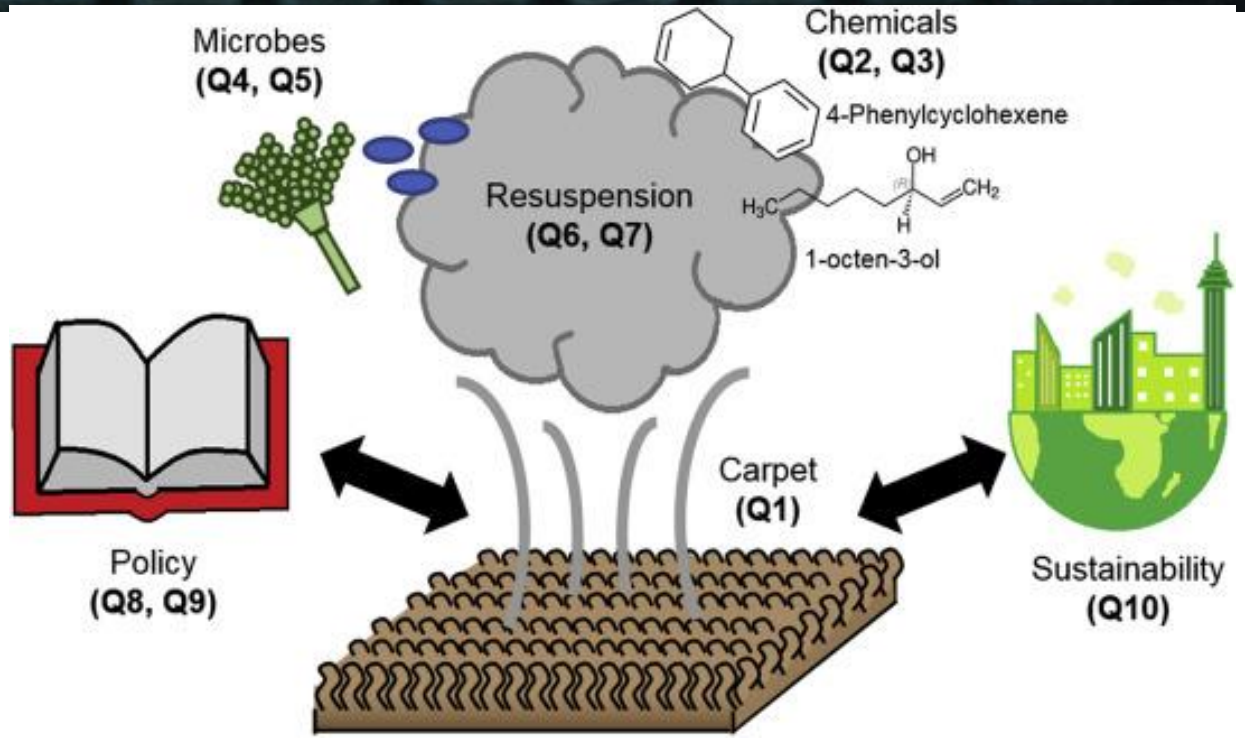
- Droplet
- Aerosol
- Direct contact
- Indirect contact




Tellier, R., Li, Y., Cowling, B.J. *et al.* Recognition of aerosol transmission of infectious agents: a commentary. *BMC Infect Dis* **19,** 101 (2019). https://doi.org/10.1186/s12879-019-3707-y

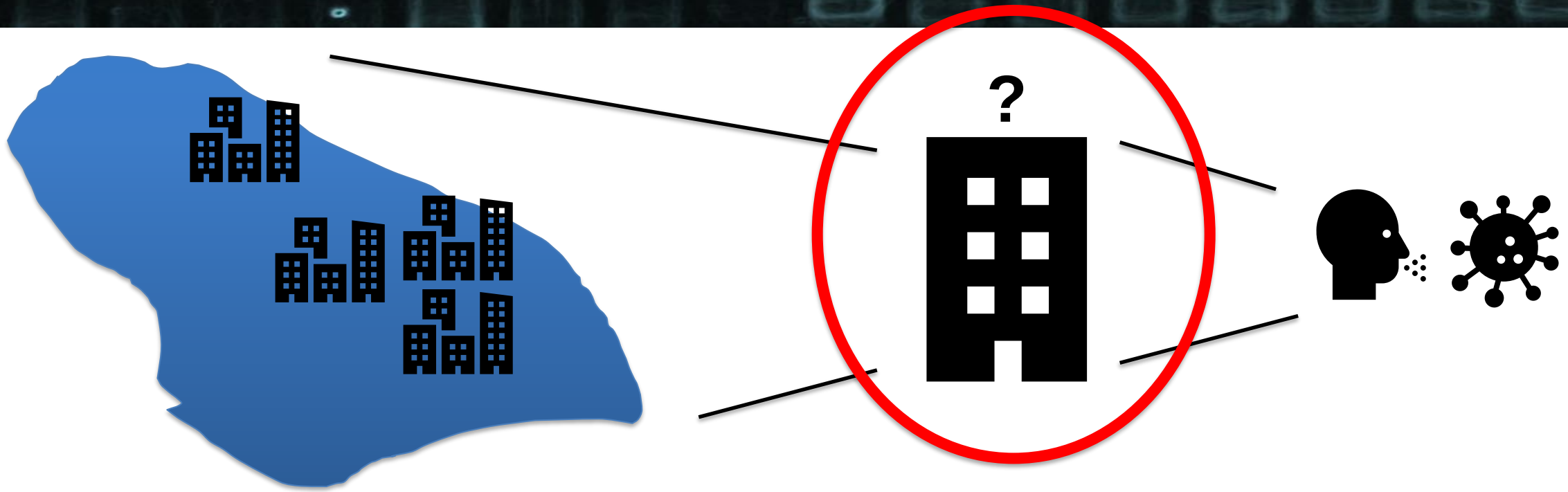
# Dust serves as a microbial reservoir

- Other viruses at high levels in floor dust (Koganti et al., 2016)
  - And SARS-CoV-2 on PM? (https://www.medrxiv.org/content/10.1101/2020.04.15.20065995v2)

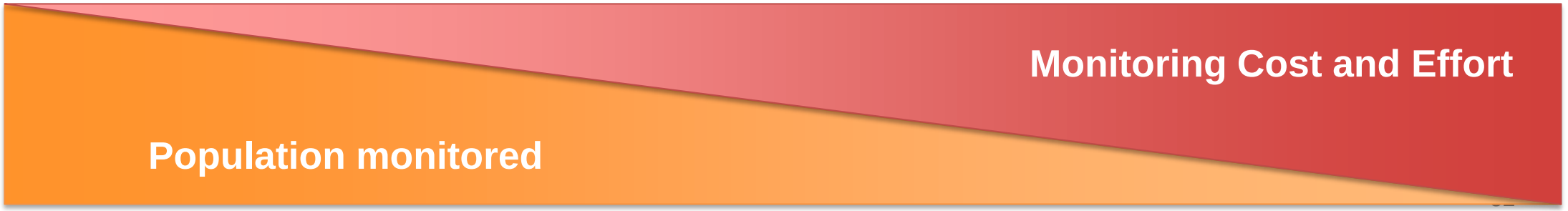



Haines et al, 2020, Building and Environment, https://doi.org/10.1016/j.buildenv.2019.106589

# We need a new long-term surveillance solution

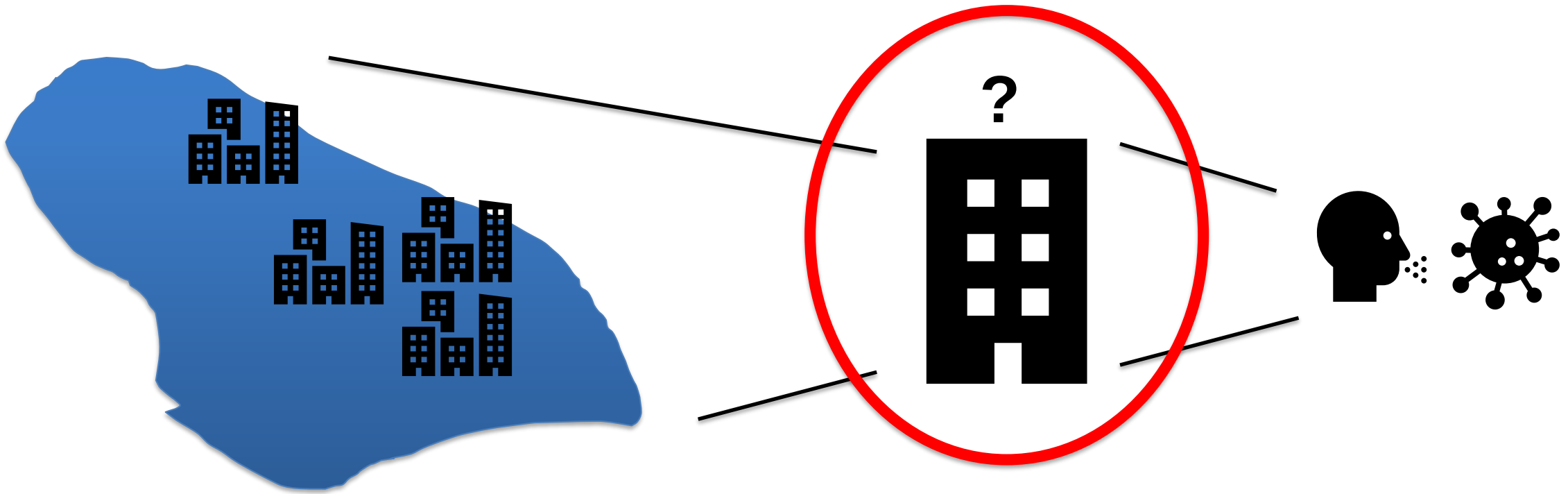



**Solutions:** Wastewater Monitoring | Building Monitoring???? | Individual Testing



# Goal: Dust as a matrix for outbreak surveillance

- Samples collected from rooms of students in isolation with positive test
- Bulk dust, surface swabs, passive sampler




**Solutions:** Wastewater Monitoring ?????????? Individual Testing

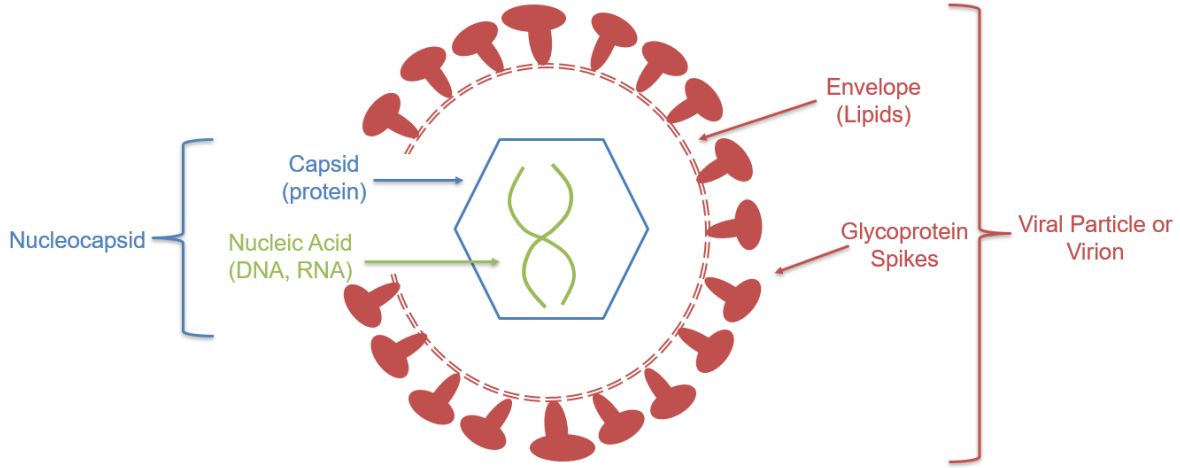
# RNA detection is different than infectivity

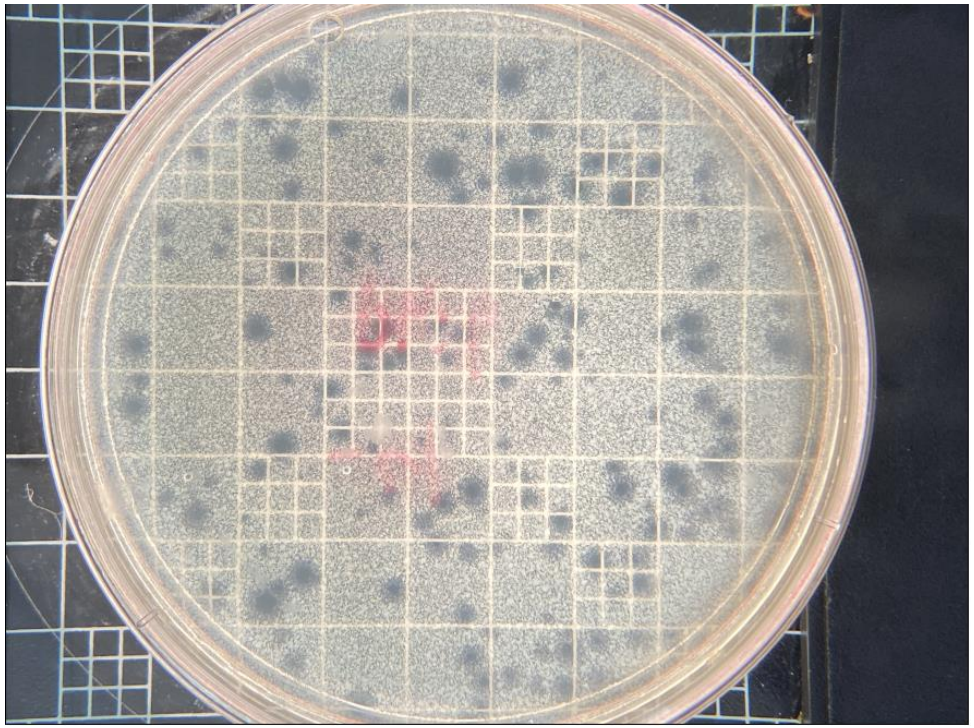
- Total viral particles → plaque forming units (PFUs)

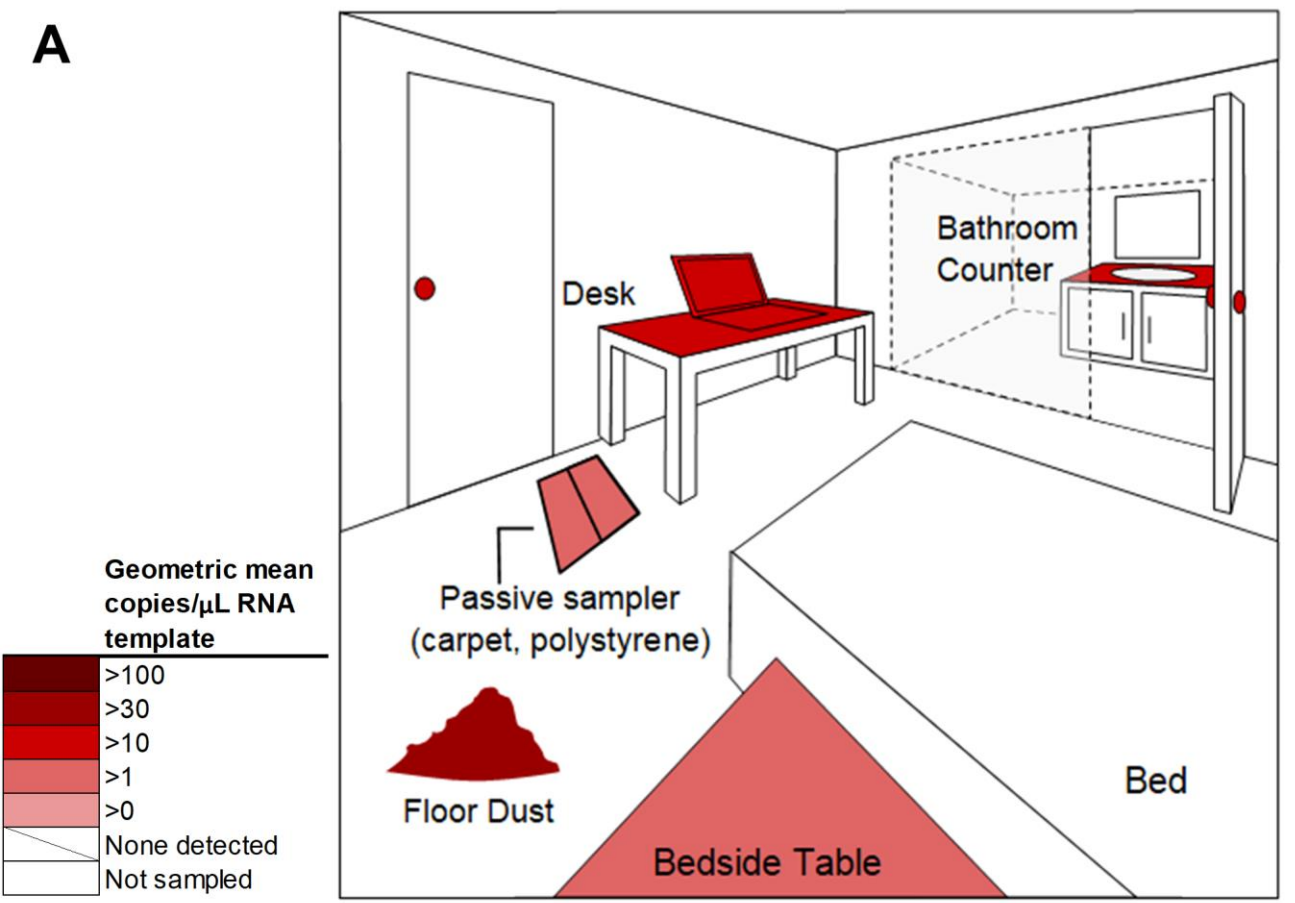
$\eta_{unknown}$ ? 

- Total viral particles → RT-qPCR value

$\eta_{unknown}$

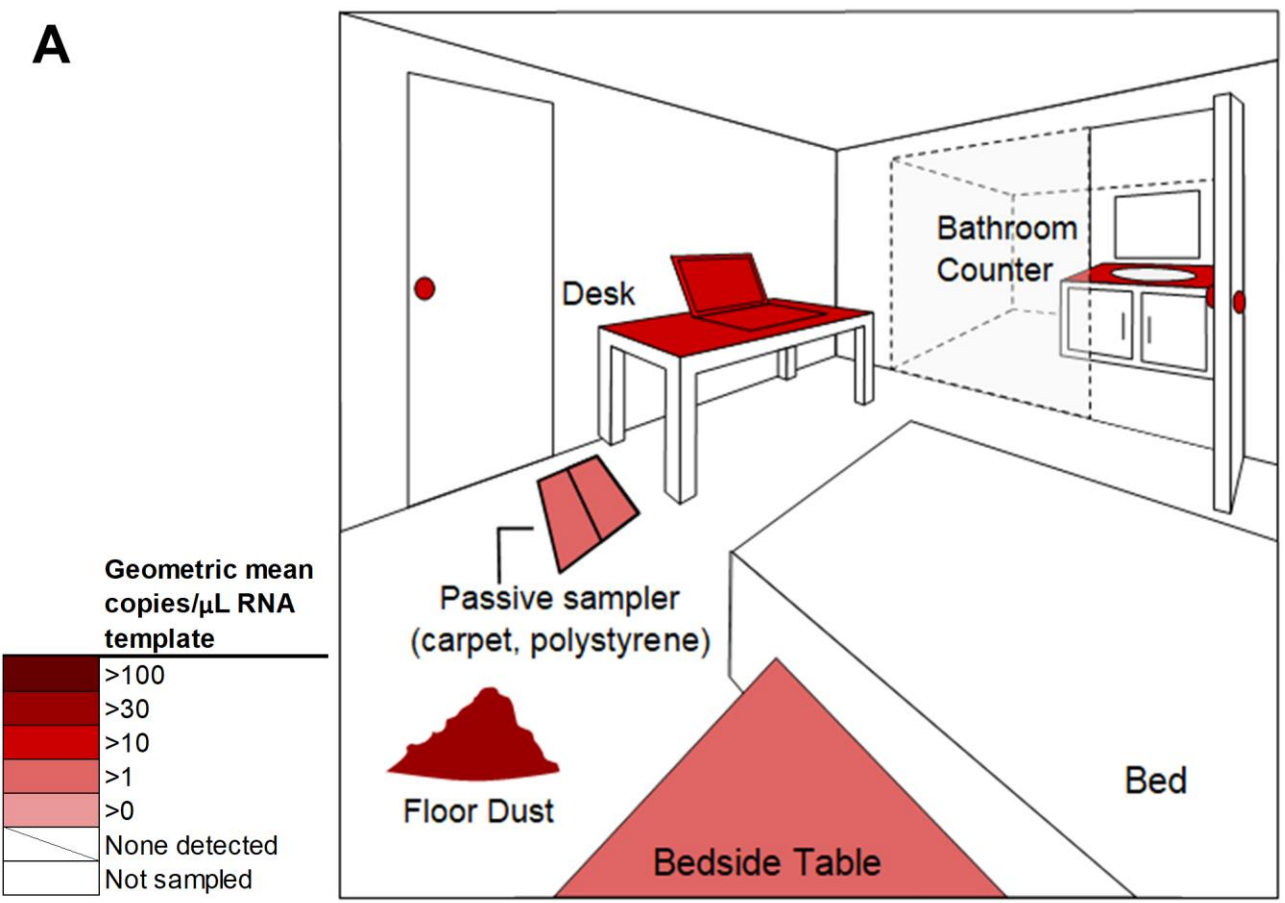


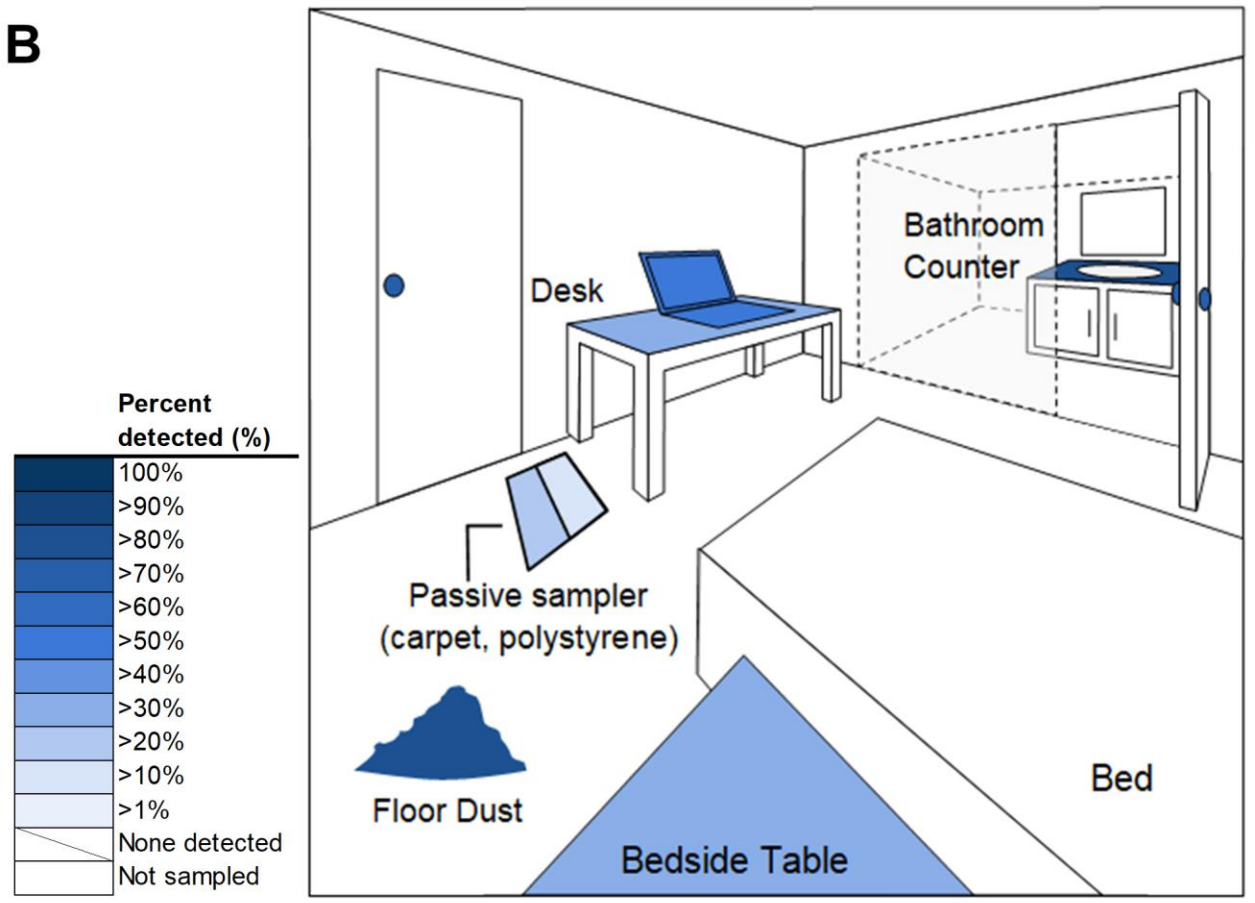





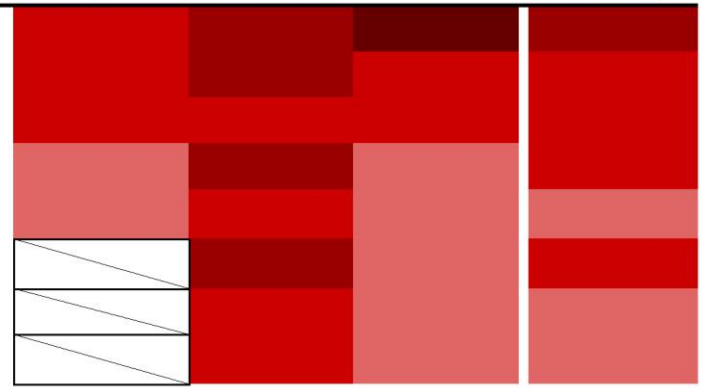

| Sample types | n (RT-qPCR, dPCR, ddPCR) | RT-qPCR | dPCR | ddPCR | Average |
|---|---|---|---|---|---|
| Floor Dust | 51,63,65 | | | | |
| Bathroom Counter | 1,2,2 | | | | |
| Door Knobs | 2,2,3 | | | | |
| Laptop | 1,4,2 | | | | |
| Polystyrene | 1,5,2 | | | | |
| Desk | 0,4,2 | | | | |
| Bedside Table | 0,2,1 | | | | |
| Carpet | 0,9,1 | | | | |

Renninger et al. *mSystems*, 2021



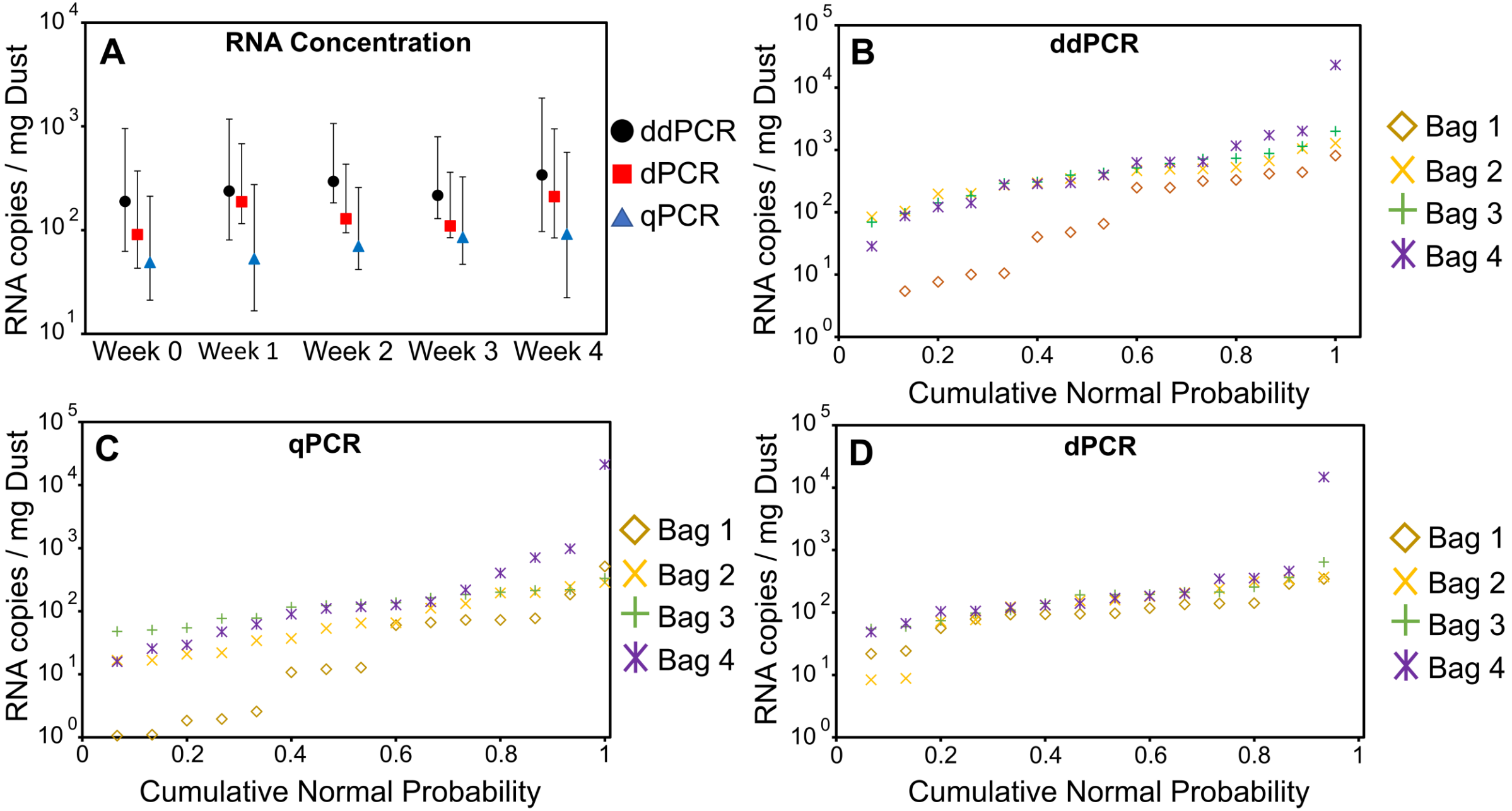




| Sample types | n (RT-qPCR, dPCR, ddPCR) |
|---|---|
| Floor Dust | 51,63,65 |
| Bathroom Counter | 1,2,2 |
| Door Knobs | 2,2,3 |
| Laptop | 1,4,2 |
| Polystyrene | 1,5,2 |
| Desk | 0,4,2 |
| Bedside Table | 0,2,1 |
| Carpet | 0,9,1 |




| Sample types | n | RT-qPCR | dPCR | ddPCR | Average |
|---|---|---|---|---|---|
| Floor Dust | 67 | | | | |
| Bathoom Counter | 2 | | | | |
| Door Knobs | 3 | | | | |
| Laptop | 4 | | | | |
| Desk | 6 | | | | |
| Bedside Table | 2 | | | | |
| Polystyrene | 7 | | | | |
| Carpet | 18 | | | | |

Renninger et al. *mSystems*, 2021

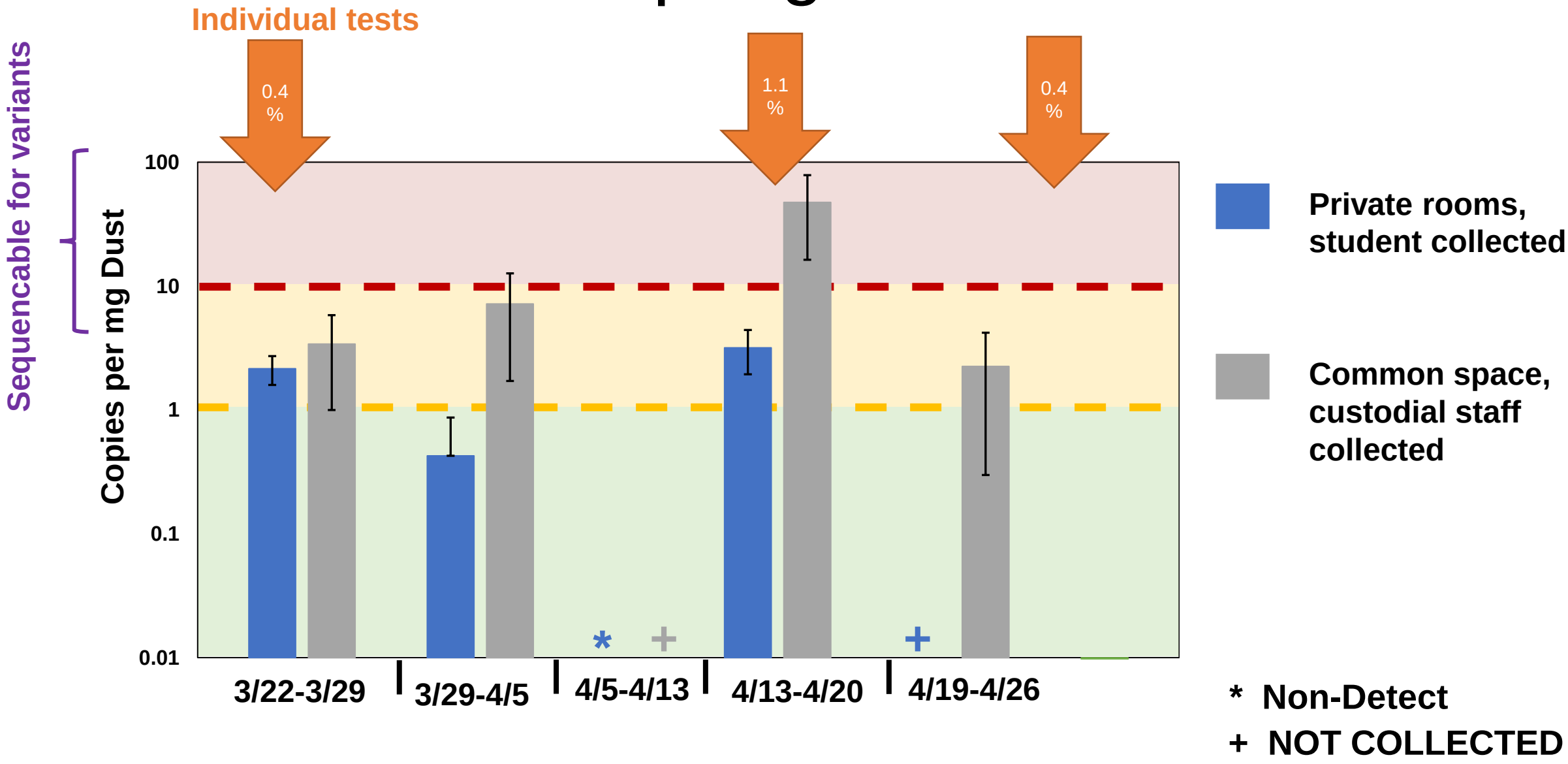



Renninger et al. *mSystems*, 2021

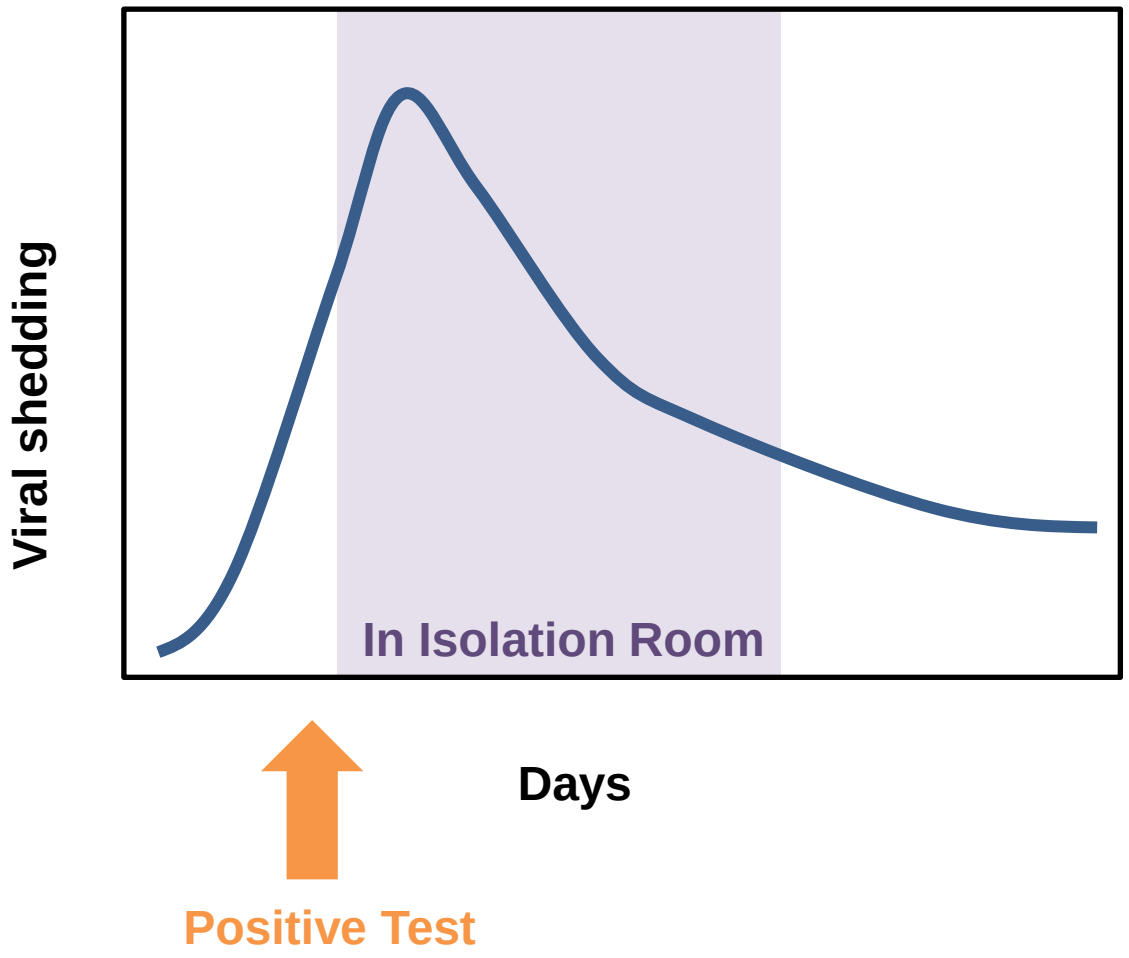
# Disinfectant

- Sodium dichloro-s-triazinetrione
- Non-selective oxidizer
  - Destroys protein
  - Mixed literature on RNA

# Pilot data from spring – Residence Hall A



# Viral shedding occurs over time



# Variant Identification

| Sample ID | clade | Lineage | substitutions | deletions | insertions |
|---|---|---|---|---|---|
| B2_T4_1 | 20C | B.1.2 | C241T,C1059T,G1358A,C14408T,A18424G,G25563T,C27964T,C28869T | | 10713:TAAGTCTGATGTGAAAGCCCACGGCT |
| B1_T4_2 | 20C | B.1.526 | C241T,C1059T,C3037T,T9867C,C14403T,C14408T,G15243T,A16500C,A20262G,C21575T,C21846T,A22320G,G23012A,A23403G,C23664T,C25517T,G25563T,C27925T,C28410T,C28869T,G28975A | 11288-11297,28274 | |

Aug 26, 2021

# Dust collected from campus buildings will help track COVID-19

*Researchers will track variants, prevalence of virus*

**Laura Arenschield**
Ohio State News
arenschield.2@osu.edu

Researchers are collecting dust from 50 buildings on The Ohio State University campus this fall to monitor the prevalence of COVID-19 and track the virus's variants. Their analyses and experiments are designed to help the university understand where COVID-19 pockets might exist as the campus opens to near-pre-pandemic levels this fall.

Their study is built on previous research that showed that RNA — part of the genetic material from inside the COVID-19 virus — can persist up to a month in dust. For that study, researchers collected dust from residence hall rooms on campus that housed students known to be infected with COVID-19.

Additional research showed viruses do not remain infectious in dust, leaving only the genetic material behind. Analyzing that genetic material offers a noninvasive, low-cost way of monitoring for COVID-19.

"The dust will show if you have even a small number of infected individuals in a building," said Karen Dannemiller, senior researcher on this study and associate professor of civil,

# So what does this mean about viability in dust and on surfaces?

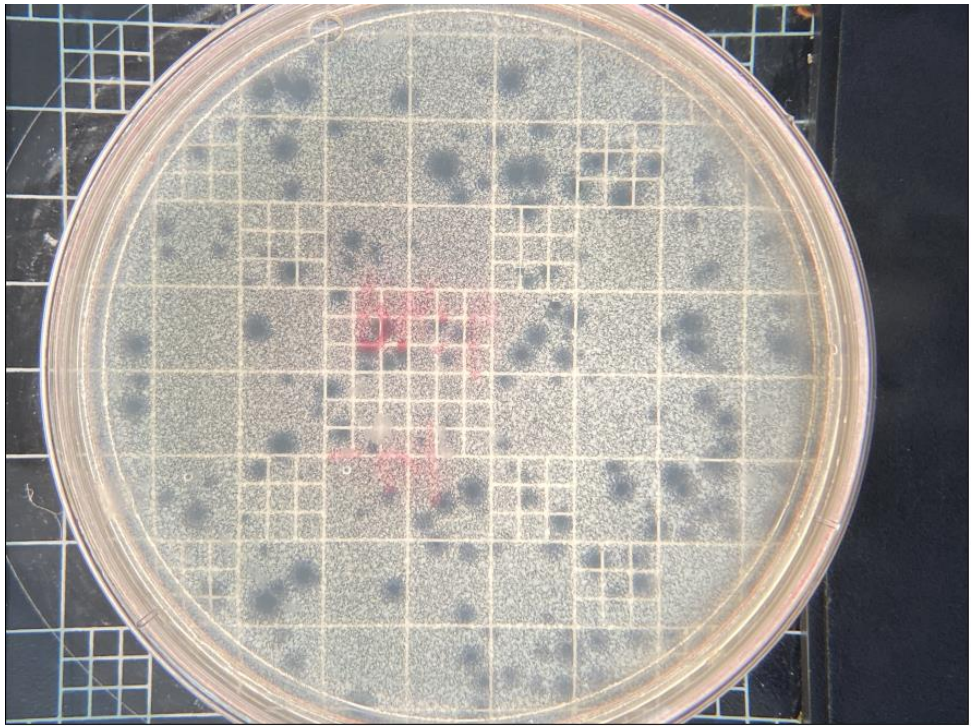
Essentially Nothing.

- Total viral particles → plaque forming units (PFUs)

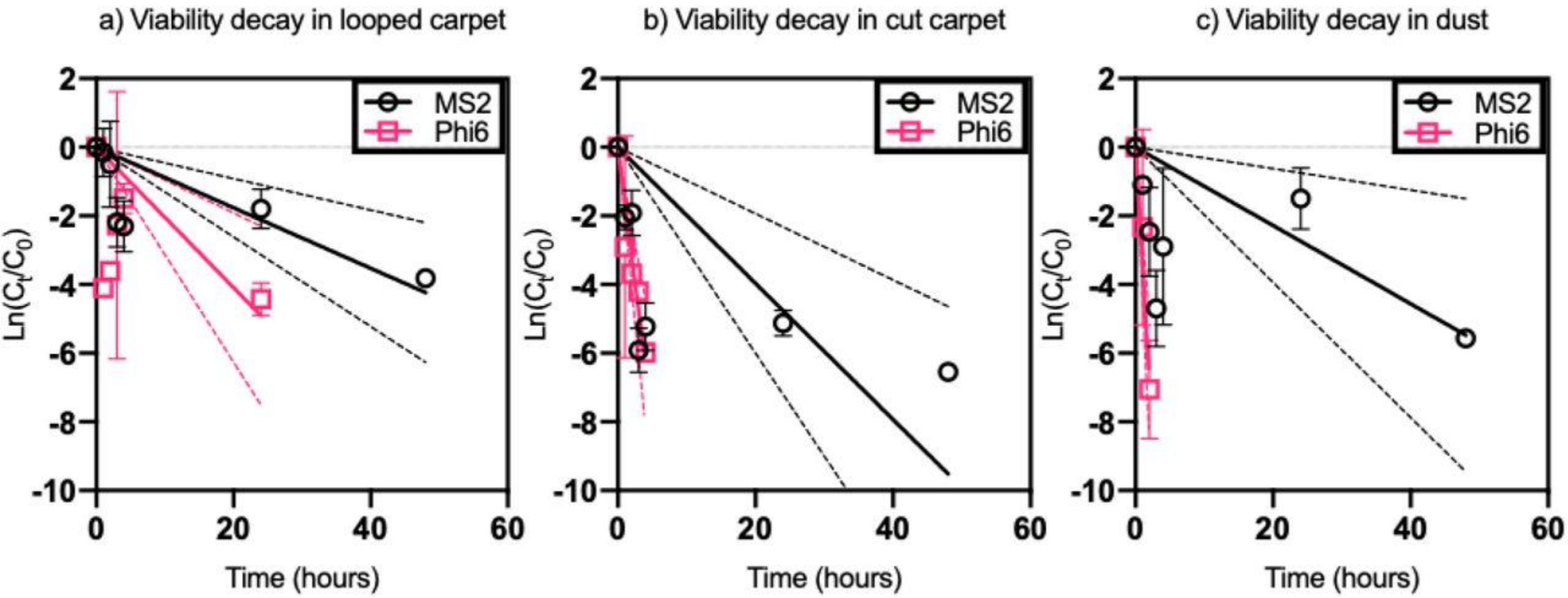
$\eta_{unknown}$ ? 

- Total viral particles → RT-qPCR value

$\eta_{unknown}$

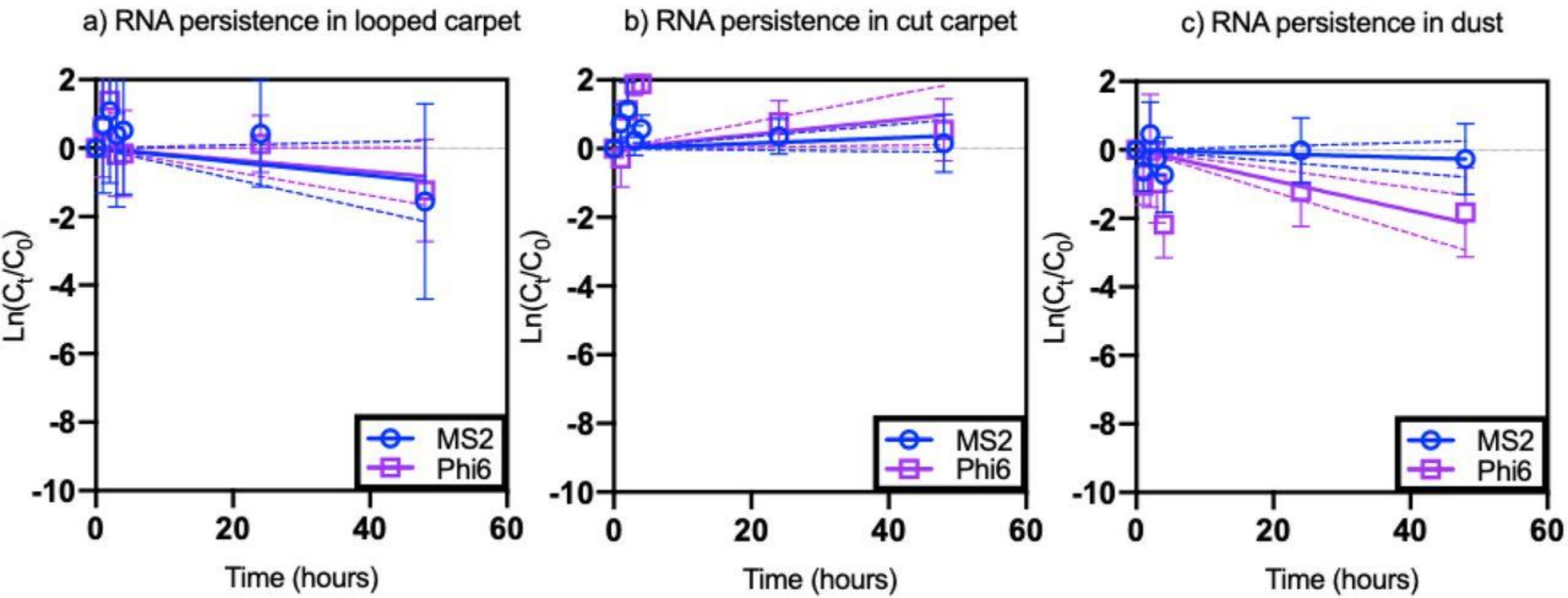


# Enveloped viruses decay more quickly than non-enveloped




https://www.biorxiv.org/content/10.1101/2021.05.17.444479v1

# RNA persists




https://www.biorxiv.org/content/10.1101/2021.05.17.444479v1

# Dust can complement Wastewater Monitoring

**Pros**

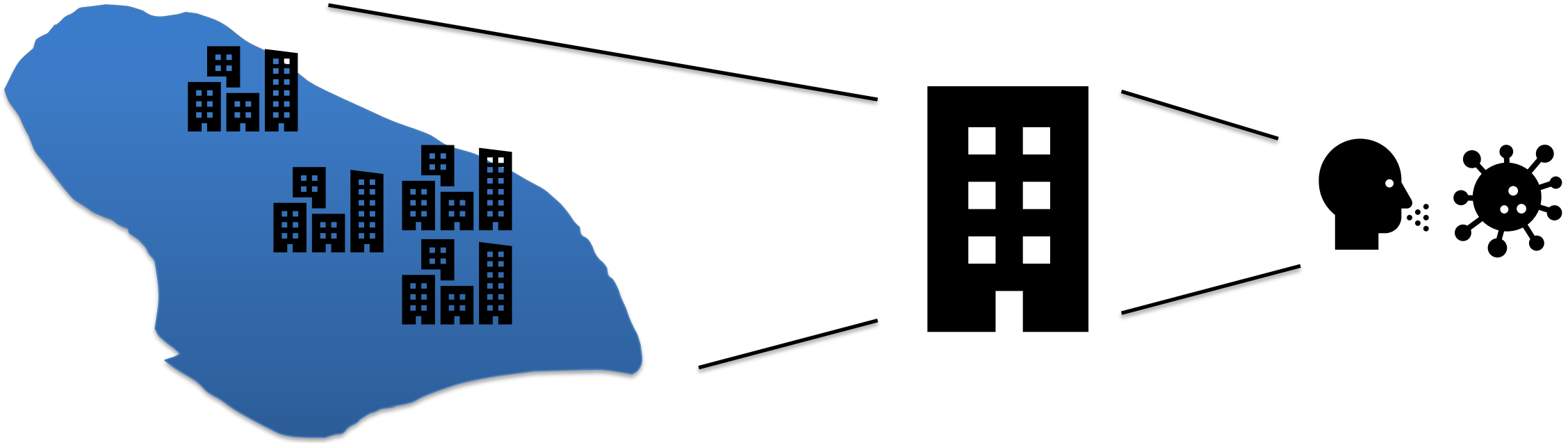
- Monitor large or small population
- 1 in 100 to 2,000,000 individuals detectable

(https://doi.org/10.1016/j.virusres.2020.198147)

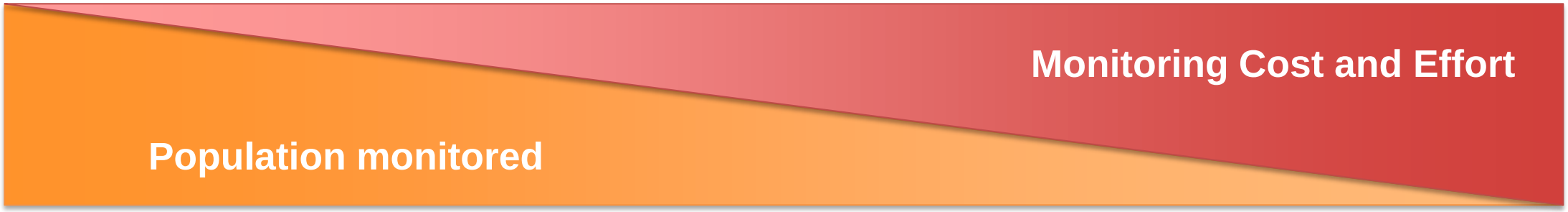
**Cons**

- Difficult sample collection
- Pre-concentration steps
- Not everyone sheds virus in feces

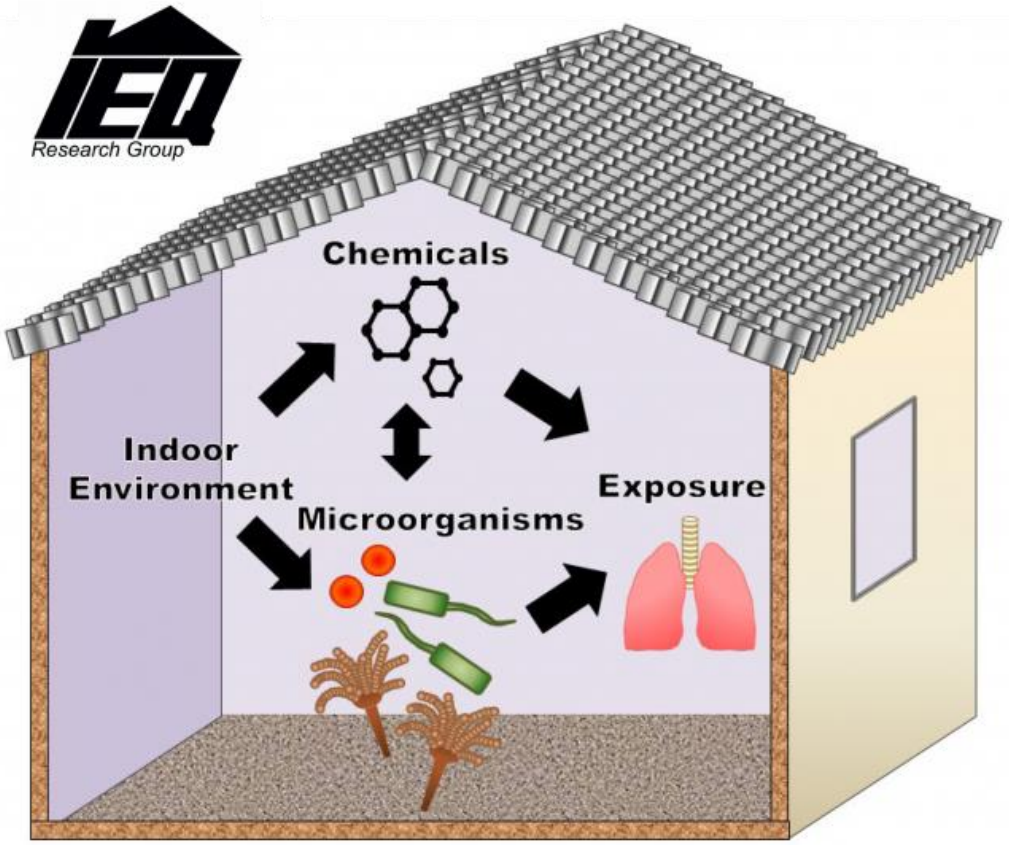
# Dust monitoring: A new long-term surveillance solution



**Solutions:** Wastewater Monitoring | Building Dust Monitoring | Individual Testing



# Summary




- Elevated moisture is sufficient to support microbial growth and function in dust
- Chemical emissions indoors are dominated by materials, but smells may come from microbes
- Dust is an efficient matrix for COVID-19 surveillance

# Acknowledgements

National Science Foundation
WHERE DISCOVERIES BEGIN

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NIEHS

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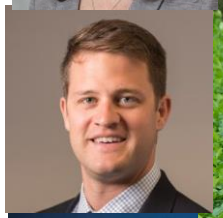
An OH·TECH Consortium Member



Colleagues
Students
Study participants

Team members not pictured: Adnan Divjan, Luis Acosta, and Rachael Rush

# Questions?

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