

THESIS

CHARACTERIZING MOLD VOCS IN RESIDENTIAL STRUCTURES IMPACTED BY FLOOD

Submitted by

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## ABSTRACT

### CHARACTERIZING MOLD VOCS IN RESIDENTIAL STRUCTURES IMPACTED BY FLOOD

Mold growth is a health concern for people re-entering their homes after a flooding event. Mold exposure can be hazardous, especially for people with asthma. Mold produces volatile organic compounds (VOCs) as it grows, and those VOCs can be used to detect the presence of mold. While VOC profiles of mold have been constructed in laboratory settings, there has been little work with samples directly from the field. VOC samples were taken from the homes of 55 Houston residents. 33 homes had been flooded, and 22 had not. The VOCs were analyzed using GCMS and identified using a NIST library of mass spectra. The VOCs found in flooded homes were compared to VOCs found in unflooded homes. There was a difference in VOCs identified, and the concentration of those VOCs, in flooded versus non-flooded homes, and some of those VOCs have been previously associated with mold growth. However, the origin of those VOCs is still not clear. Further work should include associating the VOCs found with the maximum water levels in the flooded homes, and with health data collected from the participants.

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## CHAPTER 1: INTRODUCTION

The presence of mold can be harmful for human health. Mold can impact the respiratory tract, induce inflammation and allergic response, and exacerbate asthma symptoms (Mudarri & Fisk, 2007; Portnoy et al., 2005). American's spend most of their time in their homes (Klepeis et al., 2001), so the detection of mold is important for resident's health.

Mold often grows in homes in the wake of flooding events (Emerson et al., 2015). In 2017, Hurricane Harvey made landfall on Texas' gulf coast, hitting Houston with up to 56 inches of rainfall over five days (US Department of Commerce, n.d.). Harvey produced the most rainfall of any hurricane at the time (Emanuel, 2017). The rainfall resulted in significant flooding, with 90% of River Forecast Points in southeast Texas experiencing flooding, and 46% setting records for the highest recorded river height (US Department of Commerce, n.d.). Since Hurricane Harvey, seven major hurricanes (defined as Category 3 or higher) have made landfall on the continental United States (*US Hurricane Landfalls*, n.d.). The frequency and intensity of hurricanes are expected to increase with rising global (Emanuel, 2017; Prein et al., 2017), which exacerbates extremes in precipitation (Labonté & Merlis, 2023).

Along with economic impacts, flooding can also have health impacts. Flooding of structures introduces dampness, which increases the level of microbes inside the structure (Bloom et al., 2009; Dumon et al., 2009; Emerson et al., 2015; E. K. Jones et al., 2013). Given the negative health outcomes of the presence of mold, it is important to characterize mold growth in flooded homes. As severe flooding events occur more frequently, rapid, low-cost methods for fungal detection are critical. When mold grows, the growth process produces microbial

volatile organic compounds, or MVOCs (D. Zhang et al., 2022). Characterizing these MVOCs can provide information about the presence of mold (Falzone et al., 2017). Using MVOCs to detect mold growth has advantages over traditional microbial analyses, where mold spores are captured and characterized either by culture or quantitative polymerase chain reaction (Falzone et al., 2017; Garcia-Alcega et al., 2017). Since VOCs are smaller than mold spores, they can penetrate building materials, making it easier to detect mold growing within walls and under floors.

MVOCs have been characterized from mold samples taken from flooded homes and then grown in a laboratory setting, but there is little information about MVOCs from the homes themselves (Zhao et al., 2017). By developing an association between MVOC profiles and other conditions (whether the home was flooded or not, amount of flood water, source of flood water, remediation, and microbial identification), the MVOC profiles can be used in future flooding events to provide residents with vital information about the mold conditions of their homes.

The research objectives are to characterize volatile organic compounds present in flood damaged and undamaged homes and ascertain if there are discrete MVOC profiles, or fingerprints, for identifying mold growth based on water damage. These MVOC fingerprints will be used in conjunction with health-based measurements and inhaler deployment of study participants to stratify risk provide an actionable path forward for mitigating and controlling exposures in homes impacted by future floods. To complete these objectives, we conducted exposure and health assessments with asthma cohort in Houston that experienced varying degrees of flooding (i.e., from hurricanes and other extreme precipitation events) to evaluate

the presence of MVOCS and its potential to exacerbate sub-acute markers of asthma. We hypothesize that homes that were flooded will have characteristic MVOCs, correlated with mold growth, that can be differentiated from undamaged homes.

## CHAPTER 2: REVIEW OF LITERATURE

### 2.1 Indoor Air Quality

An interest in indoor air quality started during the industrial revolution, as society moved from mostly outdoor work to work in factories and warehouses (Sundell, 2017). Now, between their homes, places of work, and recreation, it is estimated that Americans spend 90%-93% of their time indoors (Klepeis et al., 2001; Report to Congress on Indoor Air Quality. Volume 2. Assessment and Control of Indoor Air Pollution. Final Report, 1989). Of the time spent indoors, people spend most of it in their own homes. In addition to the traditional uses of the home for sleeping, family, and personal time, the COVID-19 pandemic turned the home into the workplace for many people. In 2022, 34% of workers did some or all of their work from home, an increase from pre-2020 numbers (*American Time Use Survey Summary - 2022 A01 Results*, n.d.).

The amount of time spent in the home means that the quality of the air can have a heavy impact on the health of the residents (Bonney, 2007). Contamination can be physical (particulate matter), radioactive (radon), chemical (aldehydes, nitrous oxides, volatile organic compounds) and biological (mold, infectious agents) (Bonney, 2007; A. P. Jones, 1999).

Given the role of VOCs in indoor contamination, it is important to understand all the possible sources. The materials the structure is built from may introduce VOCs to the air (Gallon et al., 2020; Kristensen et al., 2019). Human activities like cooking or cleaning also influence VOC concentrations (Kristensen et al., 2019). More houses are being built with energy efficiency

in mind, and airtightness is one strategy used. However, airtightness results in increased VOC concentrations (Blaszczok & BARANOWSKI, 2018; Kempton et al., 2022).

Increased VOCs in the air can be irritating to residents, and certain VOCs are associated with specific upper respiratory symptoms (Burge, 2001; Heseltine et al., 2009; Wang et al., 2023). High level of VOCs in the air can lead to higher rates of building related symptoms (BRS) (Suzuki et al., 2021). People with asthma experience a decline in lung function when exposed to VOCs, and VOCs in the presence of microbial growth have a synergistic effect on asthma symptoms (Becher et al., 1996; Wang et al., 2023).

Poor indoor air quality can also have psychological impacts. Perceiving a high concentration of VOCs (primarily through smell) is linked to a perceived deterioration of the environment (Becher et al., 1996). Indoor malodor is associated with negative mood, stress, and social outcomes (Dalton et al., 2020).

## **2.2 Mold Contamination**

Mold contamination is often found in flooded homes, including homes impacted by hurricane-related flooding (Emerson et al., 2015; Solomon et al., 2006). Excess water soaks into the building materials, raising the humidity and providing an environment that encourages fungal growth (Haines et al., 2021). Higher than normal levels of mold have been found in homes that experienced flooding during Hurricane Katrina (Bloom et al., 2009; Solomon et al., 2006). Several different mold species have been identified in flooded homes, the most common of which are *Cladosporium*, *Alternaria*, and *Stachybotrys chartarum* (Dumon et al., 2009). The EPA created the environmental relative moldiness index, or ERMI, as a tool to help distinguish houses with a high mold burden (US EPA, 2021). ERMI uses the ratio of

concentrations of 26 mold species related to visible mold growth and water damage (group 1) and 10 not related (group 2) to create a relative number that, when compared against a national database, can provide information about the relative mold burden of a home (S. Vesper, 2011).

### **2.3 Microbial VOCs**

When mold grows, volatile organic compounds (VOCs) are produced as a secondary metabolite, (Burge, 2001; Lemfack et al., 2018; D. Zhang et al., 2022). Compounds associated with mold growth are known as mold VOCs (MVOCs). Many different kinds of compounds are represented in MVOCs, but the most common are ketone, aldehydes, alcohols, and hydrocarbons (Alcega et al., 2017).

While MVOCs cannot quantify the amount of mold in an environment, their presence can be used to determine the presence of mold qualitatively (Fabian et al., 2005). Different species of mold release different VOCs (Erlor et al., 2018; Fischer et al., 1999). It seems unlikely that a mold species will ever be identified by just one compound, but profiles and patterns of MVOCs are species dependent, and could be used for identification (Falzone et al., 2017; Haines et al., 2021; Radványi et al., 2020). MVOCs might also be able to detect microbial growth that cannot be detected using traditional methods, due to the ability of VOCs to permeate building materials (Hachem et al., 2010; Moularat et al., 2008)

There are several factors that influence the profile of MVOCs other than the species. The substrate the mold is grown on can change the VOCs produced (Betancourt et al., 2013; Haines et al., 2021; Wheatley et al., 1997). For example, the same strain of *S. chartarum* has been shown to produce different concentrations of anisole and 3-octanone depending on if it

was grown on gypsum wallboard or ceiling tile (Betancourt et al., 2013). The age of a colony also affects the profiles of the VOCs; Bjurman et al. (1997) found that a common MVOC, 1-octen-3-ol, was only present in the later growth stages of *P. brevicompactum* (Bjurman et al., 1997). Humidity increases the amount of MVOCs produced, and mold growing in dry conditions produces different MVOCs than in wet conditions (Choi et al., 2017; Falzone et al., 2017). The season can also affect the MVOCs, as some molds grow more rapidly when temperatures are higher (Fischer et al., 2000).

MVOC analysis is usually performed by collecting a sample of the air on a tube filled with absorptive material, and then analyzing that sample using GCMS (Alcega et al., 2017; Lavine et al., 2012). The collection can be passive or active (Alcega et al., 2017; McAlary et al., 2015). The VOCs can be extracted from the absorptive material using solvents such as carbon disulfide ( $\text{CS}_2$ ), but the extraction will also dilute the target VOCs, which can be problematic when looking for trace amounts of analytes (Elke et al., 1999). Another option for extraction is thermal desorption. A thermal desorber heats the absorptive material and collects the gas-phase VOCs that evaporate off, before directing them to the analytical instrument (Vera et al., 2022). There are several combinations of analytical instruments that can be used for VOC analysis, but most consist of a separator and a detector. The separator is either a gas (GC) or liquid (LC) chromatograph. GC is the best option for VOCs. GC columns are significantly narrower than LC columns and use a temperature gradient instead of a solvent gradient to separate compounds by their physical characteristics. This is a better option for VOCs due to their small size and low boiling point. The two most common detectors are a flame ionization

detector (FID) and a mass spectrometer (MS). Both can be used, but an FID cannot provide mass information, and therefore cannot be used for unknown analysis.

While current analytical methods are reliable and convenient, faster results could be obtained in the future using real-time analysis. Electronic noses are already being used to detect VOCs in buildings (Garbacz et al., 2020; Majerek et al., 2017; Szulczyński & Gębicki, 2019). Gas sensors show promise for early detection of mold growth (Łagód et al., 2018; Suchorab et al., 2019). While there is potential for electronic noses to provide a cheap and easy way to detect MVOCs, and the presence of mold, they are currently imprecise, and have a high detection limit (Szulczyński & Gębicki, 2017)

## **2.4 Sampling and Analysis of Mold**

There are a wide variety of sampling and analysis techniques for mold. Generally, methods can be divided into two broad sampling categories: active, in which a pump is used to suck air through a capture device, and passive, where spores are not pumped, but instead settle onto the sampling media (Whitby et al., 2022). Analytical techniques can also be divided into two categories: culture based and non-culture based (Whitby et al., 2022). No technique is ideal for every situation, and it is vital that the researcher choose based on the environment and the information that they are looking to obtain (Andersen et al., 2009).

### *2.4.1 Culture Based Methods*

Sampling for culture based methods can be done using passive or active sampling, with mold spores being captured directly onto the growth media and then incubated (Martinez-Bracero et al., 2022). Culture based techniques are relatively cheap when compared to other methods, and simple to set up (Ghosh et al., 2015). Because passive sampling can be used,

sampling can occur in remote locations where powering active samplers can be challenging. Culturing only identifies viable mold, and identification can be at the species level or below (Andersen et al., 2009). However, culturing is time consuming, requiring a lengthy period of incubation. Additionally, not all molds are cultivable, so culturing might not detect all fungi present in the environment (Root et al., 2020)

#### *2.4.2 Spore Trap*

Spore traps use pumps to pull in air, depositing mold onto a sticky glass slide (Dananché et al., 2017). The slides are then evaluated optically under a microscope, and can usually be identified to the genus level (Andersen et al., 2009). The concentration of mold is extrapolated from a few sampling regions (Martinez-Bracero et al., 2022). The slides are not cultured, which makes analysis faster than culture based methods, and both viable and non-viable molds are identified (Dananché et al., 2017). Spore traps are the most common sampling technique used in research studies (Martinez-Bracero et al., 2022). However, optical analysis presents some problems. The accuracy relies highly on the skill of the operator (Martinez-Bracero et al., 2022). Overloading can also be a concern, since the sampling area is limited to the area of the slide used (Whitby et al., 2022). Additionally, the pumps are powered electrically, which can pose a problem in remote sampling locations, although battery powered pumps are available. (Martinez-Bracero et al., 2022).

#### *2.4.3 Molecular Methods*

Molecular methods for mold identification use internal transcribed spacer (ITS) regions in the mold DNA to determine the identity of the mold to the species level or below (Arunmozhi Balajee et al., 2007; Ciardo et al., 2010; Whitby et al., 2022). The tracer sequences are amplified

using polymerase chain reaction (PCR) and then referenced against a library of sequences (Martinez-Bracero et al., 2022). ITS is currently the most common molecular method for identifying mold (Ciardo et al., 2010). Molecular methods are good at identifying a wide range of molds reliably, and it can be relatively fast, especially when compared to culture methods (Arunmozhi Balajee et al., 2007). However, using PCR requires a good foundational knowledge of fungal DNA, and since primers are not universal, an understanding of what the target molds are (Martinez-Bracero et al., 2022).

#### *2.4.4 Real-time Monitoring*

Real-time information about mold concentrations can be acquired with a variety of real-time bioaerosol monitors. While there are many brands of instruments on the market, they all use the fluorescence of bioaerosols to differentiate them from other aerosols (Whitby et al., 2022). Measured concentrations have been shown to be comparable to more traditional methods like spore traps (Markey et al., 2022). Size (and sometimes shape) information can also be gathered, and identification has been demonstrated in controlled laboratory settings (Martinez-Bracero et al., 2022). However, identification has not been demonstrated in real-world settings, and the fluorescence of non-biological aerosols can interfere with analysis (Martinez-Bracero et al., 2022). While real-time monitoring is a powerful tool to monitor the changes in fungal concentrations over time, other techniques used in conjunction are necessary for accurate identification (Cheng et al., 2020).

## **2.5 Health Effects of Mold**

Mold can affect the health of those exposed (Mudarri & Fisk, 2007). The presence of mold is associated with impacts to many bodily systems (Portnoy et al., 2005) Mold can impact health through infection, allergic response, and toxicity (Curtis et al., 2004).

### *2.5.1 Respiratory Tract*

Dampness and mold have been associated with negative health outcomes such as coughing, wheezing, and other upper respiratory tract symptoms(Fisk et al., 2006; X. Zhang et al., 2019). Dampness in homes has been related to a higher prevalence of dermal and mucosal symptoms (Araki et al., 2010; Smedje et al., 2017). Mold is also correlated with reduced lung function in non-asthmatic adults (Hernberg et al., 2014). Moldy houses, especially those with molds associated with water damage, are correlated with higher degrees of childhood illness (VESPER et al., 2007).

### *2.5.2 Irritation and Inflammation*

Mold exposure is correlated with irritation and inflammatory responses. Mold exposure can cause irritation to the eyes, skin, and nose (Delanoë et al., 2020). There is significant evidence of an association between mold and allergic rhinitis (Caillaud et al., 2018). Mold exposure can also cause flu-like symptoms commonly seen with hypersensitivity pneumonitis, an inflammatory reaction of the lungs to an allergen (American Lung Association, 2022; Delanoë et al., 2020).

### *2.5.3 Asthma*

Mold has been associated with increased symptoms in asthmatics, and to asthma development in children and adults (Burge, 2001; Caillaud et al., 2018; Heseltine et al., 2009).

Higher amounts of mold in children's bedrooms is correlated with higher occurrence of childhood asthma (S. J. Vesper et al., 2006).

The cost of mold- and dampness-related asthma cases alone has been estimated to be \$3.5 billion dollars annually (Mudarri & Fisk, 2007). The American Thoracic Society recommends that everyone, but especially people with respiratory conditions (such as allergies or asthma) and people with suppressed immune symptoms avoid buildings contaminated with mold ("Mold-Specific Concerns Associated with Water Damage for Those with Allergies, Asthma, and Other Lung Diseases," 2017)

#### *2.5.4 Cognitive and Psychological Impacts*

There are cognitive impacts associated with mold exposure, especially in development. A study in Poland found that children exposed to mold in their infancy had a lower IQ than children who were not exposed (Jedrychowski et al., 2011). Children exposed to dampness at home have more behavioral problems (Baird et al., 2022). Mice exposed to mold experienced immune responses in the brain related to neural and behavioral changes (Harding et al., 2020).

Mold and dampness in the home can have psychological impacts on residents (Brooks et al., 2023). Mold in the residence is linked with higher rates of depression (Shenassa et al., 2007). The smells from mold growth can contribute to fatigue and nausea (Portnoy et al., 2005). Patients with mold exposure report higher levels of acute and post-traumatic stress (Crago et al., 2003). It's possible that that the psychological impacts are due to the loss of control that comes with a reoccurring or persistent mold problem in the home (Shenassa et al., 2007).

### 2.5.5 MVOCs and Health

The presence of MVOCs is also associated with health effects. MVOCs have been correlated with building-related symptoms (BRS) (Araki et al., 2010). High concentrations of MVOCs, alongside high humidity, is associated with higher rates of asthma (Choi et al., 2017; Kim et al., 2007). Several common MVOCs have been investigated for their toxic potential (Bennett & Inamdar, 2015; Kreja & Seidel, 2002; Wålinder et al., 2005). MVOCs in residential structures, even in very low concentrations (0.3 – 1.30  $\mu\text{g}/\text{m}^3$ ) were correlated with poor lung function, although the effects were small (Rolle-Kampczyk et al., 2008).

There are still gaps in our knowledge about the mechanisms of how mold or dampness impact human health (Nazaroff, 2022). The role of mold itself is still under investigation, and efforts to link individual microbes to health effects have been mixed. Holst et al. (2016) found a relationship between dampness in classrooms and respiratory effects in students, but was not able to correlate symptoms with the presence of any microbial agents (Holst et al., 2016). Additionally, it's unclear how much dampness and a moldy odor is associated with actual mold growth, and how much the perception of dampness and moldy odor affects health (Oluwole et al., 2017; Reponen et al., 2010). The evidence for an association between mold and asthma is more substantive, but there are still questions around mold's role in asthma development in adults (Caillaud et al., 2018). There are many, many other airborne contaminants that have been linked to health effects, and isolating mold as the sole contributor to symptoms should be a focus of future research (Kanchongkittiphon et al., 2015).

## CHAPTER 3: MATERIALS AND METHODS

### **3.1 Study Selection**

#### *3.1.1 Participant Recruitment and Study Population*

In collaboration with Baylor College of Medicine (BCM), study participants with asthma were recruited from an existing cohort that completed the Houston Home-Based Integrated Intervention Targeting Better Asthma Control for African Americans. The cohort was comprised of 178 low-income African American adults living in Harris County or those counties that border Harris County (i.e., Brazoria, Chambers, Fort Bend, Galveston, Liberty, Montgomery, or Waller counties). The median age was 50.2 years with 74.2% of the cohort being female. Because these participants provided written permission to be contacted about additional research opportunities, letters were mailed to individuals to introduce the study. Follow up phone calls were conducted with scripted screening to establish interest and eligibility for participating in the study. Eligibility criteria for participation are presented in Table 1.

All participants provided informed consent over the phone. Additionally, questionnaires were administered and completed before, during, and after the study. The research team designed the questionnaires to collect information on how they were (or were not) impacted by a flooding event, their home characteristics, the flooding status in their home, and their asthma symptoms. All study protocols and materials were approved by the Baylor College of Medicine and Colorado State University Institutional Review Boards. Data collection commenced in January 2021 and was completed in June 2023

Table 1: Eligibility Criteria for the participants.

Eligibility Criteria
<ul style="list-style-type: none"><li>• Living in Houston area in a flood-damaged or undamaged home between 18 and 45 years old</li><li>• Asthma diagnosis confirmed by medical record</li><li>• Clinical visit for respiratory health in previous 12 months</li><li>• Prescription for MDI (albuterol) in previous 12 months</li><li>• At least moderate asthma severity with daily MDI use and predicted FEV<sub>1</sub> of 60-80% (National Heart Lung and Blood Institute, 2012)</li><li>• Be able to read English at the 7<sup>th</sup> grade level</li></ul>

### 3.2 Sample Collection

Sampling campaigns were initiated in January 2021 when the COVID-19 pandemic was still a global health emergency and enforcement of universal mitigation strategies were still in effect given the emerging and more transmissible variants. As such, we developed a contactless approach for conducting exposure assessments and data collection to ensure the health and safety of the study participants and the research team. This contactless approach required shipping study kits to the participants that contained air sampling equipment and inhaler sensors. Upon receipt of these kits, study participants unpacked the kits and deployed the equipment based on a series of instructions and procedures that were developed in both written and video formats. Figure 1 shows an example of the tripod set up. Further, the research team was in frequent contact with the study participants to answer questions, troubleshoot issues, and confirmed that all equipment was operating as anticipated. Participants were instructed to install equipment in up to three separate locations inside their home. This equipment consisted of Carbograph 5 thermodesorption tubes (CAMSCO XYZ) for passive VOC sampling, Petri dishes for passive mold sampling (as previously described [REF]),

iButton (temperature and relative humidity data logger) along with field data sheets to record start/stop times, gloves, pens, Sharpie marker, roll of tape, and instructions for returning equipment back to CSU.

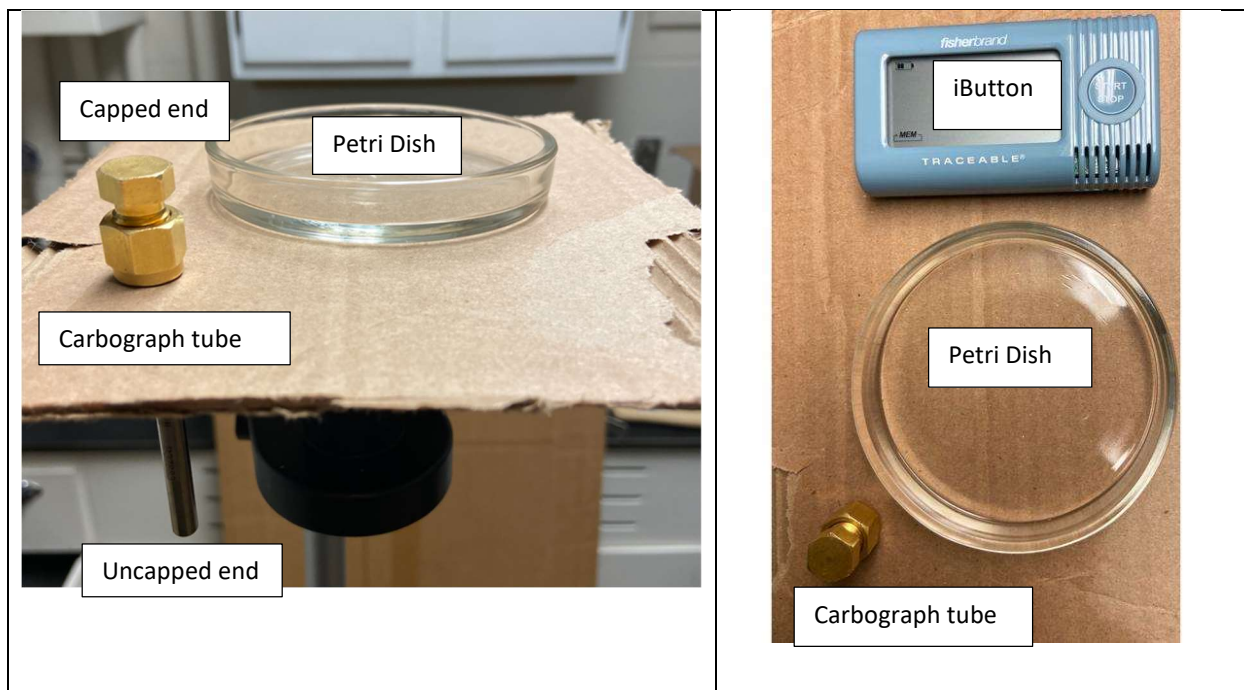


Figure 1: An example of the sampling set up. Cardboard was mounted on to a tripod. The Carbograph 5 thermodesorption tube was inserted into a premade hole in the cardboard, and a petri dish and iButton device were placed on top.

### 3.3 Lab Analysis

Sample tubes were analyzed by Eurofin using a Markes TD100-xr system for thermal desorption and an Agilent 7890A Gas Chromatograph (GC) coupled with an Agilent 5975C inert XL MSD with a Triple Axis Detector. The GC column used was a Restek Rxi-624 Sil MS, 30 m x 0.25mm x 1.4 um. Table 2 summarizes the GC temperature program. Data was collected in the full scan mode over the mass range of 35-350 amu.

Table 2: The temperature gradient used by Eurofins for GCMS Analysis.

Initial Temperature	35°C
Hold Time	4.5 minutes
Ramp	12°C per minute
Final Temperature	280°C
Hold Time	6 minutes

A NIST traceable vapor phase internal standard (IS) mix with components at a nominal concentration of 1.0 ppm was automatically loaded onto each tube prior to analysis using the TD100-xr internal standard loop option. The internal standard masses loaded onto each tube and the expected retention times are listed in Table 3.

Table 3: The internal standard mass per sample (in nanograms) and retention times as provided by Eurofins.

Internal Standard	Mass (ng)	Retention Time (minutes)
Bromochloromethane	26	5.42
1,4-Difluorobenzene	23	7.03
Chlorobenzene-d5	24	10.76
4-Bromofluorobenzene	36	12.26

### 3.4 TIC Analysis

Data files were analyzed in the software program Mass Hunter (Agilent). Mass spectrums were compared to the NIST20 Full Mass Spectral Database. Identified compounds were eliminated if they were either one of the added internal standards (see Table 3), also present in either the field or lab blank, or identified with less than 90% confidence.

### 3.5 Concentration Calculations

Concentrations of the VOCs identified were estimated using Equation 1, where RF is the response factor,  $A_s$  is the area count of the analyte peak,  $A_{IS}$  is the area count of the internal

standard,  $C_s$  is the concentration of the analyte, and  $C_{is}$  is the concentration of the internal standard.

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s} \quad \text{Equation 1}$$

To estimate the concentration of non-targeted analytes, RF was assumed to be 1, as per EPA method 8260D, section 11.7.3 (US EPA, 2019). Of the four internal standards, 4-bromoflourobenzene was chosen as it had the least amount of coelution amongst all the samples. Substituting the RF and the concentration of 4-bromoflourobenze and solving for  $C_s$ , the equation becomes

$$C_s = \frac{A_s \times 36ng}{A_{is}} \quad \text{Equation 2}$$

The concentration of the analyte in the air was calculated by first calculating the volume of the sample using

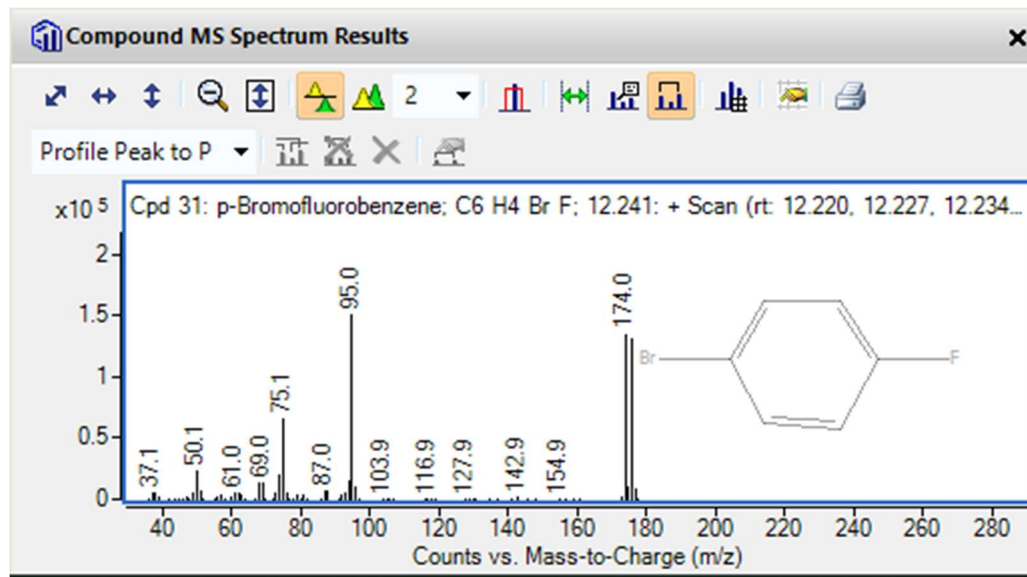
$$V = UR \times T \quad \text{Equation 3}$$

where  $V$  is the sampling volume in mL,  $UR$  is the uptake rate of the Carbograph 5 thermodesorption tube in mL/min, and  $T$  is the sampling duration in minutes. The uptake rate is dependent on the VOC, however a theoretical uptake rate of 0.5 mL/min was used for all analytes based on published rates (US EPA, 2016; Vallecillos et al., 2019; Walgraeve et al., 2011). The concentration calculated in Equation 2 was divided by the volume from Equation 3 and converted to  $\mu\text{g}/\text{m}^3$  to get the final estimated concentration in air.

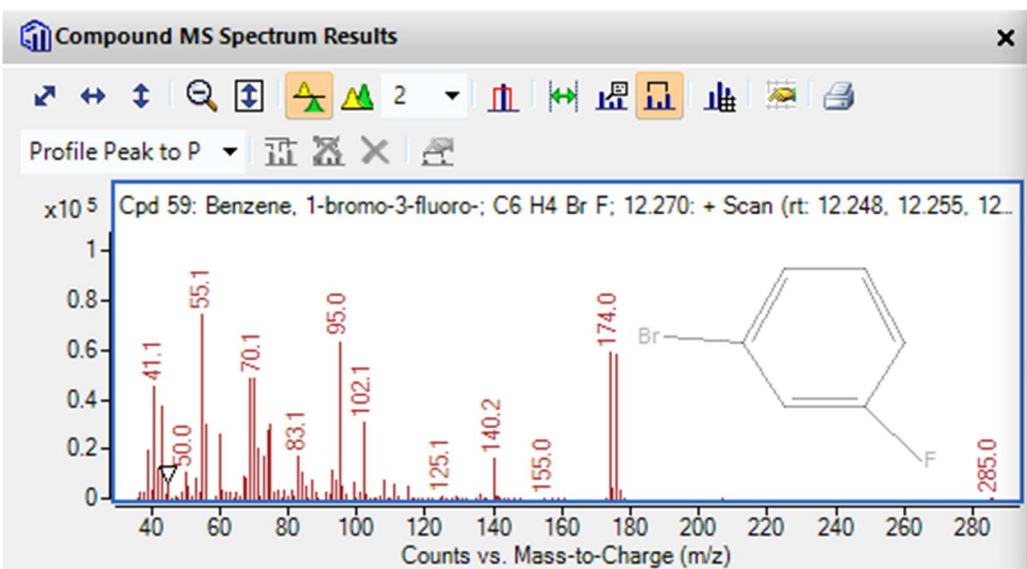
When determining the area count of the internal standard, the spectra was compared to a reference spectrum of 4-bromoflourobenzene provided by Eurofins. When calculating concentrations of a non-targeted analyte, the total ion chromatograph is used instead of an

extracted ion chromatograph (US EPA, 2019). This makes eliminating the effect of coeluting analytes more difficult. Figure 2 shows the effect of coelution on the mass spectrum. The large amount of interference mass fragments, shown as mass-to-charge peaks, contributes to a higher overall area count of the 4-bromofluorobenzene peak. Because of this, we determined that we would use a limit of 200% of the area count of the internal standard in the laboratory blank, over which the sample would be excluded from the concentration calculations. Of the 156 samples analyzed, 17 were excluded due to the high internal standard area count.

1.



2.



3.

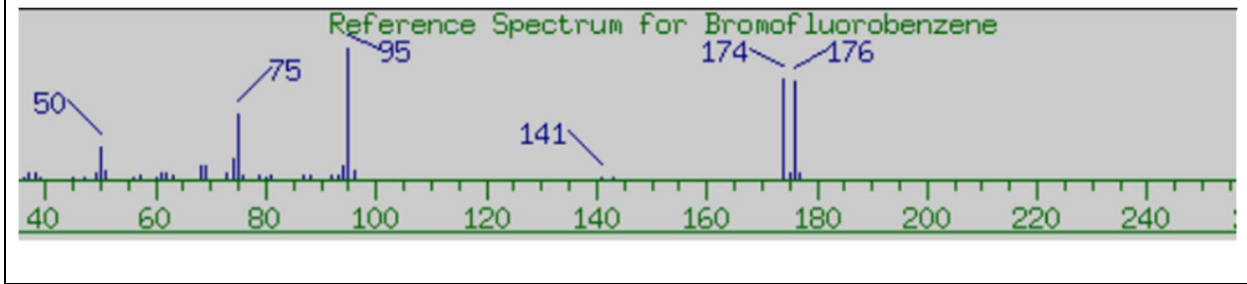


Figure 2: The mass spectra of peaks identified as 4-bromofluorobenzene. 1 shows a spectrum of a peak with low suspected coelution, evidenced by the relative lack of mass-to-charge (m/z) peaks that we would not expect to see in 4-bromofluorobenzene. 2 shows a spectrum of a peak with high suspected coelution. There are many interference peaks, especially in the 40-100 m/z range, and the 55.1 m/z peak is nearly as high as 95.0 m/z, which is the base (most abundant) peak in an ideal 4-bromofluorobenzene spectrum. 3 shows the reference spectrum provided by Eurofins.

### 3.6 Statistical Analysis

The proportion of houses that contained each VOC was determined by dividing the number of homes positive for that VOC by the total number of homes in each group. For the difference in proportions of VOCs in flooded and unflooded homes, the 95% confidence intervals for the difference were calculated using

$$CI = (\hat{p}_1 - \hat{p}_2) \pm Z_{\alpha/2} s \quad \text{Equation 4}$$

where CI is the confidence interval,  $\hat{p}_1$  is the proportion of the first group,  $\hat{p}_2$  is the proportion of the second group,  $Z_{\alpha/2}$  is 1.96 for the 95% confidence interval, and s is calculated using

$$s = \sqrt{\frac{\hat{p}_1(1 - \hat{p}_1)}{n_1} + \frac{\hat{p}_2(1 - \hat{p}_2)}{n_2}} \quad \text{Equation 5}$$

where  $n_1$  is the number of samples in the first group and  $n_2$  is the number of samples in the second group.

The average concentration of each VOC was determined and grouped by flooded and unflooded home. For the difference in average concentrations, the Wilcoxin Sum Rank test was used as a non-parametric alternative to a t-test, to construct 95% confidence intervals.

## CHAPTER 4: RESULTS

Initially, 75 participants were enrolled in the study. VOC analysis was completed for 55 participants (16 were dropped due to not completing the questionnaire or lack of follow-up and four participant's samplers were not able to be analyzed due to labeling errors. Due to the remote nature of the sampling, not all participants deployed all three samplers. 84% (n=46) of participants deployed three samplers, 9% (n=5) deployed two samplers, and 7% (n=4) deployed 1 sampler (n=4). Participants deployed different numbers of samplers based on the number of rooms in their houses and their own comfort with the equipment. The samplers were deployed for an average of 714.7 hours, or 29.8 days (minimum 550 hours, maximum 933.5 hours, standard deviation 2.54 hours).

Table 4: Home characteristics of the 55 homes included in the sampling campaign.

Housing Characteristics		
House Type	n	percent
Duplex	5	9.1%
High rise apartment	3	5.5%
Low rise apartment	18	32.7%
One family attached house	7	12.7%
One family detached house	22	40.0%
Rented	n	percent
Own	14	25.5%
Rent	41	74.5%
Home Age	n	percent
1940-1949	4	7.3%
1950-1959	9	16.4%
1960-1969	6	10.9%
1970-1979	10	18.2%

Housing Characteristics		
1980-1989	3	5.5%
2000 or later	5	9.1%
Don't know	18	32.7%
Flooded		
No	22	40.0%
Yes	33	60.0%
Water Rise		
No answer	4	7.3%
No water	5	9.1%
Ankle deep	16	29.1%
Knee deep	5	9.1%
Waist deep	3	5.5%
NA (Not flooded)	22	40.0%

In general, there was a greater variety of compounds identified in flooded homes vs unflooded homes. In total, 197 different compounds were identified across all samples from unflooded homes, and 284 compounds were identified across all samples from flooded homes. Additionally, there were more compounds identified per sample from flooded homes. In the samples from unflooded homes, an average of 27.8 (minimum of 1, maximum of 57) compounds were identified per sample, and in flooded homes, an average of 44.25 (minimum of 6, maximum of 65) compounds were identified.

The compounds were sorted into seven categories: hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, and terpenes/terpenoids based on categories of compounds commonly associated with MVOCs (Alcega et al., 2017; Betancourt et al., 2013; Buško et al., 2014; Eler et al., 2018). Hydrocarbons were the most abundant, followed by esters (Figure 3).

All seven categories of compounds were found in higher abundances in flooded homes versus unflooded homes (Figure 4).

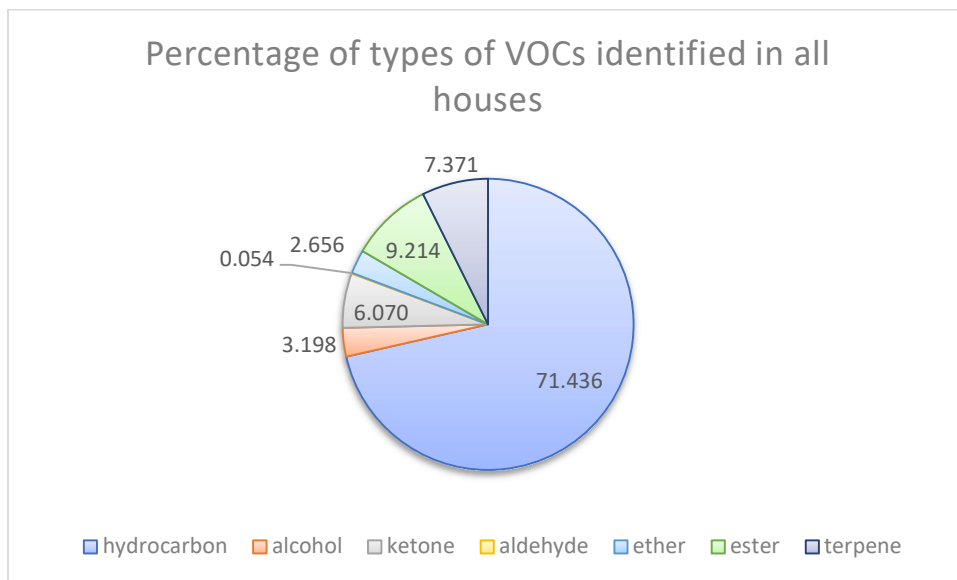


Figure 3: A pie chart of the percentages of all compounds identified that were hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, and terpenes/terpenoids. The most abundant type found was hydrocarbons.

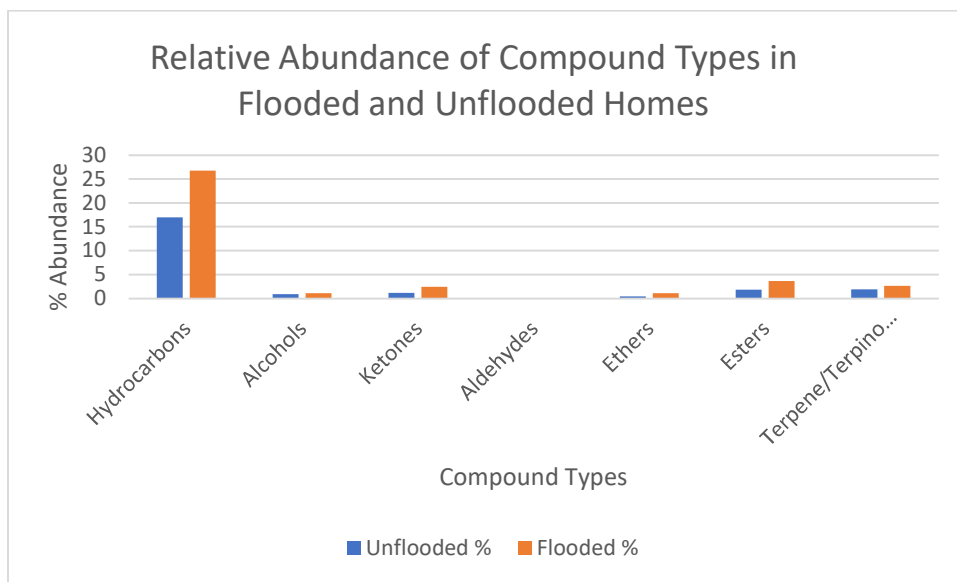


Figure 4: A bar graph of the relative abundance of the seven types of compounds categorized (hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, and terpenes/terpenoids) found in flooded (orange) and unflooded (blue) homes.

In addition to categories of compounds, 38 VOCs were chosen for further analysis based on their frequency (present in greater than or equal to 25% of all homes). All but two were

found with greater frequency in flooded homes versus unflooded homes. Of the 38 compounds, 17 were found to have a statistically significant difference in proportions between flooded and unflooded homes: butane, ethyl acetate, n-hexane, 2-butanone, 3-methyl-hexane, acetic acid butyl ester (butyl acetate), cyclohexane, methyl-cyclohexane, d-limonene, 3-methyl-pentane, 1,3-dimethyl-benzene (m-xylene), heptane, 2,2,6-trimethyl-octane, mesitylene, naphthalene, 2-borneone, and 2,2,4-trimethyl-1-pentene (the only compound to be found to be statistically less frequent in flooded homes than unflooded homes) (Table 5).

Table 5: The differences in proportions of VOCs in flooded versus unflooded homes. 95% confidence intervals were calculated, and those determined to be statistically significant are bolded.

Compound	Proportion in Flooded Homes	Proportion in Unflooded Homes	Difference in Proportions	95% Confidence Interval
<b>Butane</b>	<b>0.909</b>	<b>0.636</b>	<b>0.273</b>	<b>0.049, 0.496</b>
Pentane	0.970	0.864	0.106	-0.049, 0.261
Butane, 2-methyl-	0.970	0.818	0.152	-0.02, 0.323
Toluene	0.909	0.727	0.182	-0.029, 0.392
<b>Ethyl Acetate</b>	<b>0.909</b>	<b>0.545</b>	<b>0.364</b>	<b>0.134, 0.594</b>
<b>n-Hexane</b>	<b>0.848</b>	<b>0.545</b>	<b>0.303</b>	<b>0.062, 0.544</b>
<b>2-Butanone</b>	<b>0.848</b>	<b>0.500</b>	<b>0.348</b>	<b>0.106, 0.591</b>
<b>Hexane, 3-methyl-</b>	<b>0.818</b>	<b>0.455</b>	<b>0.364</b>	<b>0.117, 0.61</b>
<b>Acetic acid, butyl ester</b>	<b>0.788</b>	<b>0.455</b>	<b>0.333</b>	<b>0.083, 0.584</b>
<b>Cyclohexane</b>	<b>0.758</b>	<b>0.500</b>	<b>0.258</b>	<b>0.003, 0.513</b>
Isobutane	0.697	0.500	0.197	-0.064, 0.458
<b>Cyclohexane, methyl-</b>	<b>0.727</b>	<b>0.409</b>	<b>0.318</b>	<b>0.063, 0.574</b>
Camphene	0.667	0.455	0.212	-0.051, 0.475
p-(1-Propenyl)-toluene	0.636	0.409	0.227	-0.036, 0.49
<b>D-Limonene</b>	<b>0.636</b>	<b>0.364</b>	<b>0.273</b>	<b>0.013, 0.532</b>
Ethylbenzene	0.606	0.409	0.197	-0.068, 0.462
Octane	0.606	0.409	0.197	-0.068, 0.462
Isopropyl Alcohol	0.515	0.500	0.015	-0.255, 0.285

<b>Pentane, 3-methyl-</b>	<b>0.636</b>	<b>0.273</b>	<b>0.364</b>	<b>0.115, 0.612</b>
<b>Benzene, 1,3-dimethyl-</b>	<b>0.576</b>	<b>0.227</b>	<b>0.348</b>	<b>0.105, 0.592</b>
Isoprene	0.424	0.409	0.015	-0.251, 0.281
Cyclopentane, methyl-	0.394	0.409	-0.015	-0.28, 0.249
<b>Heptane</b>	<b>0.515</b>	<b>0.182</b>	<b>0.333</b>	<b>0.099, 0.568</b>
.alpha.-Pinene	0.455	0.273	0.182	-0.07, 0.434
Benzene, 1,4-dichloro-	0.455	0.273	0.182	-0.07, 0.434
Acetic acid, methyl ester	0.424	0.318	0.106	-0.151, 0.364
Methyl Isobutyl Ketone	0.455	0.227	0.227	-0.017, 0.471
Hexanal	0.424	0.273	0.152	-0.1, 0.403
<b>Octane, 2,2,6-trimethyl-</b>	<b>0.455</b>	<b>0.182</b>	<b>0.273</b>	<b>0.039, 0.507</b>
Pentane, 2-methyl-	0.364	0.318	0.045	-0.209, 0.3
<b>Mesitylene</b>	<b>0.424</b>	<b>0.182</b>	<b>0.242</b>	<b>0.009, 0.476</b>
<b>Naphthalene</b>	<b>0.424</b>	<b>0.182</b>	<b>0.242</b>	<b>0.009, 0.476</b>
Cyclotrisiloxane, hexamethyl-	0.242	0.455	-0.212	-0.466, 0.042
Benzene, 1-ethyl-4-methyl-	0.394	0.182	0.212	-0.02, 0.444
Benzene, 1-ethyl-2-methyl-	0.394	0.182	0.212	-0.02, 0.444
p-Xylene	0.303	0.318	-0.015	-0.265, 0.235
<b>(+)-2-Bornanone</b>	<b>0.394</b>	<b>0.136</b>	<b>0.258</b>	<b>0.038, 0.477</b>
<b>1-Pentene, 2,4,4-trimethyl-</b>	<b>0.030</b>	<b>0.636</b>	<b>-0.606</b>	<b>-0.815, -0.397</b>

The concentrations of all identified compounds were estimated. The maximum concentration estimated was 230.9  $\mu\text{g}/\text{m}^3$ , and the minimum was 0.02  $\mu\text{g}/\text{m}^3$ .

Of the 38 compounds that were present in over 25% of the samples, almost 60% (n=22) had higher average estimated concentration in samples from flooded homes versus unflooded homes. Using the Wilcoxin rank sum test, the difference in concentrations were found to be

statistically significant for seven compounds: pentane, 2-methyl butane, toluene, isobutane, ethyl benzene, isopropyl alcohol (2-propanol), and 1,4-dichloro-benzene (Table 6).

Table 6: The difference in average concentrations of 38 VOCs identified in flooded versus unflooded homes. The confidence intervals were calculated, and those determined to be statistically significant are bolded.

Compound	Average concentration, Flooded (ug/m3)	Average concentration, Unflooded (ug/m3)	Difference (ug/m3)	95% Confidence Interval
Butane	15.52	12.58	2.94	-2.33, 3.79
<b>Pentane</b>	<b>18.97</b>	<b>10.80</b>	<b>8.17</b>	<b>1.33, 5.21</b>
<b>Butane, 2-methyl-</b>	<b>12.55</b>	<b>6.98</b>	<b>5.57</b>	<b>1.98, 7.48</b>
<b>Toluene</b>	<b>16.27</b>	<b>7.39</b>	<b>8.88</b>	<b>3.3, 13.22</b>
Ethyl Acetate	3.41	3.56	-0.15	-0.8, 0.7
n-Hexane	1.62	1.80	-0.18	-0.67, 0.27
2-Butanone	3.74	3.95	-0.21	-0.83, 1.03
Hexane, 3-methyl-	1.41	1.32	0.09	-0.13, 0.35
Acetic acid, butyl ester	2.51	2.69	-0.18	-1.14, 0.54
Cyclohexane	3.33	2.51	0.83	-0.11, 1.74
<b>Isobutane</b>	<b>17.40</b>	<b>10.30</b>	<b>7.10</b>	<b>0.2, 7.73</b>
Cyclohexane, methyl-	1.60	1.60	0.00	-0.29, 0.47
Camphene	1.44	1.56	-0.12	-0.19, 0.54
p-(1-Propenyl)-toluene	4.52	4.85	-0.33	-2.49, 1.76
D-Limonene	13.54	17.49	-3.96	-10.36, 1.99
<b>Ethylbenzene</b>	<b>2.90</b>	<b>2.04</b>	<b>0.86</b>	<b>0.16, 1.83</b>
Octane	0.93	0.84	0.09	-0.16, 0.32
<b>Isopropyl Alcohol</b>	<b>32.05</b>	<b>11.25</b>	<b>20.80</b>	<b>0.58, 11.25</b>
Pentane, 3-methyl-	1.06	1.05	0.00	-0.43, 0.05
Benzene, 1,3-dimethyl-	9.43	10.56	-1.13	-6, 2.71
Isoprene	2.68	3.19	-0.52	-1.24, 0.37
Cyclopentane, methyl-	1.43	1.21	0.22	-0.13, 0.39
Heptane	2.24	1.72	0.52	-0.74, 2.31

.alpha.-Pinene	4.98	4.13	0.86	-1.03, 1.58
<b>Benzene, 1,4-dichloro-</b>	<b>8.03</b>	<b>1.65</b>	<b>6.38</b>	<b>1.32, 11.74</b>
Acetic acid, methyl ester	0.63	1.54	-0.91	-0.9, 0.34
Methyl Isobutyl Ketone	2.05	2.44	-0.40	-1.7, 0.47
Hexanal	1.94	1.90	0.04	-1.23, 0.47
Octane, 2,2,6-trimethyl-	2.22	2.12	0.10	-0.96, 0.45
Pentane, 2-methyl-	3.86	2.86	0.99	-0.62, 1.85
Mesitylene	2.46	2.24	0.21	-1.7, 0.76
Naphthalene	5.07	2.16	2.90	-0.88, 2.32
Cyclotrisiloxane, hexamethyl-	1.52	1.16	0.36	-0.6, 0.67
Benzene, 1-ethyl-4- methyl-	1.13	2.62	-1.49	-3.03, 0.05
Benzene, 1-ethyl-2- methyl-	3.76	4.04	-0.28	-1.59, 0.8
p-Xylene	3.90	3.61	0.28	-2.39, 2.59
(+)-2-Bornanone	1.61	5.67	-4.06	-11.39, 0.44
1-Pentene, 2,4,4- trimethyl-	0.22	0.26	-0.04	-0.56, 0.15

The houses were stratified based on flood level (no water, ankle deep, knee deep, and waist deep). While no statistical analysis was performed, the waist deep houses do seem to have a higher concentration of VOCs when compared to the houses with lower water levels (Figure 5).

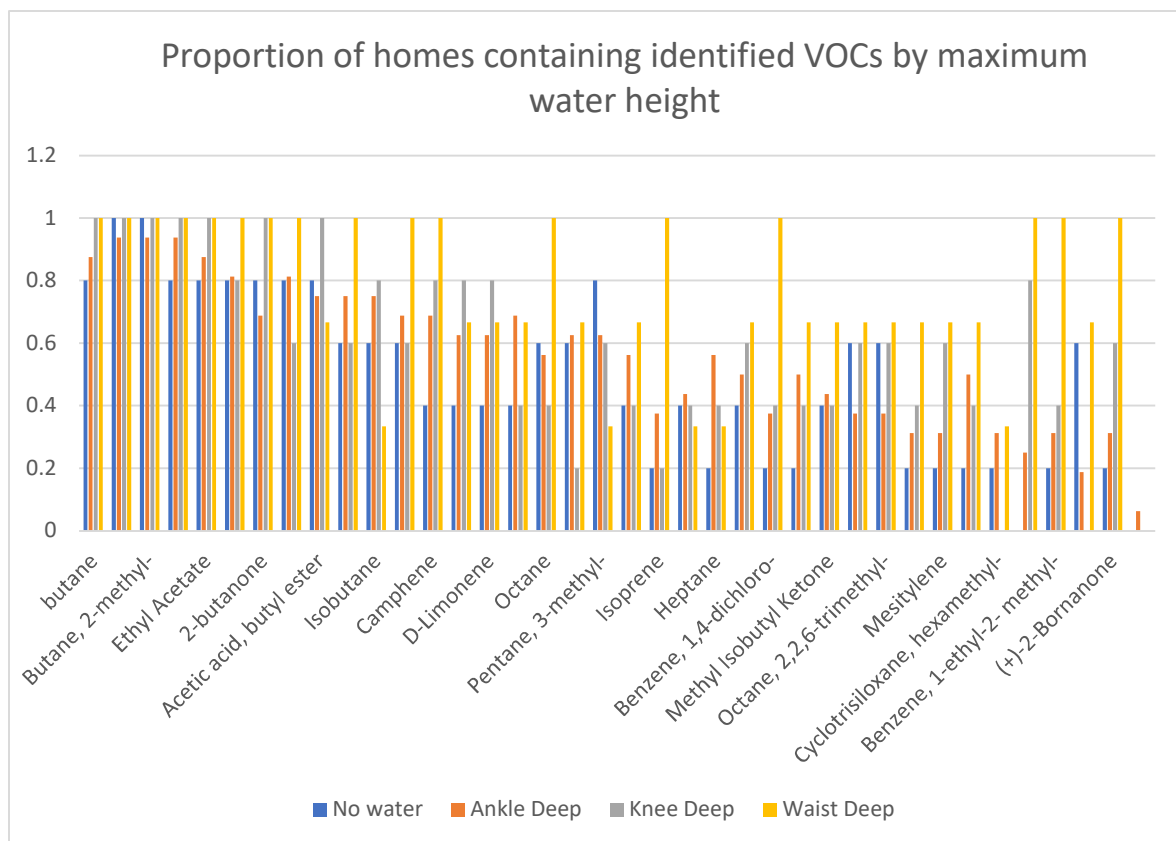


Figure 5: The proportion of homes that contain each of the 38 VOCs identified, stratified by maximum water height. There appears to be some correlation between waist deep water and a higher proportion of homes, but it is not yet known if that difference is statistically significant.

The estimated concentrations of VOCs were compared to odor thresholds (PubChem, n.d.). Of the 19 compounds with published odor thresholds, 12 of them were over the limit (Table 7).

Table 7: A comparison of the estimated concentrations of 19 of the VOCs identified and their odor thresholds. Compounds found in concentrations greater than their threshold are highlighted in bold.

Compound	Average Concentration ( $\mu\text{g}/\text{m}^3$ )	Low Odor Threshold ( $\mu\text{g}/\text{m}^3$ )	Proportional Difference
<b>Butane</b>	<b>15.52</b>	<b>2.852515</b>	<b>5.440812</b>
<b>Pentane</b>	<b>18.97</b>	<b>6.492025</b>	<b>2.922047</b>
<b>Toluene</b>	<b>16.27</b>	<b>6.029611</b>	<b>2.69835</b>
Ethyl Acetate	3.41	23.06356	0.147852
n-Hexane	1.62	229.1084	0.007071
2-Butanone	3.74	5.898569	0.634052

<b>Acetic acid, butyl ester</b>	<b>2.51</b>	<b>0.285055</b>	<b>8.805312</b>
Cyclohexane	3.33	86.05317	0.038697
<b>Ethylbenzene</b>	<b>2.9</b>	<b>0.390799</b>	<b>7.420699</b>
Octane	0.93	70.08098	0.01327
<b>Isopropyl Alcohol</b>	<b>32.05</b>	<b>2.458078</b>	<b>13.03864</b>
<b>Benzene, 1,3-dimethyl-</b>	<b>9.43</b>	<b>0.217096</b>	<b>43.43698</b>
<b>Acetic acid, methyl ester</b>	<b>0.63</b>	<b>0.515076</b>	<b>1.223121</b>
<b>Methyl Isobutyl Ketone</b>	<b>2.05</b>	<b>0.409652</b>	<b>5.004243</b>
Pentane, 2-methyl-	3.86	288.6	0.013375
Mesitylene	2.46	9.831493	0.250216
<b>Naphthalene</b>	<b>5.07</b>	<b>0.0498</b>	<b>101.8068</b>
<b>p-Xylene</b>	<b>3.9</b>	<b>0.217096</b>	<b>17.96439</b>
<b>(+)-2-Bornanone</b>	<b>1.61</b>	<b>0.016188</b>	<b>99.45604</b>

## CHAPTER 5: DISSCUSION

Several compounds were found with greater frequency in flooded homes versus unflooded homes: butane, ethyl acetate, n-hexane, 2-butanone, 3-methyl-hexane, acetic acid butyl ester (butyl acetate), cyclohexane, methyl-cyclohexane, d-limonene, 3-methyl-pentane, 1,3-dimethyl-benzene (m-xylene), heptane, 2,2,6-trimethyl-octane, mesitylene, naphthalene, and 2-borneone. Buško et al. (2014) found that *Fusarium graminearum*, a type of rot that target produce, releases p-xylene and naphthalene when it grows. They also identified several VOCs that we found were more frequent in flooded homes, but the difference was not significantly different: methyl isobutyl ketone, 1-ethyl-3-methylbenzene (we found both 1-ethyl-2-methyl- and 1-ethyl-4-methylbenzene), hexanal, and alpha pinene (Buško et al., 2014). Alpha pinene was also identified in the VOCs emitted from a compost facility, along with camphene, limonene, and camphor (2-borneone) (Fischer et al., 2000). While these VOCs were not able to be directly connected to fungi growth, previous work by Fischer et al. (1999) found that camphene was produced by *Asperfillus fumigatus* (Fischer et al., 1999). A study identified VOCs that might be related to microbial growth downwind of a waste water treatment plant (isopropyl alcohol, pentane, heptane, ethyl acetate, o- and p-xylene, and 2-butanone) although again the VOCs could not be solely connected to fungal growth (Alcega et al., 2017). Sunesson et al. (1995) identified heptane, 2-butanone, alpha pinene, camphene, limonene, and ethyl and methyl acetate (acidic acid methyl ester) with the growth of *Penicillium commune*, xylene and 2-propantol (isopropyl alcohol) with the growth of *Paecilomyces variotii* (Sunesson et al., 1995). Wheatley et al. (1997) identified 2-methyl-pentane, hexane, ethyl acetate, 2-butanone,

heptane, octane, hexanal, p-xylene, and limonene with the growth of *Trichoderma viride* and *T. pseudokoningii* (Wheatley et al., 1997).

Several VOCs we identified as more frequent in flooded homes are not yet identified in the literature as having microbial origin. While it is possible that these compounds do have microbial origin and just haven't been found yet, VOCs in the home have many other sources. One possible source is new building materials (Gallon et al., 2020; A. P. Jones, 1999). Toluene, alpha pinene, limonene, and 2-butanone have been identified in construction and newly built homes (Gallon et al., 2020; Hodgson et al., 2000). Given that every participant who reported on their remediation progress (n=22) were either in the middle of it (n=4) or had already completed (n=18), the contribution of VOCs from new building materials should be considered. In the future, efforts should be made to collect the VOC samples before remediation starts.

Other home characteristics gathered in the questionnaire might also be associated with VOCs. Future work should be done associating products used in the home, pets, and tobacco use with the VOCs identified. Additionally, insights into the contribution of flooding could be gathered by stratifying the flooded houses by maximum flood height. While statistical analysis has not yet been completed, there does seem to be some pattern emerging in the proportion of homes with the 38 VOCs identified, with homes reporting waist deep water having the highest proportion (Figure 5). Further analysis should be done to determine if there is a relationship between water level and MVOCs.

The concentrations of VOCs calculated were all low relative to published exposure limits. However, Rolle-Kampczyk et al. (2008) found that a total VOC concentration as low as  $8.21 \mu\text{g}/\text{m}^3$  could have a negative impact on lung function (Rolle-Kampczyk et al., 2008). There

are many possible explanations to this, but Rolle-Kampczyk et al. points out that VOCs in general have a low odor threshold, and the odor of the VOCs might be contributing to psychological effects (Rolle-Kampczyk et al., 2008). Of the 38 compounds we identified, 19 have published odor thresholds and 12 of them were over the threshold (Table 7) (*Haz-Map - Hazardous Chemicals and Occupational Diseases Information*, n.d.). Odor levels can have a psychological effect on residents (Becher et al., 1996; Dalton et al., 2020; Portnoy et al., 2005), and the relationship between odor thresholds and the resident's perception of their home could provide additional insights to the health data collected.

VOCs have been shown to impact asthma symptoms, although the evidence is inconsistent (Nurmatov et al., 2015). Rumchev et al. (2004) found that the presence of toluene and dichlorobenzene were risk factors for asthma in children (Rumchev, 2004). In contrast, Smedje and Norbäck (2001) found no association between total VOCs in schools and asthma rates (Smedje & Norbäck, 2001). Exposure to indoor VOCs seem to also have an impact on pulmonary health, although again the evidence is inconsistent (Alford & Kumar, 2021). More work needs to be done on associating VOCs in homes with adverse health outcomes.

## CHAPTER 6: CONCLUSION

Mold is a human health hazard, especially for those with asthma. Wet conditions facilitate the growth of mold, like those that exist after a structure has been flooded. There are many methods of detecting mold growth, one of which is analyzing for MVOCs. There have been many studies determining the MVOCs produced from cultured molds in labs, there have been less examining MVOCs in situ, and very few that specifically include flooding. We sampled for VOCs in homes that were and were not flooded and identified several as significant based on their frequency in homes. While, in line with the hypothesis, there were compounds identified as more likely to be present in flooded versus unflooded homes, the origin of those compounds is unclear. Some of the identified compounds have been associated previously with microbial growth, but not all. Additionally, many of those compounds are associated with various sources, including building materials. Further work should build off these results to examine the relationship between the MVOCs and other flood characteristics such as maximum water rise. As a part of the larger study, the MVOC data can be associated with the health data obtained from participants to draw conclusions about the role of MVOCs in indoor air on the health of adults with asthma.

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