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Supplemental Information for:

The Chemical Assessment of Surfaces and Air (CASA) Study: Using chemical perturbations in a test house to investigate indoor processes.

Authors: Delphine K. Farmer[1*], Marina E. Vance[2*], Dustin Poppendieck[3], Jon Abbatt[4], Michael R. Alves[5], Karen C. Dannemiller[6,7], Cholaphan Deeleepojananan[8], Jenna Ditto[4◊], Brian Dougherty[3], Olivia R. Farinas[6], Allen H. Goldstein[5], Vicki H. Grassian[8], Han Huynh[4§], Deborah Kim[8], Jon C. King[6], Jesse Kroll[9], Jienan Li[1], Michael F. Link[3], Liora Mael[2⸙], Kathryn Mayer[1⁑], Andrew B. Martin[2†], Glenn Morrison[10], Rachel O'Brien[11], Shubhrangshu Pandit[8], Barbara J. Turpin[10], Marc Webb[10], Jie Yu[4], Stephen M. Zimmerman[3]

Affiliations:
1. Department of Chemistry, Colorado State University, Fort Collins, CO, USA
2. Department of Mechanical Engineering, University of Colorado Boulder, CO, USA
3. National Institute of Standards and Technology, Gaithersburg, MD, USA
4. Department of Chemistry, University of Toronto, Toronto ON
5. Department of Environmental Science, Policy and Management, University of California Berkeley, Berkeley CA
6. Department of Civil, Environmental, and Geodetic Engineering, Division of Environmental Health Sciences, The Ohio State University, Columbus, OH
7. Sustainability Institute, The Ohio State University, Columbus, OH
8. Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, USA
9. Massachusetts Institute of Technology, Cambridge, MA
10. Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA
11. Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI, USA

◊Now at: Department of Energy, Environmental, and Chemical Engineering, Washington University of St. Louis, St. Louis, MO, USA
⁑Now at: Department of Chemistry, Southwestern Community College, Chula Vista, CA
§Now at: Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, USA and NOAA Chemical Sciences Laboratory, Boulder, CO, USA
⸙Now at: Winterthur Museum, Garden and Library, University of Delaware, Newark, DE, USA
†Now at: Colorado Department of Public Health and Environment, Denver, CO, USA

SI Section 1. List of Acronyms, not including chemical names

AFM-IR – atomic force microscopy – infrared (spectroscopy)
ATR-FTIR – attenuated total reflection element with Fourier transform infrared (spectroscopy)
CASA – Chemical Assessment of Surfaces and Air study
CFM – cubic feet per minute
CIMS – chemical ionization mass spectrometer (e.g., I-CIMS is an iodide ionization mass spectrometer)
CPC – condensation particle counter

CSU – Colorado State University
CU – University of Colorado
DMA – differential mobility analyzer
EC – elemental carbon
GC-MS – gas chromatograph with mass spectrometry detection
HEPA – high efficiency particulate air filter
HOMEChem –House Observations of Microbial and Environmental Chemistry study
LOD – limit of detection
Lpm – liters per minute
MC – mist chamber
MERV – minimum efficiency reporting value, refers to a filter rating
NZERTF – Net-Zero Energy Residential Test Facility
NIST – National Institute of Standards and Technology
OPC – optical particle counter
PCO – photocatalytic oxidation
PFA – perfluoroalkoxy, referring to the type of synthetic fluoropolymer tubing
PTFE – polytetrafluoroethylene, referring to the type of synthetic fluoropolymer tubing
PILS – particle into liquid sampler
PM – particulate matter
PTR-MS – proton transfer reaction mass spectrometer
ppb – parts per billion; defined as the moles of an analyte divided by the number of moles of air and multiplied by 10^9
ppm – parts per million; defined as the moles of an analyte divided by the number of moles of air and multiplied by 10^6
ppq – parts per quadrillion; defined as the moles of an analyte divided by the number of moles of air and multiplied by 10^{15}
ppt – parts per trillion; defined as the moles of an analyte divided by the number of moles of air and multiplied by 10^{12}
QCM – quartz crystal microbalance
RH – Relative Humidity
SMPS – scanning mobility particle sizer
SVOC – semi-volatile organic compounds
SVTAG – semi-volatile thermal desorption gas chromatography system
UC – University of California
HRV – heat recovery ventilation
UV – ultraviolet
VOC – volatile organic compounds
WE-CAN – Western Wildfire Experiment for Cloud Chemistry, Aerosol, Absorption and Nitrogen study
WSOC – water soluble organic carbon

SI Section 2. Figures and Tables

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Figure S3. Comparison of SV-TAG signal (normalized and in arbitrary units) versus temperature during low RH and high RH events during CASA.

Figure S4. The emissions ratios for selected VOCs from the cocktail smoker compared to aircraft observations from wildfire smoke.

Figure S5. Timeseries showing stacked signals from five observed ions for the iodide-CIMS and PTR-MS instruments across several days.

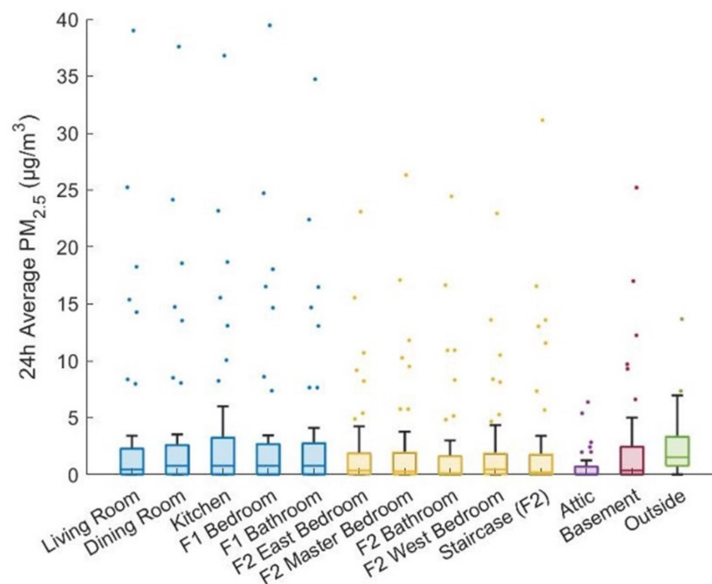


Figure S1. Indoor 24hr-average $PM_{2.5}$ concentrations measured throughout the house during the entire CASA field campaign using low-cost sensors (data corrected with subsequent laboratory calibrations). Each floor's sensors are grouped into three colors. This includes the first floor (blue), second floor (yellow), and basement (red), attic (purple), basement (red), and outside (green). Error bars represent one standard deviation.

Throughout the duration of the CASA campaign, sensors located on the same floor had similar agreements in their 24-h averages. Outliers in the boxplot represent smoke injection days. Of all the indoor sensors, the kitchen saw the highest median concentration while the attic saw the lowest. While the test house had a low air change rate, the first-floor bedroom and the second-floor main bedroom observed the strongest infiltration of outdoor $PM_{2.5}$ during background periods.

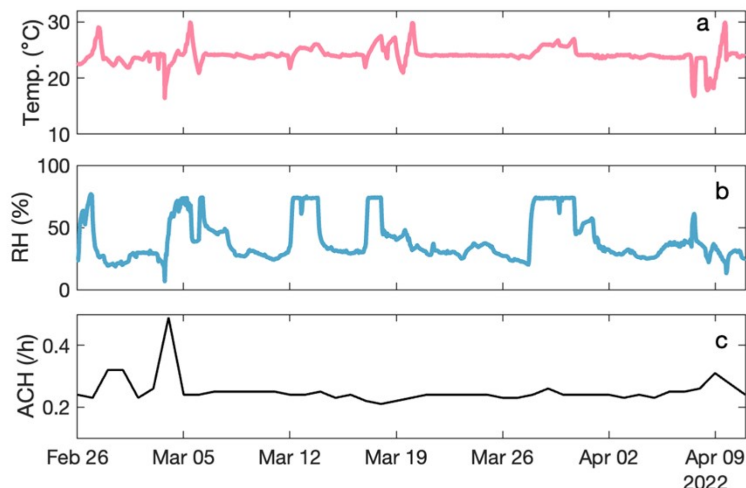


Figure S2. Indoor environmental conditions during the CASA experiment. Temperature (a) and relative humidity (RH, b) are shown as hourly average values for the first floor of the NZERTF. The air change per hour (ACH, c) values represent daily sulfur hexafluoride (SF_6) decay rates. Note the y-axes have different scales between panels.

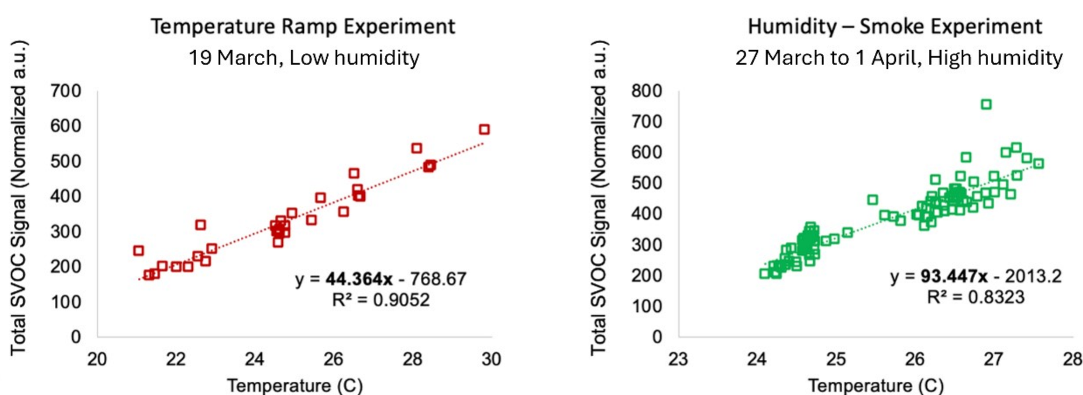


Figure S3. Comparison of SV-TAG signal (normalized and in arbitrary units) versus temperature during low RH and high RH events during CASA. The humidity ranged between 30% and 50% on 19 March, and between 65% and 80% between 27 March and 1 April.

Table S1. CASA cooking experiments (3/13/2022 – 3/15/2022).

Cooking Event	Date	Cook type	Canola oil added (g)	Green peppers added (g)	Tater tots added (g)	Bacon added (g)	Highest pan temperature (°C)
C1	March 13 (high RH)	Pan	10	118	197	103	249
C2		Pan	12	124	197	111	238
C3	March 14 (low RH)	Pan	10	111	199	112	271
C4		Air fryer	10	103	201	109	N/A*
C5		Pan	10	102	197	109	260
C6	March 15 (low RH)	Pan	11	103	199	112	260
C7		Air fryer	10	101	199	103	N/A*
C8		Pan	10	104	201	109	277

**Cooking events 4 and 7 did not have a recorded pan temperature because cooking was performed with an air fryer.*

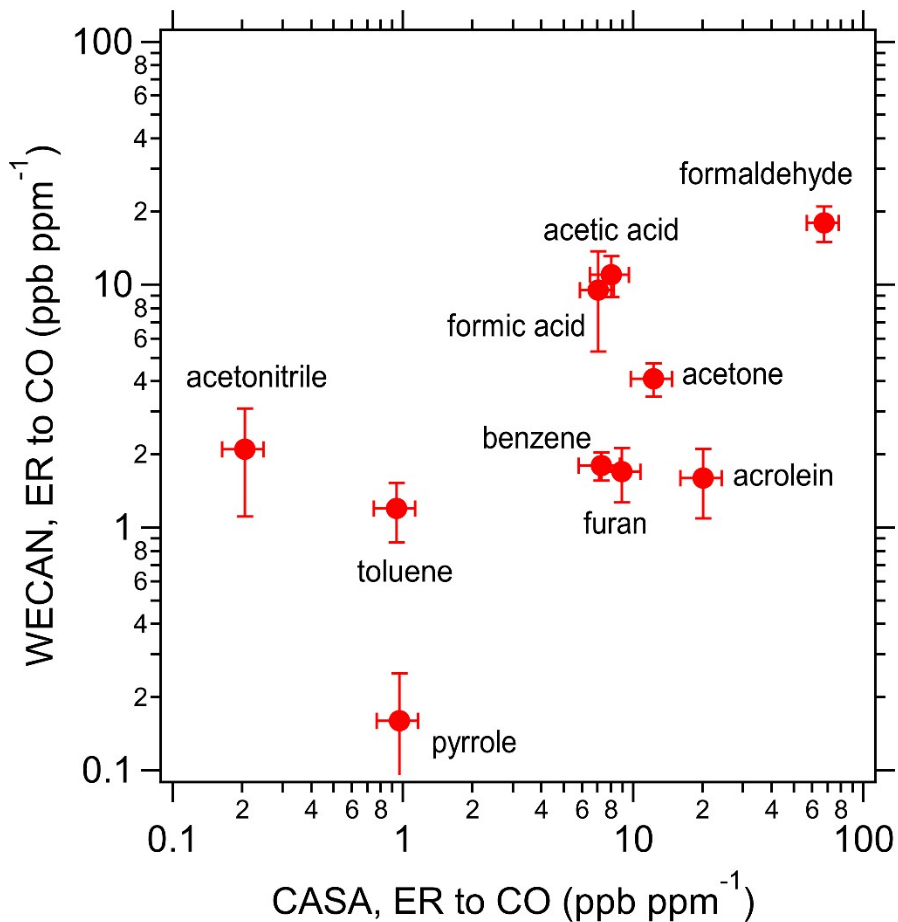
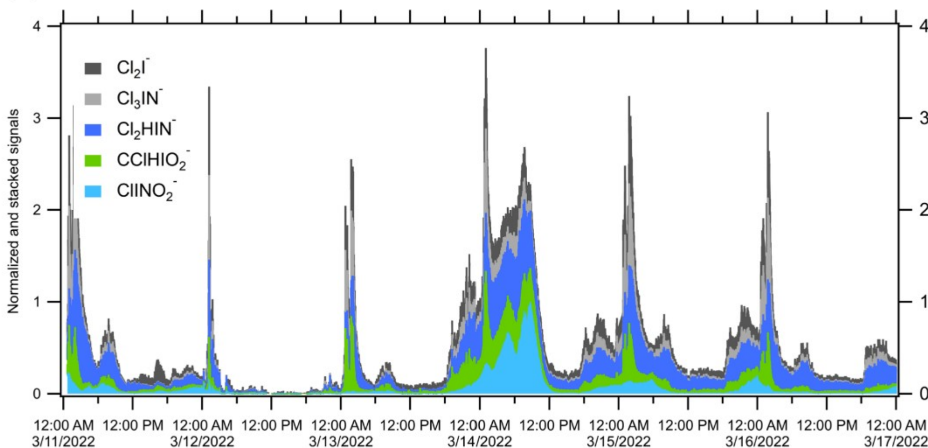


Figure S4. The emissions ratios for selected VOCs using PTR-MS and laser absorption spectrometer data (formic acid, formaldehyde) are compared to literature data from Permar et al.[1] This figure was adapted from data initially presented by Li et al.[2]



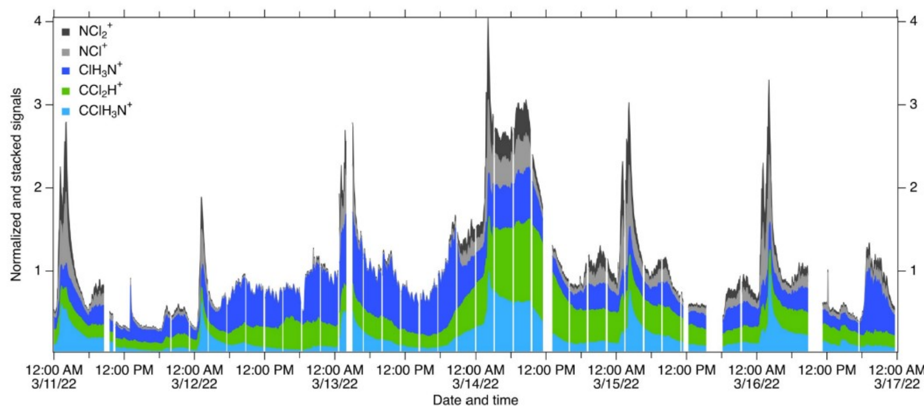


Figure S5. Timeseries showing stacked signals from five observed ions for the (A) iodide-CIMS and (B) PTR-MS instruments across several days (11-17 March). Signals are normalized to their maximum value during the time period. Each instrument showed clear spikes in concentration late at night (midnight to 2am local time) when the automated laundry cycles were running.

SI Section 3. Materials and methods for microbial analysis.

3.1 Microbial analysis

Bacterial and fungal communities in floor dust (n = 3) were measured with quantitative polymerase chain reaction (qPCR) and next-generation DNA sequencing. Dust samples were stored at 4 °C and then sieved to remove particles with diameter > 300 µm (Retsch GmbH, Haan, Germany). DNA was extracted using the Maxwell® RSC PureFood GMO and Authentication Kit (Promega Corp., Madison, WI, USA) with 5 min of bead beating to release spore contents (Biospec Products, Inc., Bartlesville, OK, USA). Bead tubes contained 0.3 g of 100 µm glass beads, 01 g of 500 µm glass beads, and 1 g of garnett particles (Biospec Products, Inc.).[3] Fungal concentration was estimated using a universal fungi qPCR assay targeting the 18S rRNA gene.[4] After elution, samples were diluted 100x in Tris-EDTA to minimize PCR inhibition, then measured in triplicate against a standard curve made with *Aspergillus fumigatus* AF293 spores.

Measured concentrations were expressed in spore equivalents of DNA. Sample wells received 12.5 µL of PowerUp™ SYBR™ Master Mix (Applied Biosystems, Waltham, MA, USA), 9 µL of DNAse free water, 2 µL of extracted DNA, and 0.75 µL each of the primer pair FF2 (5'-GTTAAAAAGCTCGTAGTTGAAC-3') / FR1 (5'-CTCTCAATCTGCAATCCTTATT'-3')[5] at 10 µM concentration. Thermal cycling parameters were as follows: 50 °C for 2 min followed by 95 °C for 10 min and then 40 alternations between 95 °C for 15 s and 60 °C for 1 min. Bacterial concentration was not measured.

Sequencing of extracted DNA was performed at Research and Testing Laboratory (Lubbock, TX, USA) using an Illumina MiSeq equipped with 2x300 bp chemistry (Illumina Inc., San Diego, CA, USA) at a depth of at least 30,000 sequences per sample. The following adapters were selected to target the bacterial 16 S rRNA and fungal ITS1 regions: 515F (5' – GTGCCAGCMGCCGCGGTAA – 3'), 806R (5' – GGACTACHVGGGTWTCTAAT – 3'), ITS1F (5' – CTTGGTCATTTAGAGGAAGTAA – 3'), and ITS2aR (5' – GCTGCGTTCTTCATCGATGC – 3').[6] Sequence data were uploaded to GenBank (PRJNA1072831). Bacterial sequences were processed using a QIIME2-based bioinformatics pipeline.[7-11] Specifically, adapter sequences were removed using *Cutadapt*[12] and denoising was performed with *DADA2*[13] truncating the forward and reverse reads at 240 and 160 base pairs respectively. Taxonomy was assigned using the naïve bayes feature classifier[14, 15] trained on the Greengenes database[16]. Fungal sequences were denoised using a DADA2-based pipeline customized for ITS sequences[13]. *Biostrings* version 2.66.0[17], *Cutadapt* version 4.4[12], and *ShortRead* version 1.56.1[18] were used to remove adapters. The *maxEE* and *truncQ* parameters of the *filterAndTrim* function in DADA2 were both set to eight following Rolling et al.[19]. The UNITE version 9.0 database was used for taxonomic identification.[20]

Absolute abundances of fungal ASVs were calculated by multiplying relative abundance values from sequencing with fungal concentration data from qPCR[21]. For bacteria, relative abundance data were used for analysis. Sixteen bacterial ASVs detected in an extraction blank were considered contaminants and removed from all samples. Attributes of fungal genera were retrieved from the FungalTraits database[22]. R version 4.3.0 and the following packages were used for data exploration: *phyloseq* version 1.44.0[23], *readxl* version 1.4.3[24], *tidyverse* version 2.0.0[25], *vegan* version 2.6.4[26] and *writexl* version 1.4.2[27]. Fungal measures were compared with nine dust samples from occupied homes across the United States incubated for 1 week at 50% relative humidity.[28] These latter samples were processed, extracted, sequenced, and then denoised using an identical fungal protocol except that sequencing was performed at a depth of at least 10,000 reads per sample. Sequence data from occupied homes are available from GenBank (PRJNA1072816). The following samples were used specifically: SRR27861756, SRR27861752, SRR27861749, SRR27861746, SRR27861742, SRR27861739, SRR27861734, SRR27861731, SRR27861728.

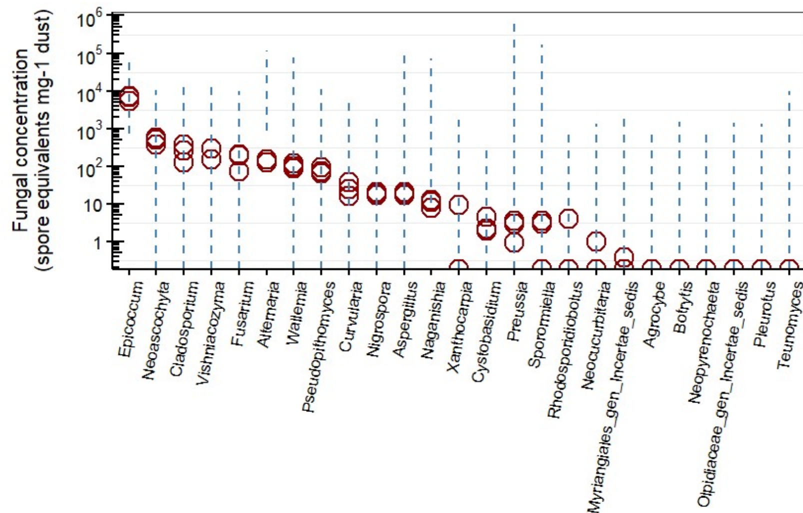


Figure S6. Concentration (spore equivalents mg^{-1} dust) of most abundant fungal genera in floor dust of test house (circles, n = 3) and occupied homes (error bars, n = 9). All genera comprising ≥ 2.5 % of reads in at least one dust sample (i.e., test house or occupied home) are displayed.

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Certain equipment, instruments, software, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement of any product or service by NIST, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose. The policy of NIST is to use the International System of Units in all publications. In this document, however, some units are presented in the system prevalent in the relevant discipline.

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