Bad air and good air revisited

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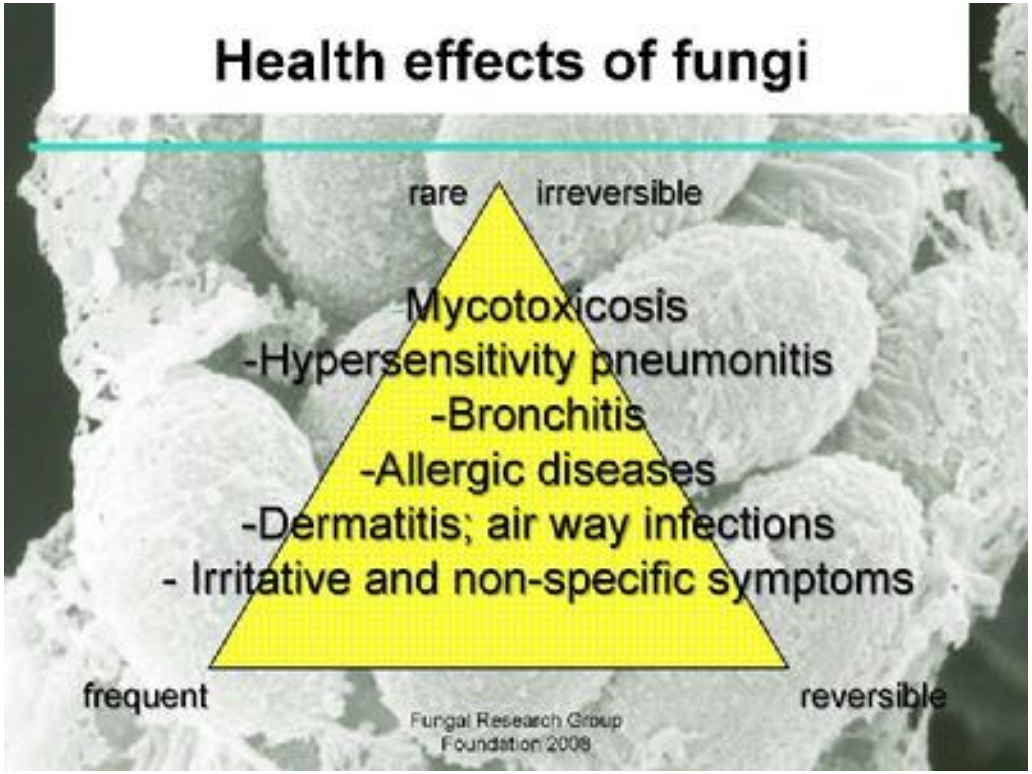
Known health risks associated with fungi

-- Fungal pathogens, especially in immuno-compromised individuals

-- Asthma

-- Allergy

--Toxins



http://www.johanningmd.com/moldandwaterdamage.html

Building-Related Illnesses

"**Building-related illnesses (BRIs)** are a heterogeneous group of disorders whose etiology is linked to the environment of modern airtight buildings"

- Legionella infection
- Occupational asthma
- Hypersensitivity pneumonitis
- Inhalational fever (humidifier fever, grain fever, swine confinement fever, organic dust toxic syndrome, mycotoxicosis)

Nonspecific BRIs:

"Nonspecific BRIs are those for which a link between building-related exposure and illness is more difficult to prove. The term **sick building syndrome** has been used to refer to illnesses that occur in clusters within a building and that cause often nonspecific symptoms.

http://www.merckmanuals.com/professional/pulmonary_disorders/environmental_pulmonary_diseases/building-related_illnesses.html

http://www.home-air-purifier-expert.com/sick-building-syndrome.html#introduction

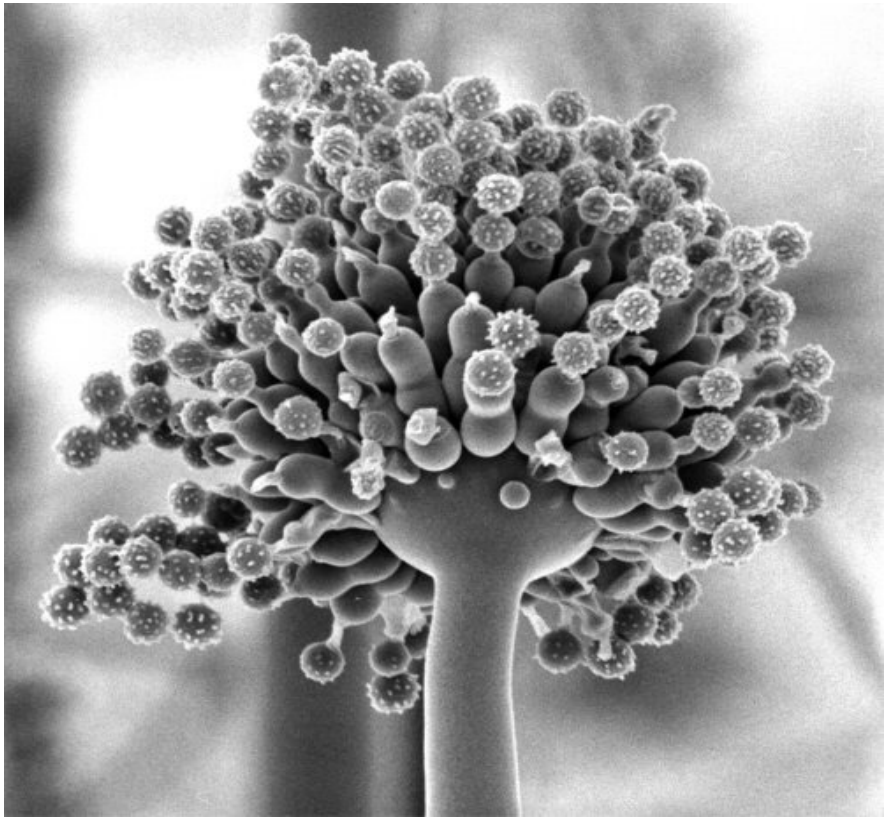
“Sick building syndrome”

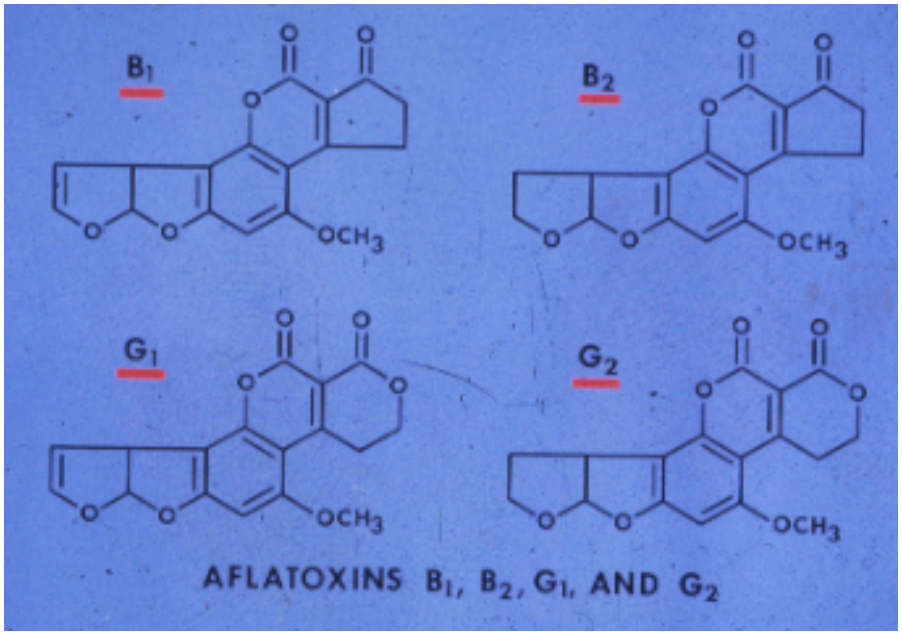
A controversial medical diagnosis consisting of a variety of non-specific symptoms that include eye irritation, sore throat, cough and shortness of breath, depression, fatigue, headache, dry itchy skin, insomnia, dizziness, immune dysfunction, nausea, nosebleeds, numbness, diarrhea, genitourinary changes, and tremors.

Building related illnesses

Diseases and Exposure

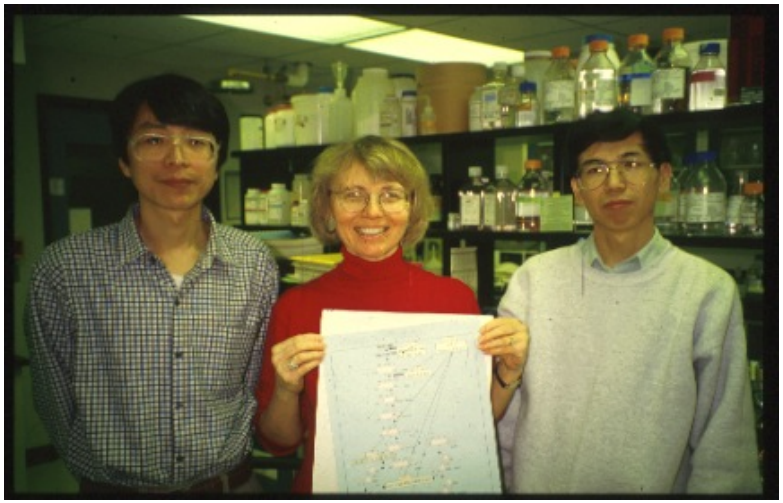
http://www.johanningmd.com/images/300_buildin_related_disease_comic02.jpg



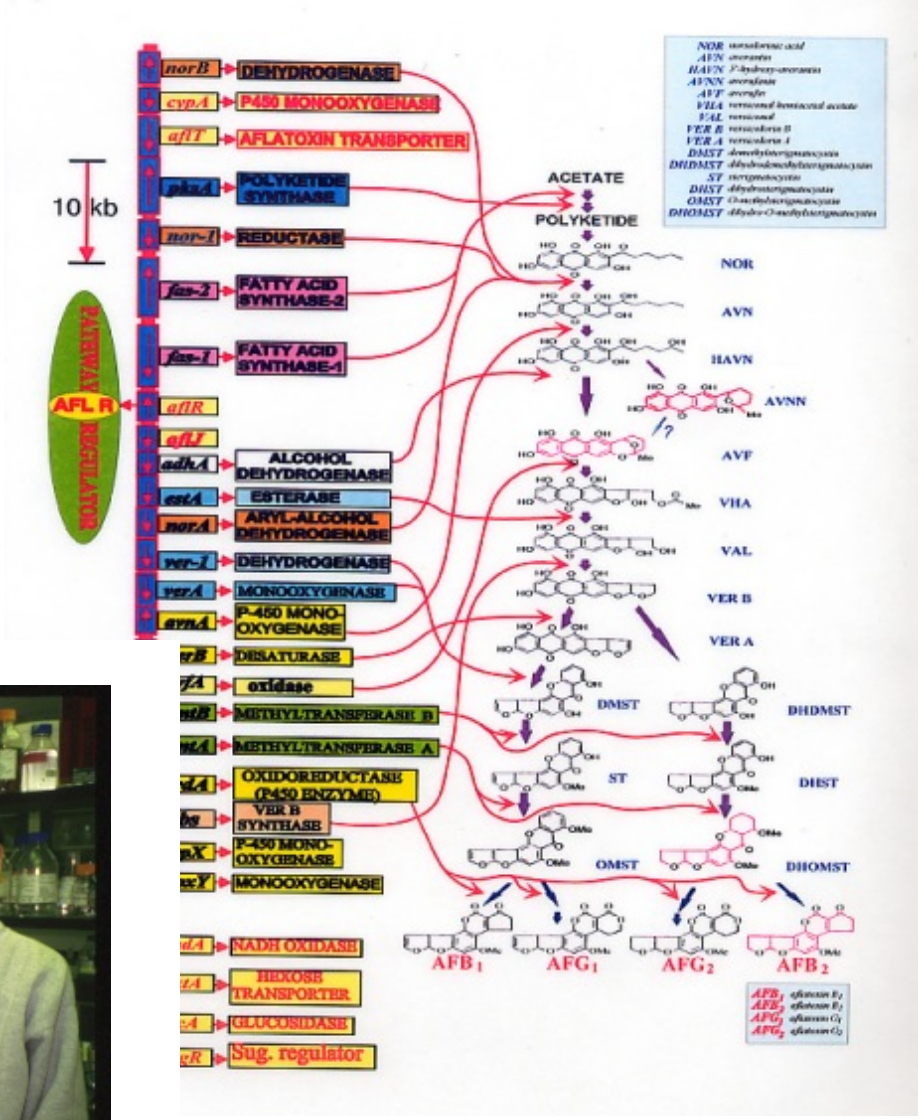


For much of my professional life I worked at Tulane University in New Orleans, Louisiana where I taught genetics, studied aflatoxin biosynthesis, and collaborated with colleagues at the Southern Regional Research Center (U.S. Dept. Agriculture) on mycotoxin research.

Aflatoxin biosynthesis

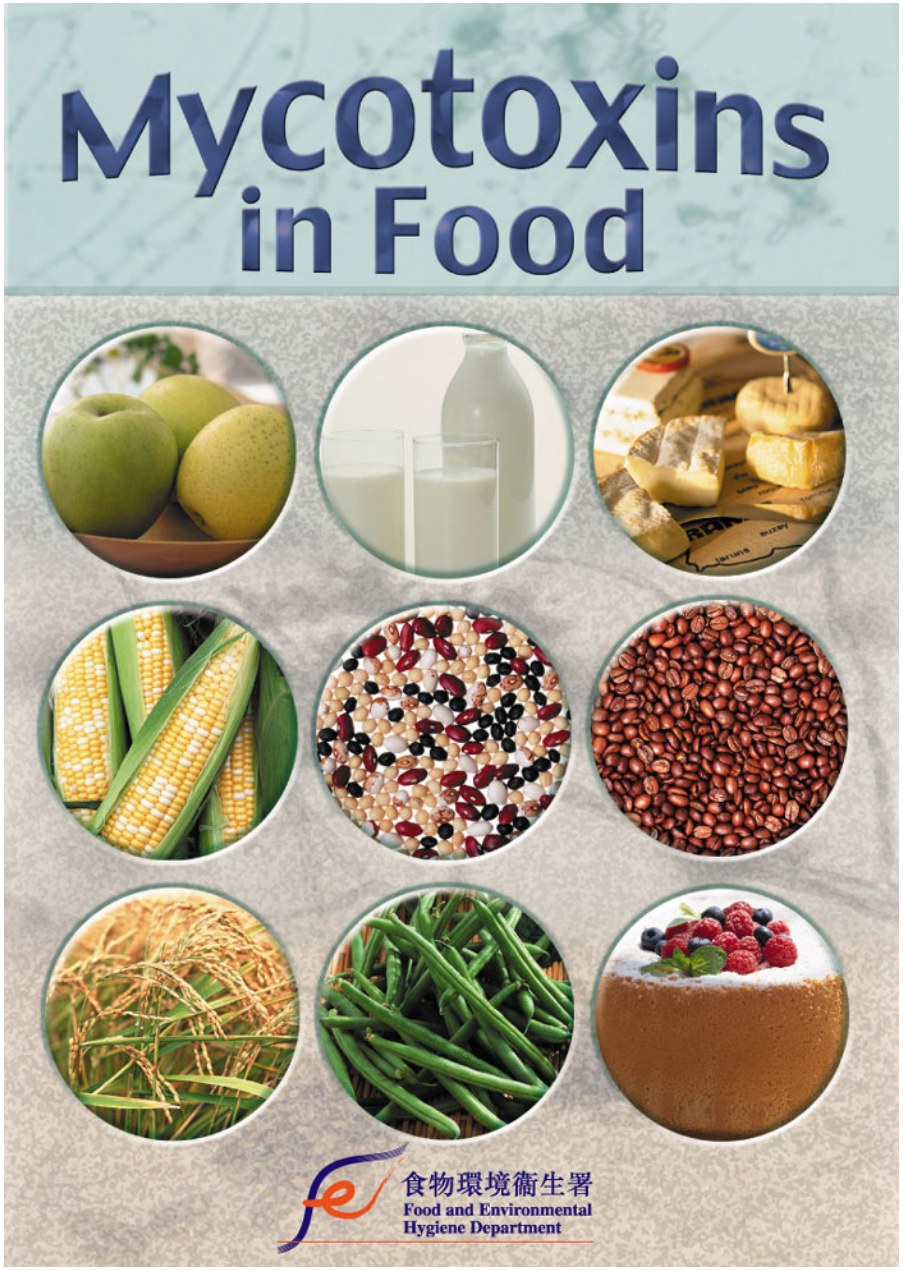


P.-K. Chang J. Yu



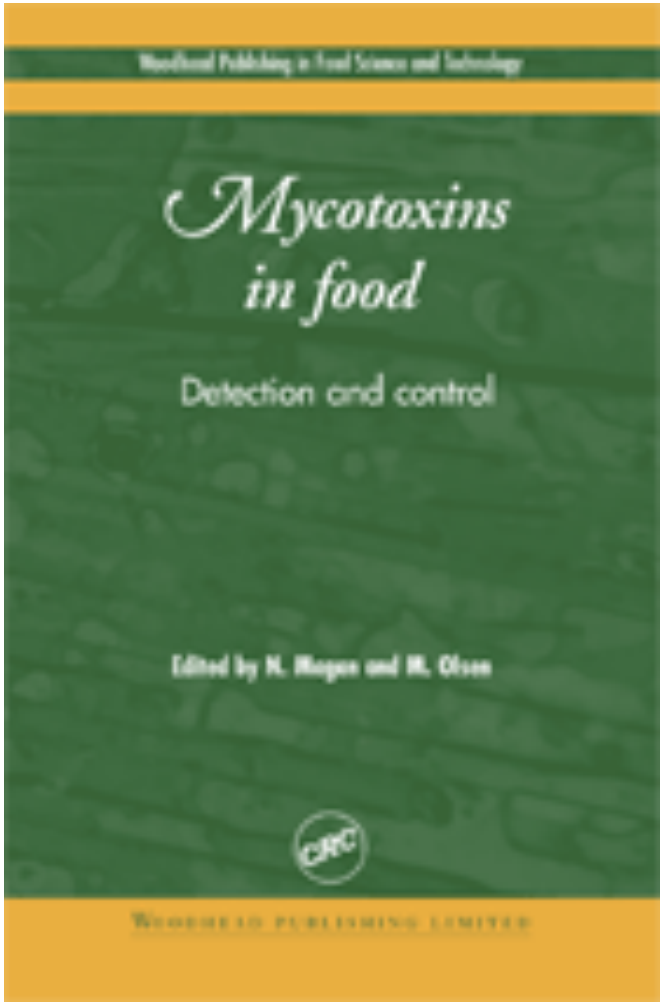
Biosynthetic pathway gene cluster

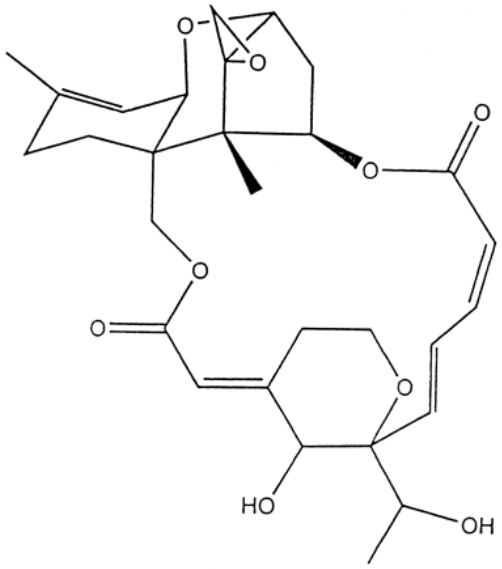
Louise Lee, Ed Cleveland, Deepak Bhatnagar, Maren Klich, Jeff Carey, Nancy Keller



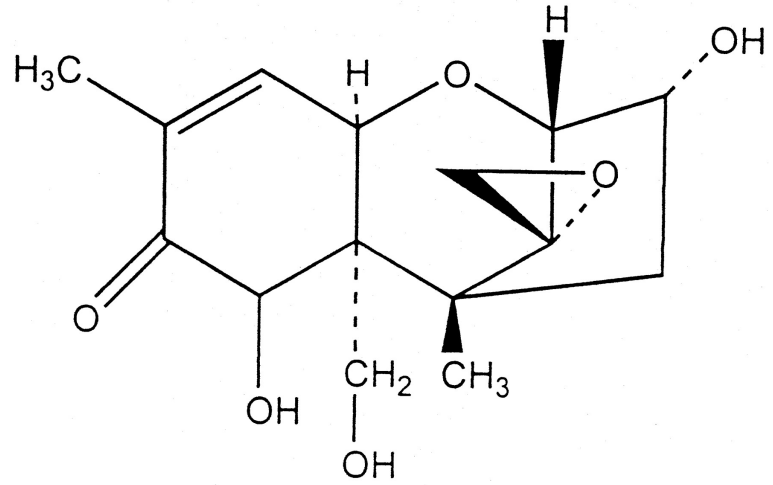
Government of Hong Kong

Food and Environmental Hygiene Department





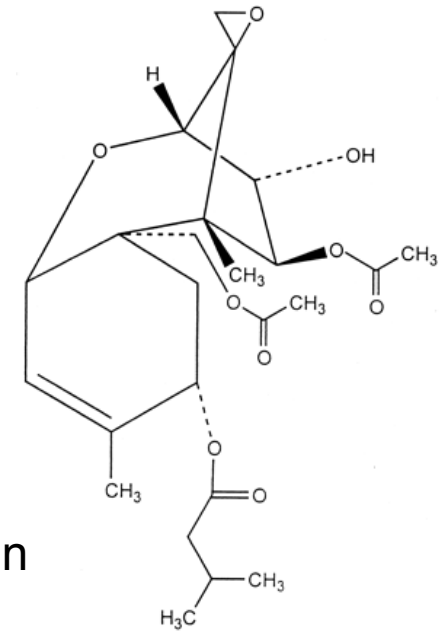
Satratoxin H



Deoxynivalenol

Trichothecene mycotoxins

More than sixty of these sesquiterpenoids are known. All contain a common 12,13 epoxytrichothene skeleton. Consumption of these mycotoxins results in alimentary hemorrhaging and vomiting; direct contact causes dermatitis.



T-2 Toxin

“Black mold” and pulmonary hemorrhage

During the 1990s, eight infants in Cleveland, Ohio suffered pulmonary hemorrhage. They all lived in mold damaged homes. When *Stachybotrys* was isolated from these homes it was hypothesized that fungal toxins (trichothecenes) were associated with the deaths. Later the CDC concluded that a cause-and-effect relationship had not been proven.

Public fear of “black mold”

Public fear of molds is high. Unscrupulous lawyers and “remediation” companies have taken advantage of the lack of sound science.

In many states, insurance companies stopped issuing coverage for mold associated damage

My perspective: most mycotoxin research has been conducted in an agricultural context and focuses on dietary exposure.

--Inhalation of mycotoxins in spores is a possible health hazard but it is difficult to breathe in high levels of mycotoxins, or absorb them through the skin, in sufficient quantity for toxicity.

--No single mycotoxin is known to cause anything like the array of symptoms associated with sick building syndrome.

Fungal genomics

In August, 2005, I was about to start a sabbatical leave and was scheduled to go to The Institute for Genomics Research (TIGR – now Craig Venter Institute) to work on the **annotation of the genome of the aflatoxin-producing fungus *Aspergillus flavus.***

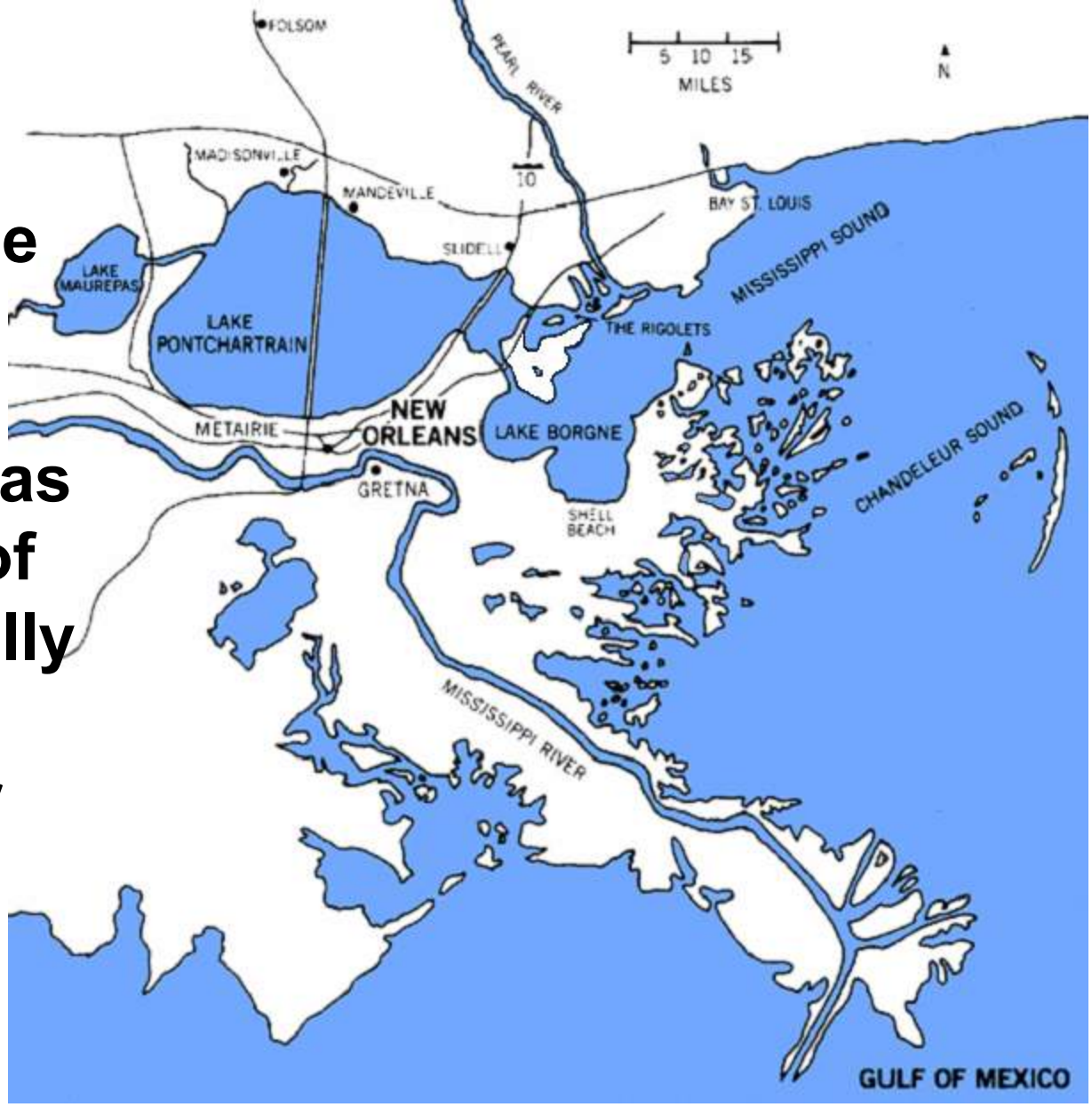
Hurricane Katrina

Sunday
August 28, 2005

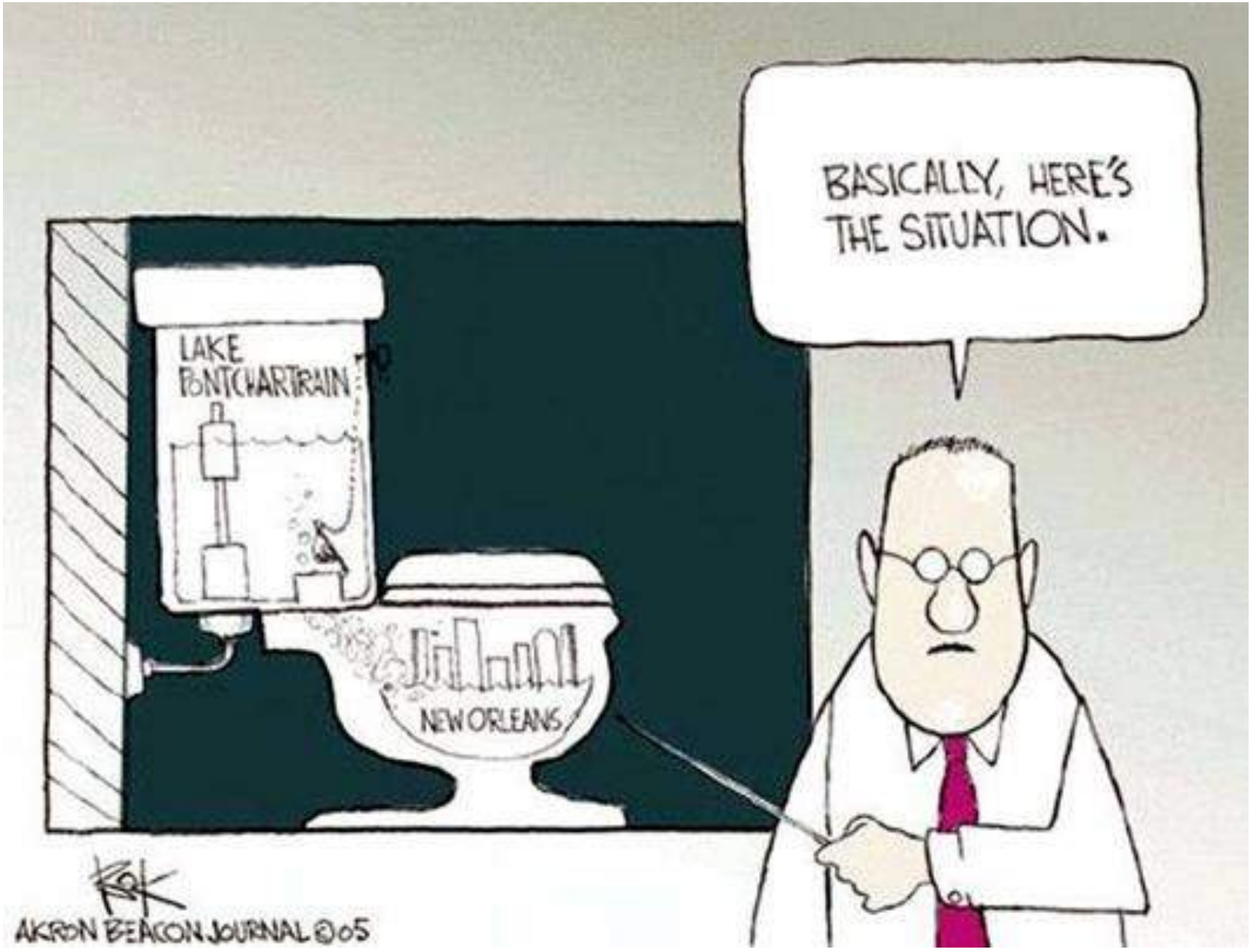
A Category 5 Storm

(National Geographic)

New Orleans is surrounded by water. Most of the city is below sea level and for its entire history it has faced the threat of flooding, especially from the Mississippi River



http://www.mpcnetwork.com/marinersinfo/charts/gm/2neworleans.gif


BASICALLY, HERE'S THE SITUATION.
LAKE PONTCHARTRAIN
NEW ORLEANS
AKRON BEACON JOURNAL ©05

The failure of the levees following Hurricane Katrina flooded the city.

http://www.hurricanekatrina.com/

http://www.floodsite.net/juniorfloodsite/html/en/student/thingstoknow/geography/katrina2.html

After Hurricane Katrina, 80% of the buildings and their contents spent weeks filled with flood water. After the floods receded, filamentous fungi grew on all organic surfaces.

Photo courtesy of Bob Thomas, Loyola University

Post-Katrina mold in the built environment

http://tulane.edu/news/newwave/images/100610_poe_mold_2.jpg

http://media2.s-nbcnews.com/j/msnbc/Components/Photos/051117/051117_hurricaneMold_hmed_5a.grid-6x2.jpg

http://www.a-ztech.us/content/mold-testing-and-consultation

Home of Dr. Jiujiang Yu

(Southern Regional Laboratory)

My story

My husband and I had left the city before the storm, and eventually drove to New Jersey. The government did not let us return to New Orleans for over a month . Before returning home, I asked a mycologist friend at Rutgers University, Dr. Jim White, if I could use his lab to make some media on which to cultivate molds.

On Oct. 14, 2005 I returned home for the first time . . .

As I walked through my house, I took pictures and then used a sterile swab to sample different parts of my flooded home.

The samples I took from my flooded home have formed the basis of my new research.

Our home after flood receded, October 2005.

Family room

Entry way to our house with “honeymoon rugs” and 18th century clock

The pictures do not convey the terrible odor

The entire time I was taking those pictures (wearing a mask), I felt that something was very wrong and that I should not be breathing that air.

The horrible, horrible smell in my flooded home made me feel sick. I re-examined my view about "sick building syndrome."

Back in New Jersey at Rutgers University I subcultured my Katrina molds

The University invited me to complete my sabbatical at Rutgers during the spring semester of 2006 and then offered a permanent job.

biotech.rutgers.edu/images/homephoto-foranhall.jpg

“

Fungi and non specific building related illness

The number one suspect is black mold and mycotoxin (trichothecene) exposure . However, some researchers have hypothesized the volatile organic compounds (VOCs) might be involved.

Mølhave, L. (2009). Volatile organic compounds and the sick building syndrome. In Environmental Toxicants: Human Exposures and their health effects. (M. Lippmann Ed) 3rd ed., pp. 241-256, Wiley-Interscience, New York.

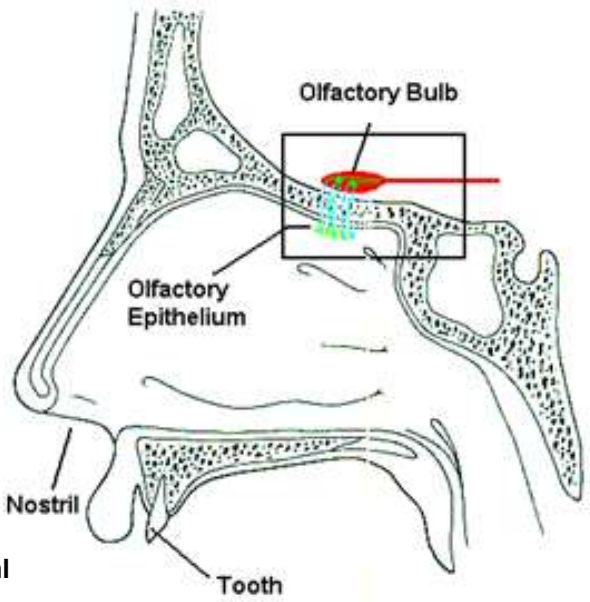
Volatile organic compounds (VOCs) are low molecular mass compounds with high vapor pressure that easily evaporate from liquid form at room temperature.

Among the best known VOCs are industrial solvents such as ethyl acetate, chloroform and toluene.

Our sense of smell depends on volatile organic compounds

A chemical compound has a **smell** when two conditions are met: the compound needs to be volatile, so it can be transported to the olfactory system in the upper part of the nose, and it needs to be in a sufficiently high concentration to be able to interact with one or more of the olfactory receptors.

http://en.wikipedia.org/wiki/Aroma_compound



http://faculty.washington.edu/chudler/chems.html

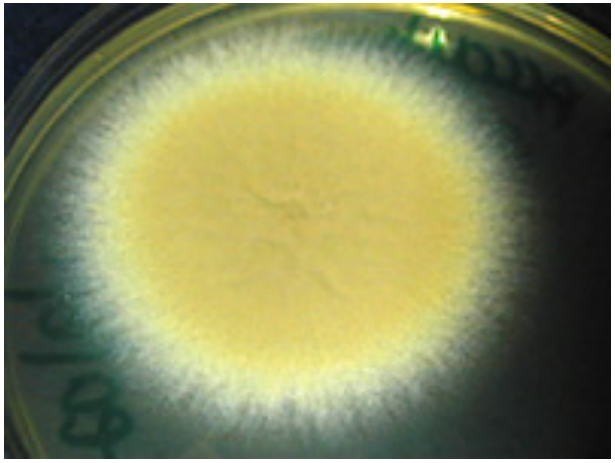
My post-Katrina lab set out to answer these questions:

1. What fungi were in my flooded home? What VOCs did they make?

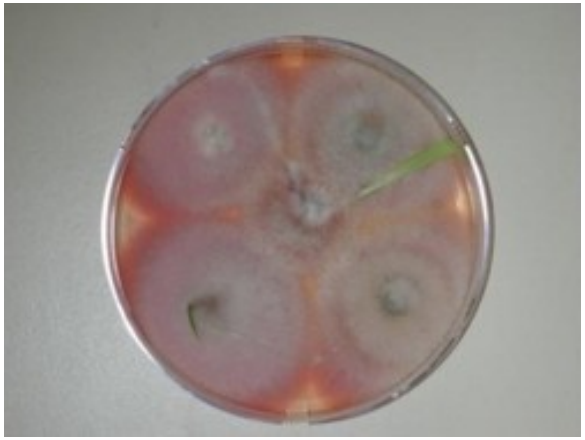
2. Can the VOCs made by these fungi be tested in genetic models for their possible toxicity?

3. If toxigenic VOCs can be found, do they affect human health?

Cladosporium

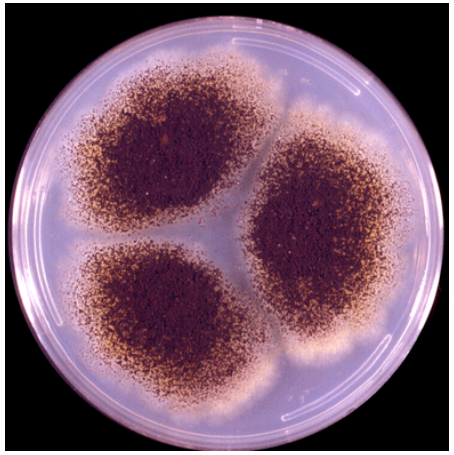


Paecilomyces

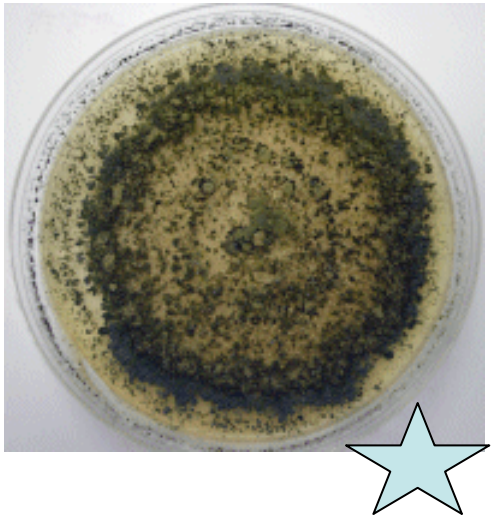


Phoma

Penicillium



Aspergillus

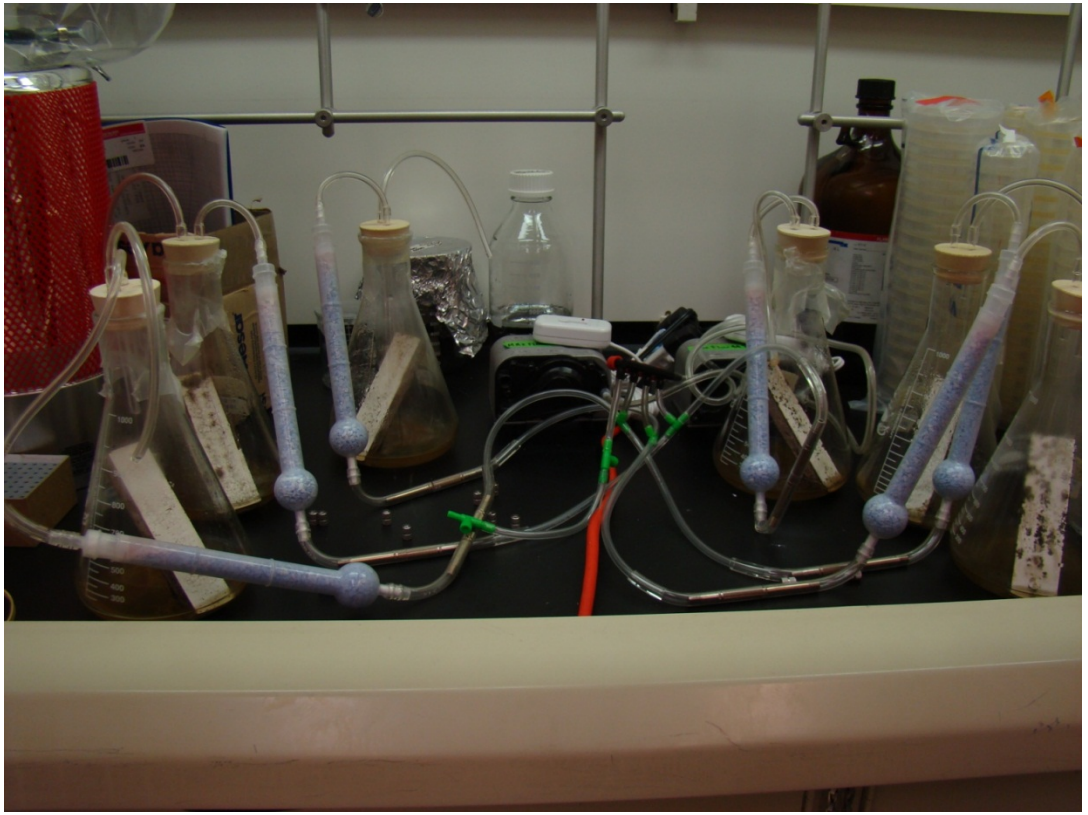


Trichoderma

We used GC-MS to identify volatile organic compounds (VOCs) from Katrina molds, developed model habits, and tested individual VOCs in these model habits and systems.

Assaying volatile organic compounds (VOCs)

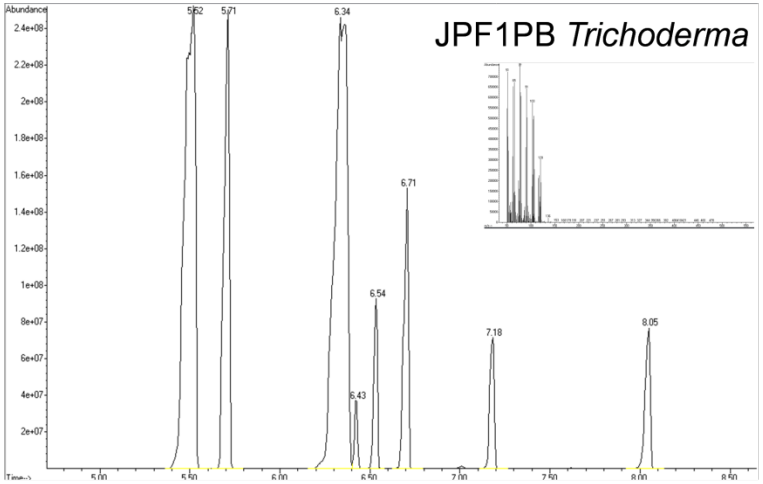
Strains grown on water saturated “dry wall” (gypsum board) in a flask at 25^{o}C. VOCs were adsorbed on a Tenax TA resin and analyzed by GS-MS.



Richard Hung

VOCs produced by molds as analyzed by GC-MS

Mold produce complex mixtures of alcohols, ketones, esters, small alkenes, monoterpenes, and sesquiterpenes. Each species emits a unique profile of different VOCs, the proportion and concentration of each individual VOC varies depending on the substrate, length of fungal growth, and other environmental parameters.



Gas Chromatograph with Mass Spectrum Inset

We did not find any new, unusual VOCs.

Four genetic models:

Caenorhabditis elegans

Arabidopsis thaliana

Drosophila melanogaster

Saccharomyces cerevisiae

Two VOC approaches:

Expose models to growing fungi

Expose models to low amounts of authentic standards of individual VOCs

(we bought 14 of the cheapest ones)

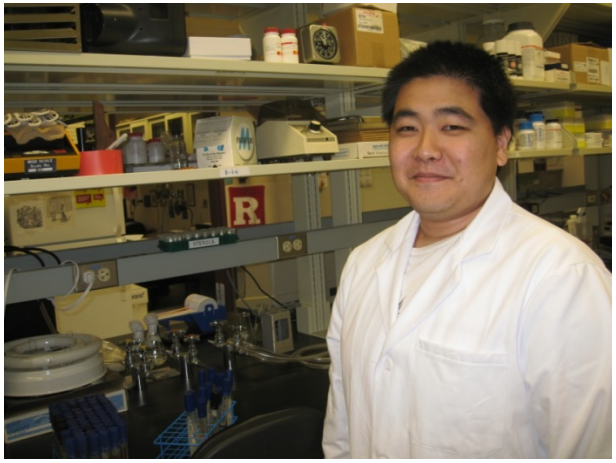
http://www.kiwicrossing.com/drrussell/images/worm.jpg

In two years of rather futile preliminary experiments, we learned that *C. elegans* tends to crawl out of the Petri dish when exposed to VOCs from growing Katrina molds.

(Thanks to Monica Discoll and members of her lab for helping us.)

Arabidopsis thaliana

pioneered by Richard Hung and Samantha Lee



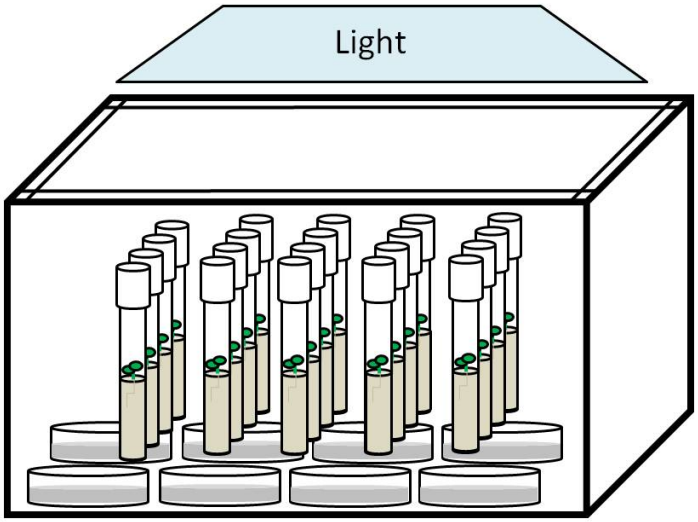
Spent a lot of time developing exposure systems

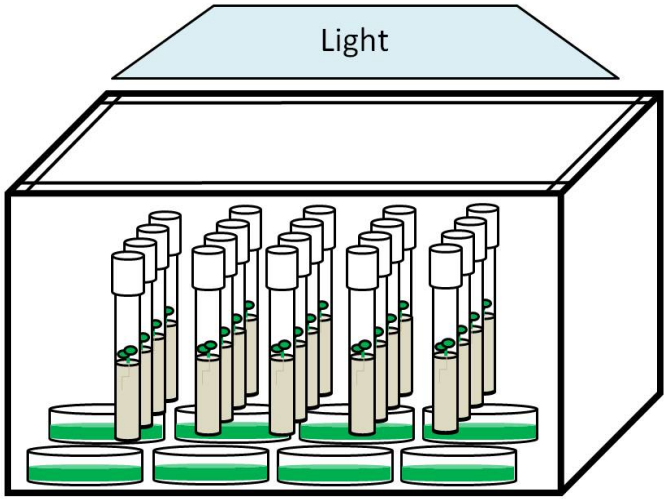
1-octen-3-ol

Control 3 ppm 4 ppm♪

♪

Exposure of 14 day old plants for 24 hours♪



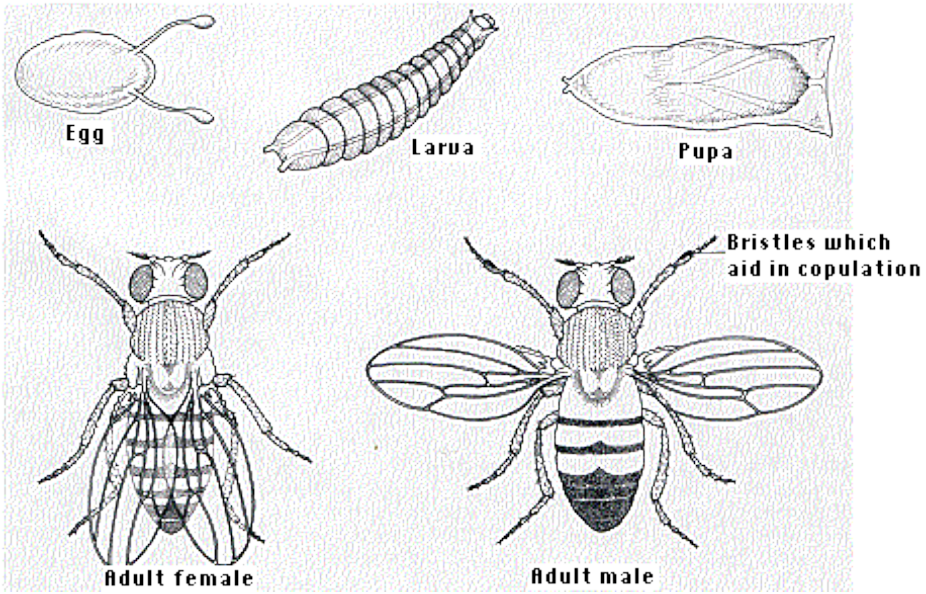


Arabidopsis exposure chambers: control and Trichoderma exposed

Arabidopsis thaliana **exposed to** ***Trichoderma viride*** **VOCs for two weeks. The visible indicators of growth promotion in volatile exposed plants were observed including larger leaves, root mass, and lateral root development.**

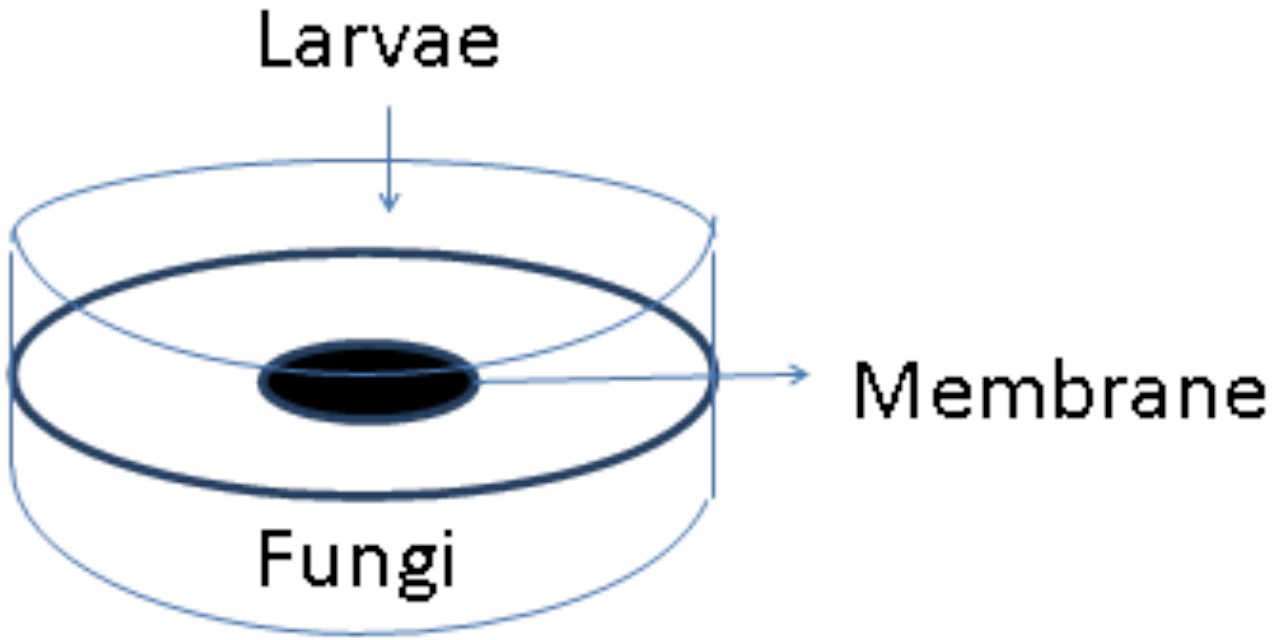
Today will focus on other experiments using other models in which we found toxigenic effects.

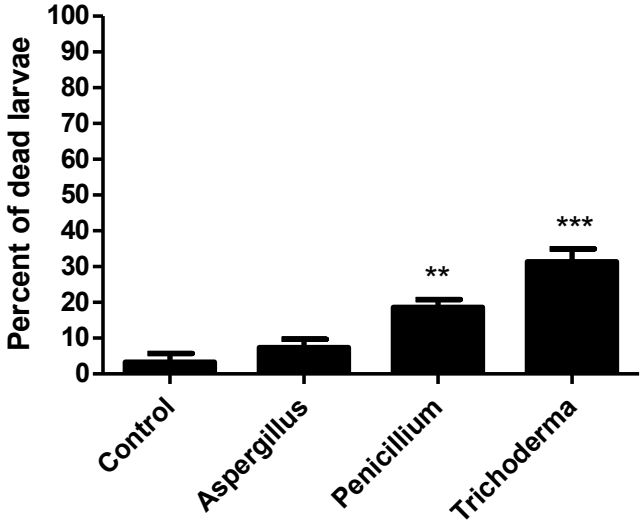
We also have developed a *Drosophila melanogaster* model for testing the toxicity of mold and industrial VOCs. Dr. Arati Inamdar has led the project, using both adult and larval exposure systems.

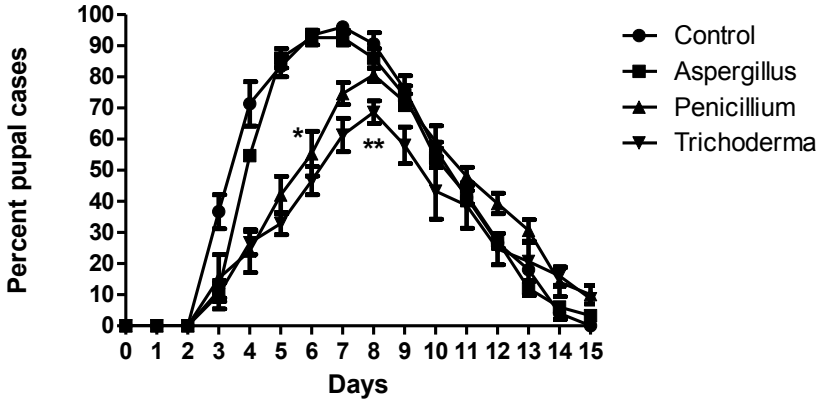


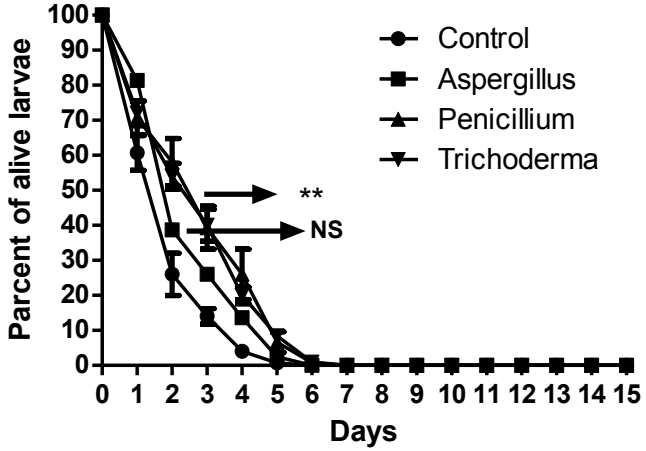
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/Drosophila.html

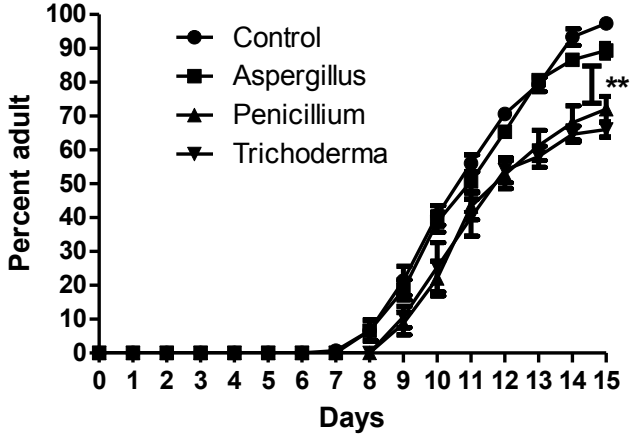
Double Petri plate system to test VOCs emitted from a living culture of mold: larvae on one plate and fungus on the other





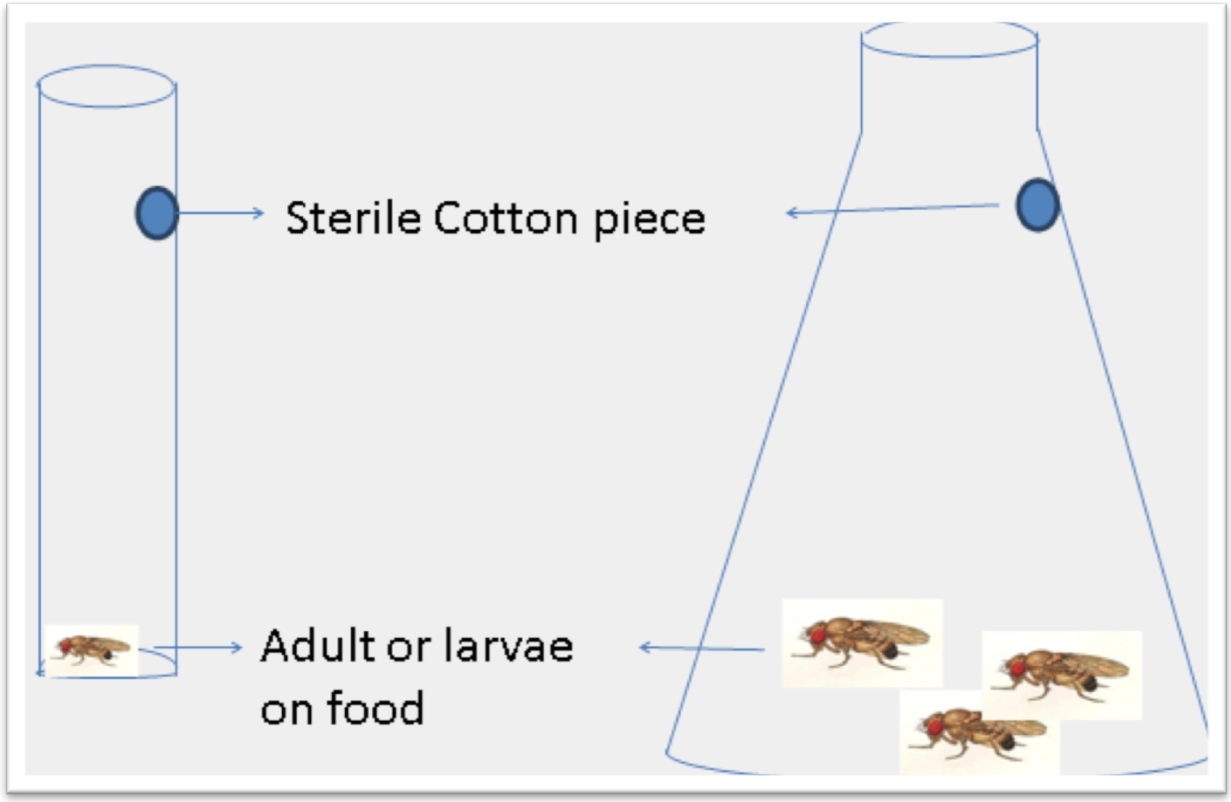






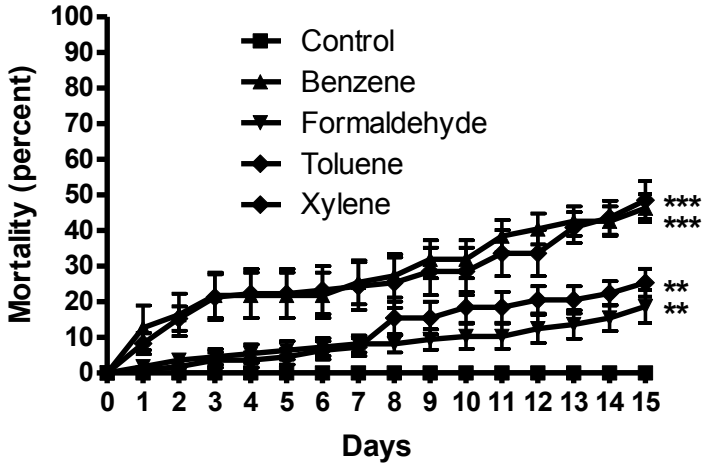
Exposure to volatile metabolites from *Aspergillus niger*, *Penicillium chrysogenum* and *Trichoderma viride* induces differential toxicity to 3rd instar larvae of *Drosophila melanogaster* (Indamdar et al 2012)

Exposure method for chemical standards



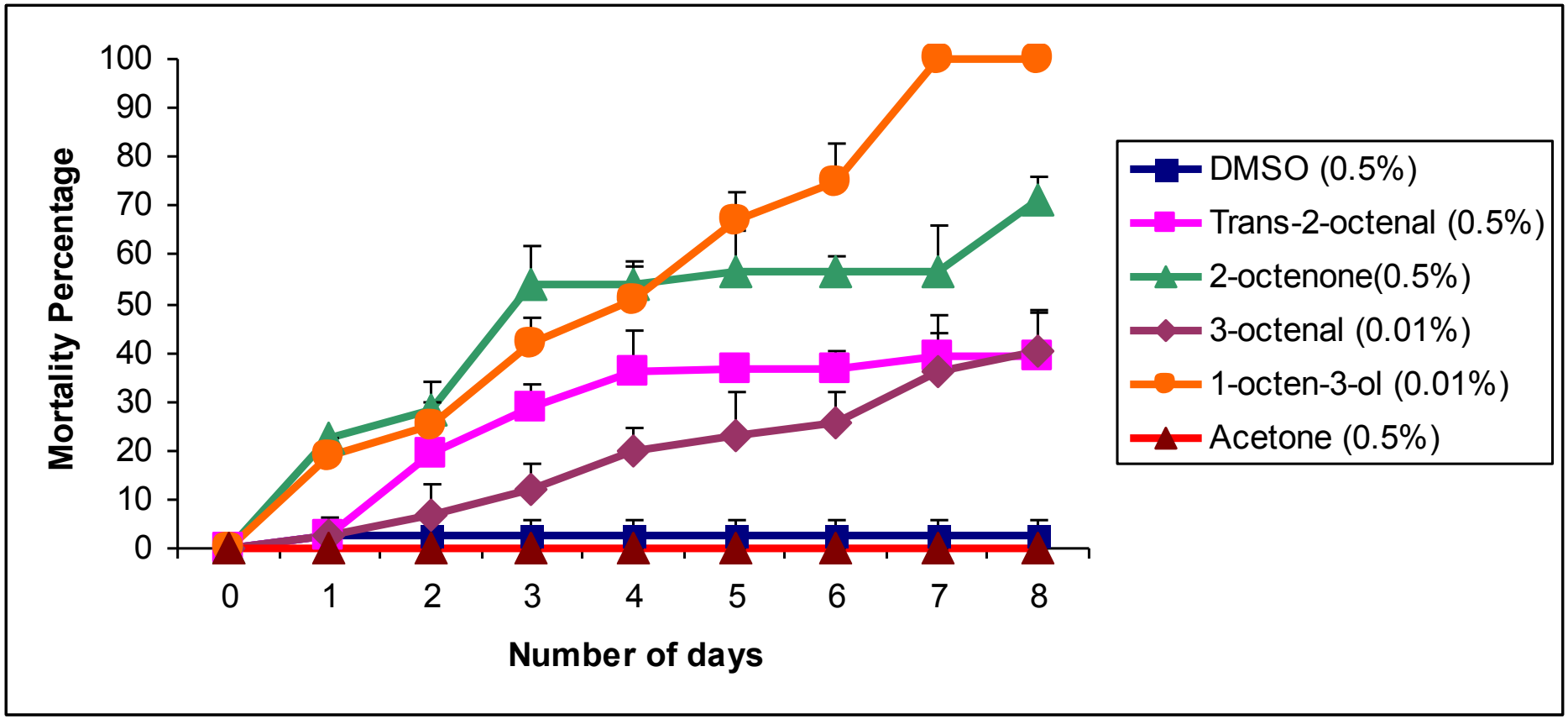
Simple apparatus to facilitate intake of vapor phase VOCs by Drosophila flies. Chemical exposure is calculated in ppm based on v/v (liquid chemical/area of exposure vessel)

Industrial solvents



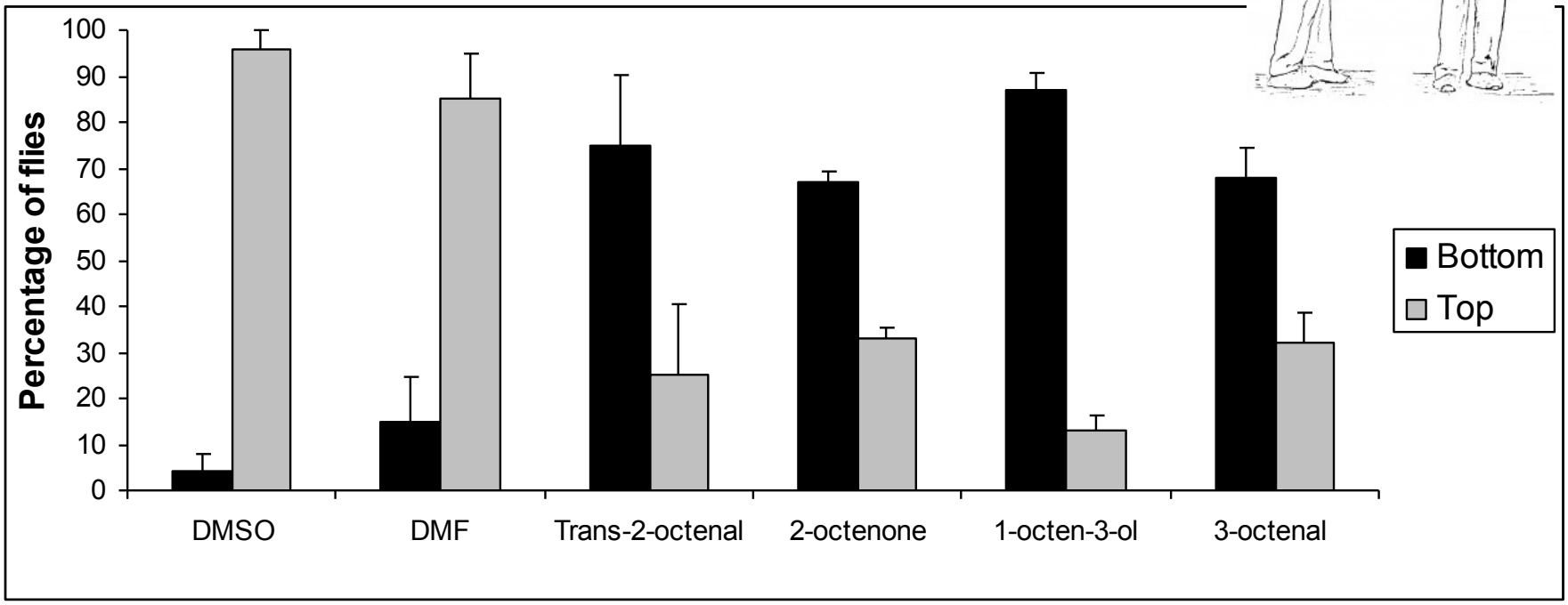
Exposure of adult wild type flies to industrial solvents. We exposed adult flies to benzene, formaldehyde, toluene and xylene at 2.8 ppm concentration for 15 days. The percent mortality was calculated from 10 replicates of 10 flies (N=100) from two individual experiments. **Error bars represent standard error of the mean. * represents the significant difference between control and VOCs exposed flies where **=P<0.005 and ***=P<0.001. (Inamdar et al 2012)**

Adult Drosophila exposed to C-8 VOCs



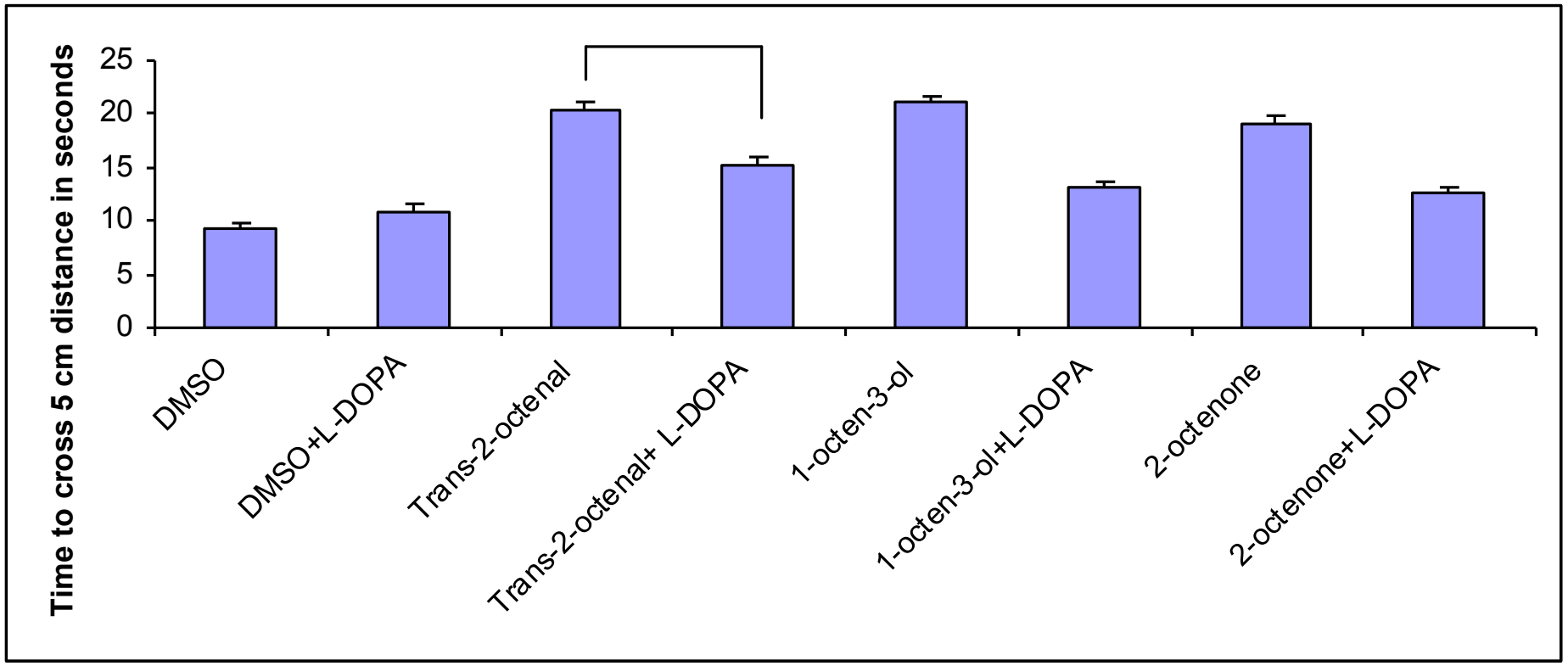
Effect of standard volatile organic compounds on the survival of flies expressed in percentage mortality. DMSO and acetone lack any deleterious effect on flies. Error bars represent SEM (N=60-100 depending on degree of toxicity).

Feany, M. B. and Bender, W. W. (2000). A *Drosophila* model of Parkinson's disease. *Nature* 404: 394-398



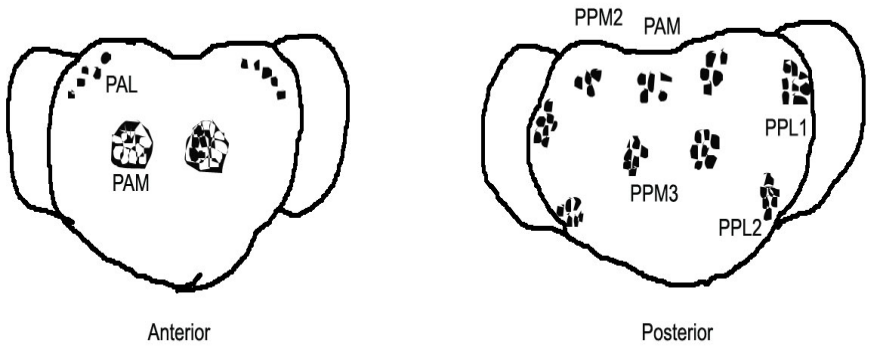
Effect of DMF, trans-2-octenal, 2-octenone, 3-octenal or 1-octen-3-ol on the mobility of flies exposed for 8 sec. (Fifty flies were tested in each trial, however due to toxicity effects N= 30-50)

***Drosophila* negative geotaxis assay (developed for studying Parkinson's disease).**
Flies are scored for their ability to crawl up the sides of a 9 cm vial within 8 seconds. Control flies all reach the top within this time period. Flies that remain at the bottom or crawl less than 5 cm are scored as "bottom," those that crawl more than 5 cm in 8 seconds as scored as "top." (*Inamdar, A. et al. Toxicol. Sci. 117: 418-426***).**



Mobility of *Drosophila melanogaster* exposed to 1-octen-3-ol, trans-2-octenal, 3-octenal and 2-octenone with and without the addition of L-DOPA .

Flies were fed the precursor of dopamine, 3, 4-dihydroxy-L-phenylalanine (L-DOPA) at a concentration of 1 mM for 72 hrs. Then both L-DOPA fed and non-L-DOPA fed flies were exposed to selected VOCs. L-DOPA partially rescued the mobility defect.
(Inamdar, A. et al. Toxicol. Sci. 117: 418-426).

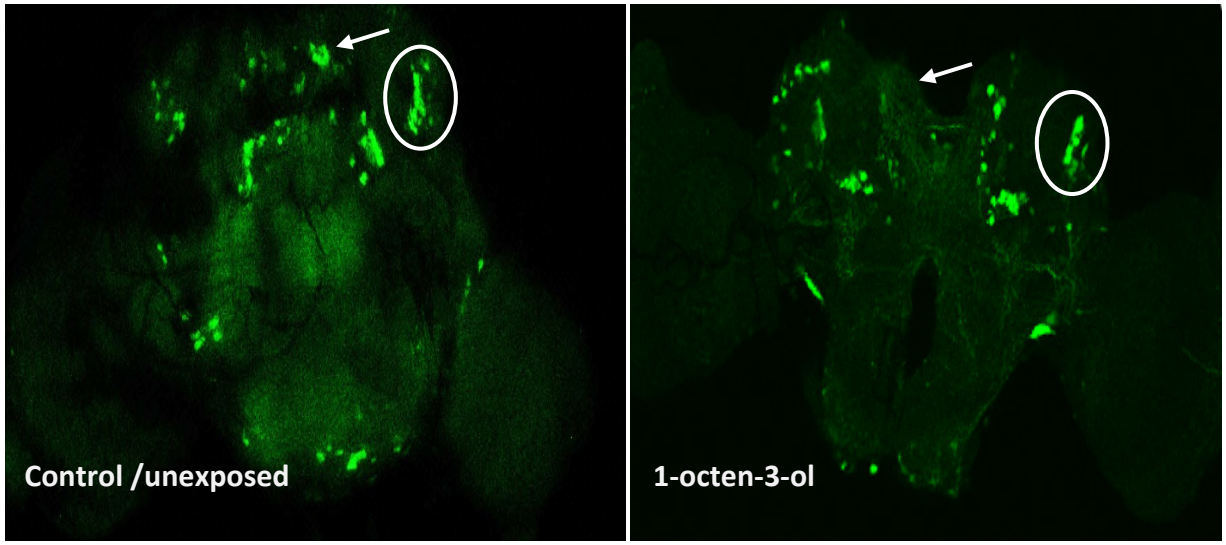


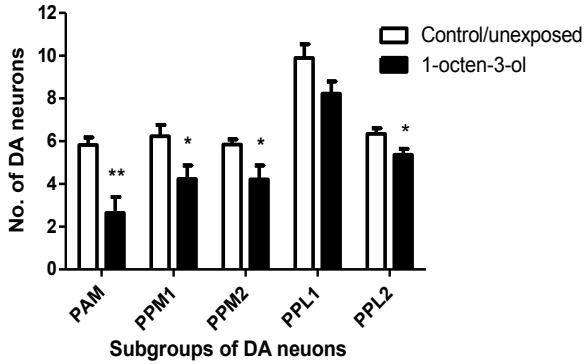
A schematic diagram showing the location and number of dopaminergic neurons in adult *Drosophila* brain viewed from anterior and posterior aspects. (A) The anterior aspect of brain consists of two subgroups, PAL (protocerebral anterolateral) and PAM (protocerebral anteromedial). (B) The posterior aspect consists of five subgroups, unpaired PPM1, paired PPM2, paired PPM3 (protocerebral posterior medial); paired PPL1 and paired PPL2 (protocerebral posterolateral).

Lundell, M. J. and Hirsh, J. (1994) Temporal and spatial development of serotonin and dopamine neurons in the Drosophila CNS, Dev Biol. 165: 385-396.

These dopaminergic neurons can be visualized by driving the expression of Green Fluorescent Protein (GFP) in dopaminergic neurons using the GAL4-UAS-P system

The exposure of 1-octen-3-ol (0.5ppm) on TH-GAL4; UAS-GFP fly brains

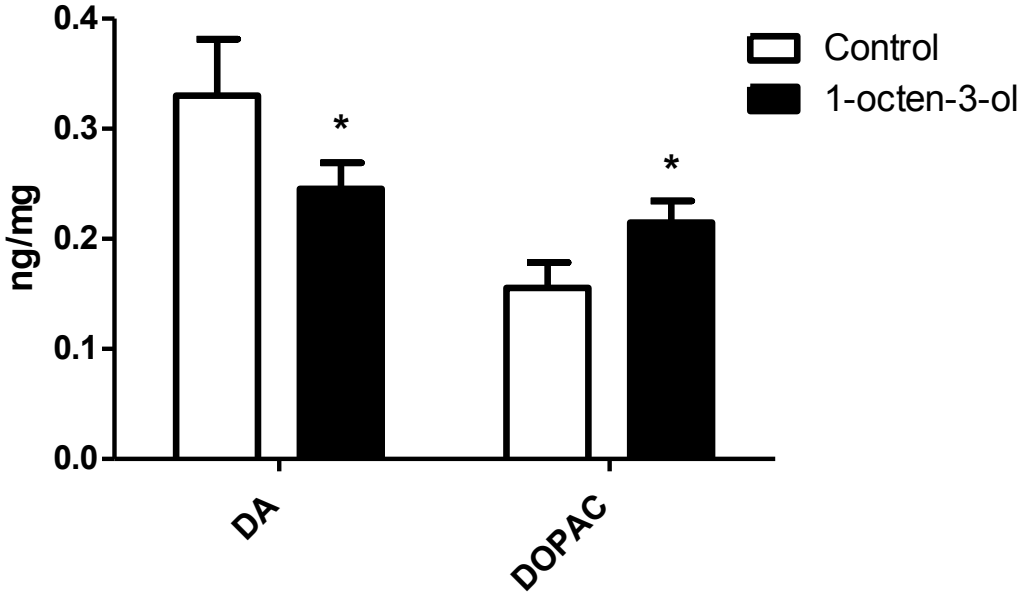




Exposure for 24 hr led to a decrease of GFP-expressing TH neurons.

The PPM1 and PPL1 subgroup of DA neurons are indicated by the arrow and circle, respectively. Except for PPL1, there was a significant decrease in all the subgroups of dopaminergic neurons
(Inamdar et al, 2013 PNAS)

Effect of 1-octen-3-ol on dopamine and DOPAC (a metabolic product of dopamine)

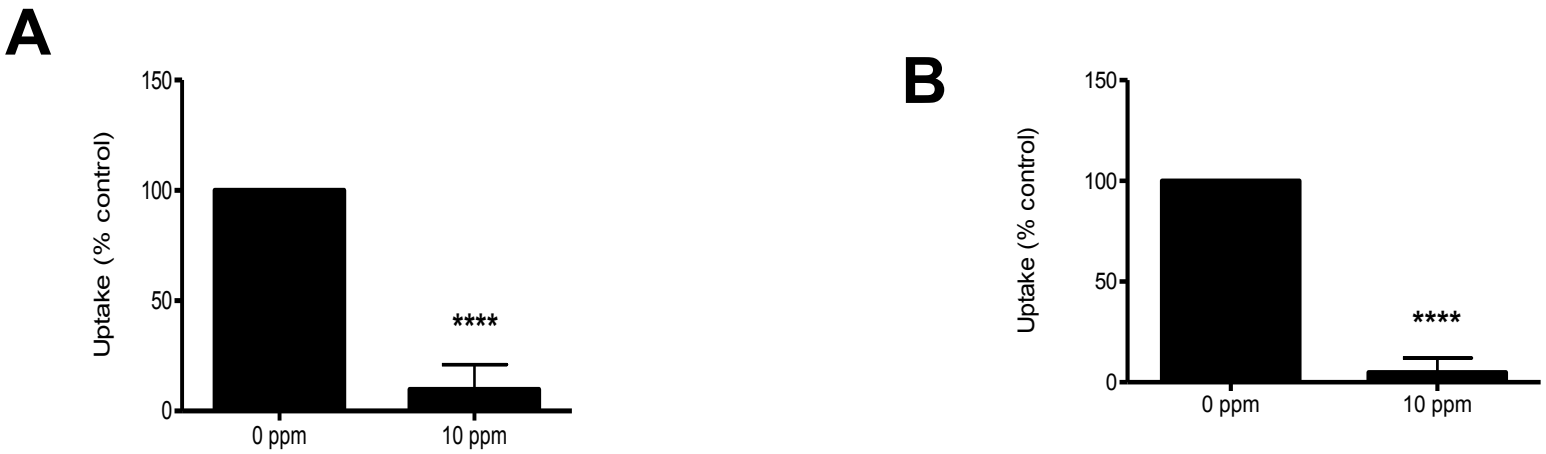


Exposure to 0.5 ppm of 1-octen-3-ol for 24 hr caused a decrease in dopamine pools with a subsequent increase in DOPAC levels in head extracts

(N = 200 for each group and data represents values from three independent experiments). (Inamdar et al 2013 PNAS)

Vesicular monoamine transporter 2 (VMAT2)

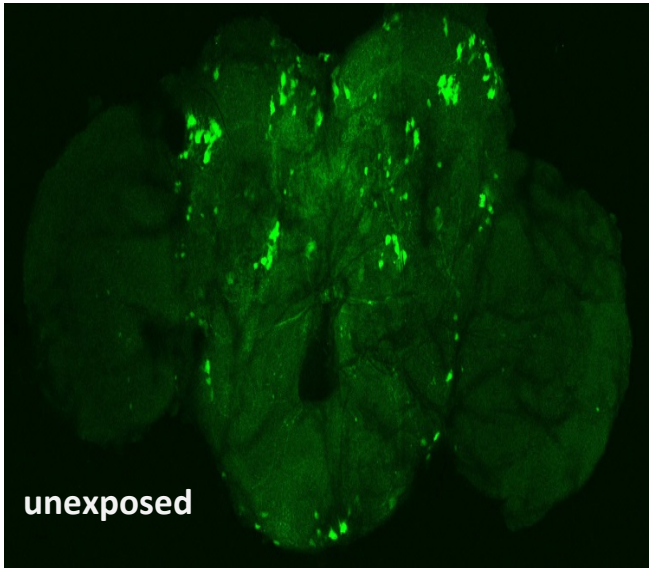
VMAT2 is essential in the presynaptic neuron's ability to facilitate the release of neurotransmitters into the synaptic cleft. If VMAT2 function is inhibited or compromised, neurotransmitters such as dopamine cannot be released via normal transport (exocytosis, action potential) into the synapse. Impaired VMAT2 function/activity may contribute to symptoms of depression, anxiety, Parkinson's disease, social anxiety, and many other conditions, via inhibition of normal dopamine release into the synapse. Long-term use of amphetamine and methamphetamine causes long-lasting reductions in VMAT2 expression/activity, similar to chronic use of cocaine.

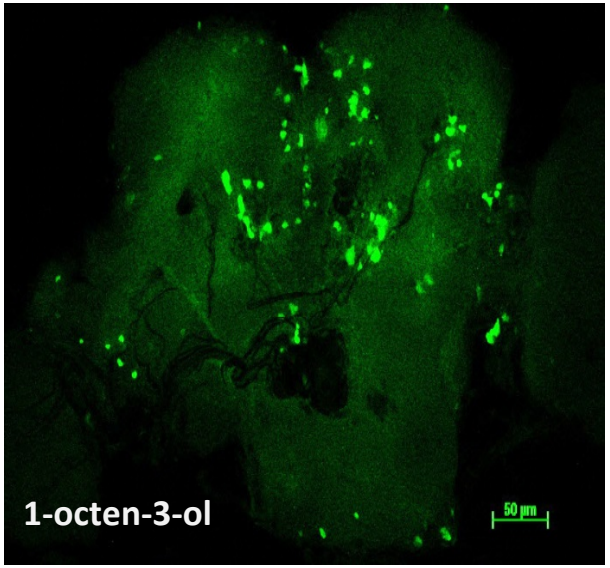


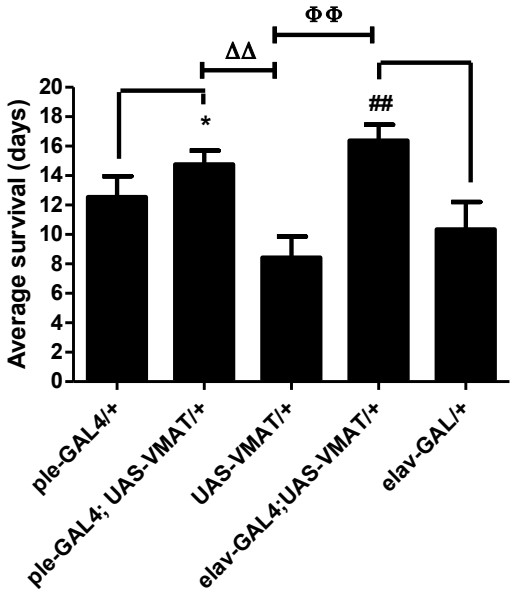
Radioactive dopamine uptake in transgenic human embryonic HEK-DAT (A) and HEK-DAT/VMAT2 cells (B). Cells were exposed to 10 ppm 1-octen-3-ol by the airborne exposure method for 2 h. Radioactive dopamine uptake was significantly reduced by 95% in HEK-DAT/VMAT2 cells and 90% in HEK-DAT cells as assessed with two-way ANOVA where * represents the significant difference between the unexposed and 1-octen-3-ol exposed cells and ****=P<0.0001.

(Inamdar et al 2013)

Effect of 1-octen-3-ol on transgenic and mutant dVMAT flies.







Exposure of 0.5 ppm of 1-octen-3-ol for 24 hr to TH-GAL4; UAS-dVMAT overexpression caused restoration of dopamine and DOPAC pools in head extracts

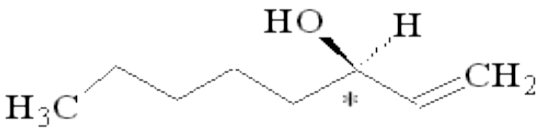
(N=160 for each group and data represents values from two independent experiments).

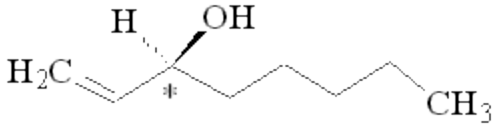
Inamdar et al, 2013 PNAS

Take a closer look at 1-octen-3-ol (= mushroom alcohol)

Why are they selling it so cheaply?

Mushroom alcohol = 1-octen-3-ol





***R*)-(-)-1-octen-3-ol**

Mushroom smell

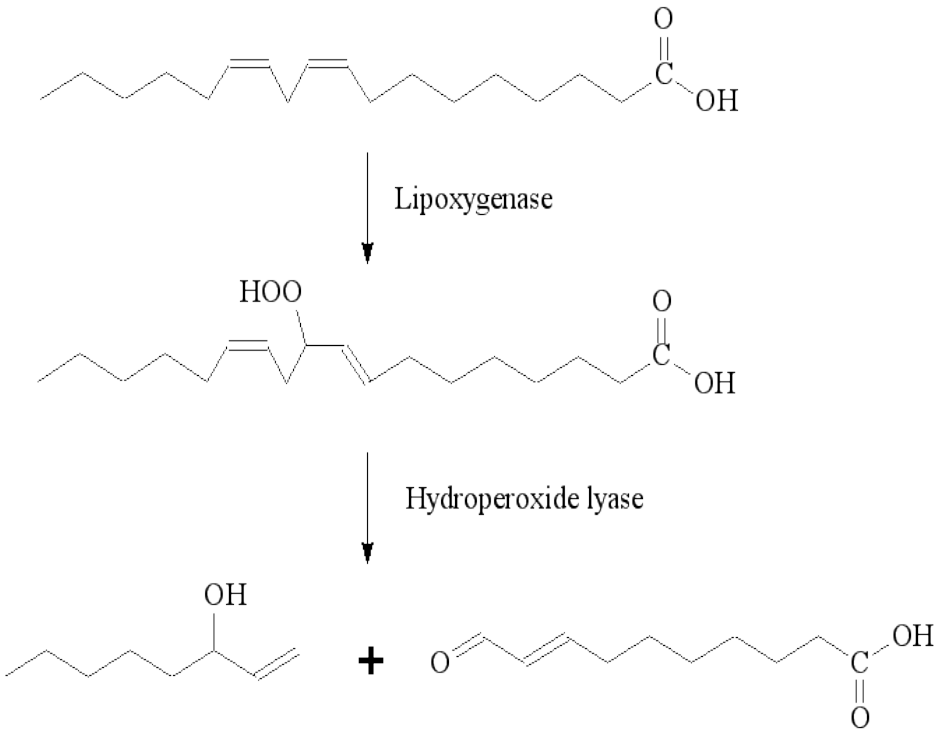
***S*)-(+)-1-octen-3-ol**

Moldy smell

www.chm.bris.ac.uk/motm/octenol/octenoljm.htm

1-octen-3-ol ($C_8H_{16}O$)

is a breakdown product of linoleic acid and the most ubiquitous of fungal VOCs.



Matsui K, et al. 2003. Linoleic acid 10-hydroperoxide as an intermediate during formation of 1-octen-3-ol from linoleic acid in *Lentinus decadetes.* Biosci Biotechnol Biochem. 67 :2280-2**.**

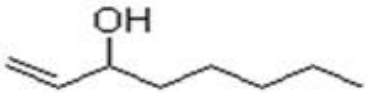
(Fwww.chm.bris.ac.uk%252Fmotm%252Foctenol%252Foctenolh.htm%3B722%3B477)

There is a large literature on the use of 1-octen-3-ol as a flavoring agent in foods and as an ingredient of various perfumes.

http://www.google.com/imgres?q=warhol+campbells+mushroom+soup&um=sch&tbnid=UxNG09

1-octen-3-ol is found in human sweat-
It attracts mosquitoes and other insects

w.chm.bris.ac.uk/motm/octenol/octenoljm.htm



Studies have shown that octenol, when combined with carbon dioxide, is highly attractive to many species of mosquitoes, sand flies and biting midges. American Biophysics offers octenol, which when used in conjunction with carbon dioxide, enhances catch rates for the Mosquito Magnet®. Our research shows that octenol can increase the catch rates by 3 to 4 times. http://omar.fm/2010/11/will-mosquitoes-rule-earth

1-octen-3-ol is what entomologists call a semiochemical

Definition: "A chemical emitted by a plant or animal that evokes a behavioral or physiological response in another organism." Examples: pheromones (same species), allelochemicals (different species).

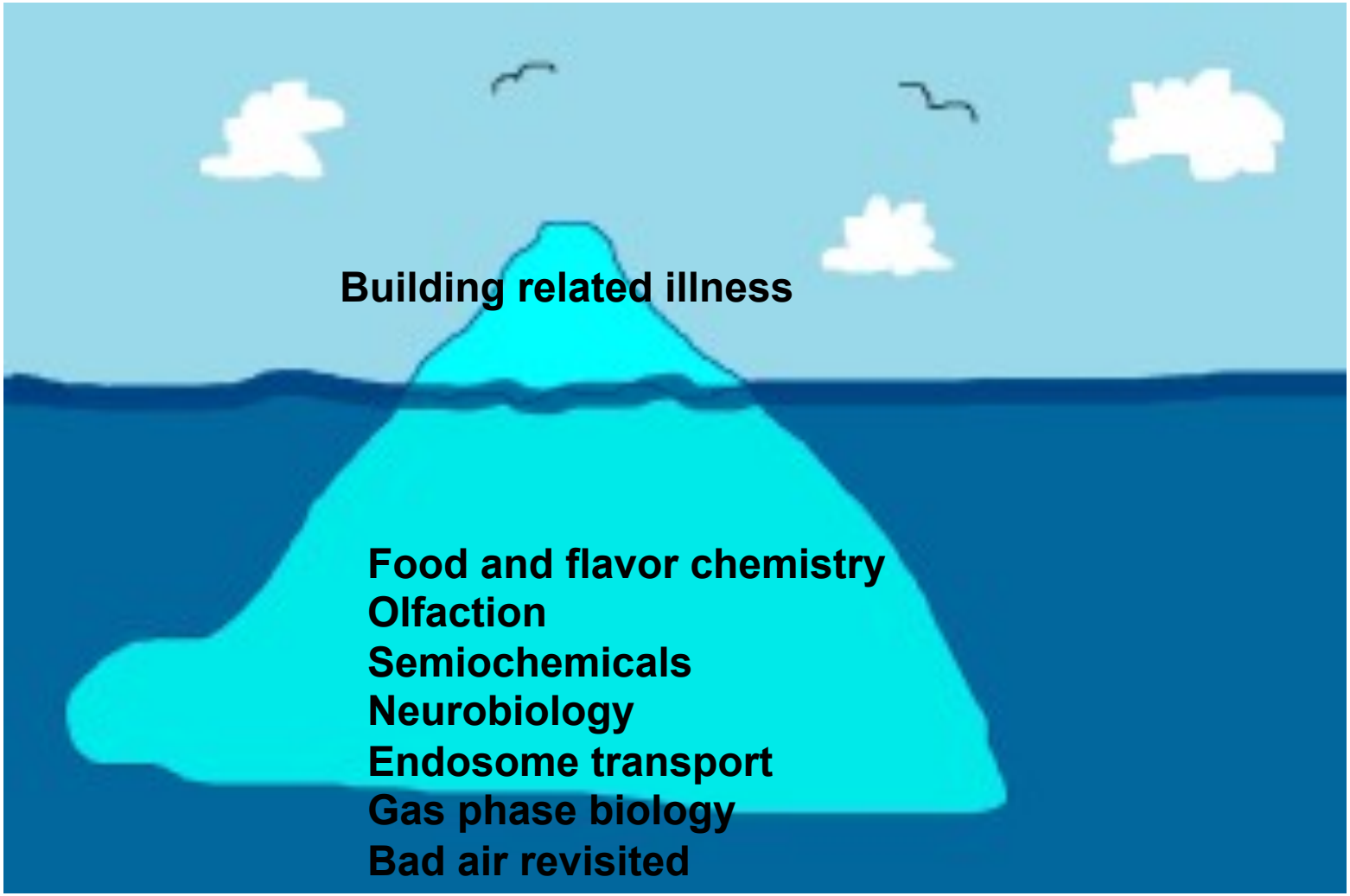
VOCs are used as semiochemicals in cellular signaling And interspecific communications in many ways by many organisms.

http://insects.about.com/od/s/g/semiochemicals.htm

Bridging disciplines through genetics

The group of scientists who study **mycotoxins**, **fungal genomics**, **entomology**, **sick building syndrome, indoor air quality, flavor chemistry** and **volatile organic compounds** do not see themselves as belonging to a single scientific community. They attend different conferences, read and publish in different journals, use different scientific approaches and often communicate using different scientific jargon. This means that these scientific communities rarely have a chance to learn from one another. Our lab is attempting to link the study of fungal compounds circumscribed by their volatility and/or their toxicity to these other fields using genetic models.

“Tip of the iceberg”



http://goodhorsemanship.ca/Goodhorse_wordPress/wp-content/uploads/2013/05/iceberg-300x199.jpg

Bad air and good air revisited

In teaching the history of microbiology, we often mention early theories of disease – before germ theory – only to dismiss them.

In particularly, the idea of “bad air,” often associated with erroneous ideas about the causes of cholera and malaria, is discredited.

noun: mi·as·ma (mīˈazmə,mē-/)

“a highly unpleasant or unhealthy smell or vapor”

synonyms: stench, stink, reek, fume, vapor, odor, gas, fetor, smell (https://www.google.com/?gws_rd=ssl#q=miasma)

,

Bad air revisted

http://micareme.tvpilotlight.com/files/2012/01/cholera_art_340x340.png

Pure fresh air- "to keep the air he breathes as pure as the external air without chilling him.“ Florence Nightingale

The Daily Mirror

THE MORNING JOURNAL WITH THE SECOND LARGEST NET SALE

No. 2,122. MONDAY, AUGUST 15, 1910 One Halfpenny.

MISS FLORENCE NIGHTINGALE, "THE ANGEL OF THE CRIMEA," WHOSE DEATH IS ANNOUNCED, WITH SOME OF THE NURSES WHOSE PROFESSION SHE CREATED.

Good air revisited

http://spartacus-educational.com/REnightengale7.jpg

As we address the microbiology of the built environment, we should pay attention to the metabolites those microbes make, and how those metabolites may be affecting humans, for good and for bad.

Acknowledgements

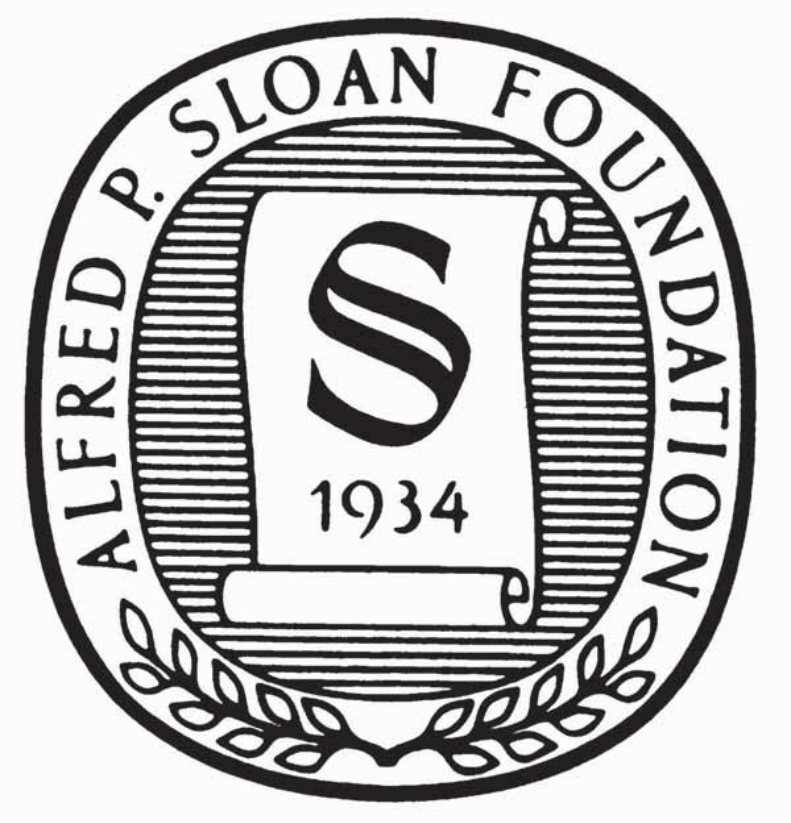
Bennett lab at Rutgers University: S. Padhi, A. Inamdar, S. Morath, P. Masurakar, R. Hung, S. Lee, K Prianana, A. Schinck, D. Pu

Jason Richardson

Collecting Sandy Molds --January 3, 2013

Our toxicological studies are continuing with molds isolated in the aftermath of Super Storm Sandy in collaboration with Environmental Health and Occupational Heath Sciences Institute (EOHSI) at Rutgers.

Sloan Foundation and Univ. California-Berkeley



Paula J. *Olsiewski*
Rachel Adams

The End!

http://www.commonsenseevaluation.com/wp-content/uploads/2012/12/Out-Of-Odor.jpg