Keene STATE COLLEGE

Wisdom to make a difference.

Preventing and Managing the Most Likely Lab Accidents

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Chemical Hygiene Officer
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My Lab Safety History

- I worked as a lab tech in environmental chemistry labs for 5 years, first at **Cornell University** and then at the **University of Vermont**.
- I starting working in Environmental Health and Safety at **UVM** in 1985 as new OSHA regulations led to increased concern about compliance in labs.
- In 2011, I moved back to **Cornell** to be the Laboratory Ventilation Specialist and then Chemical Hygiene Officer there.
- In 2014, I moved to **Keene State** to be the Chemical Hygiene Officer and then became the Environmental Safety Manager as well.
- I have been involved in the **American Chemical Society**'s Division of Chemical Health and Safety programs for 25 years and served as chair of the national Committee on Chemical Safety.

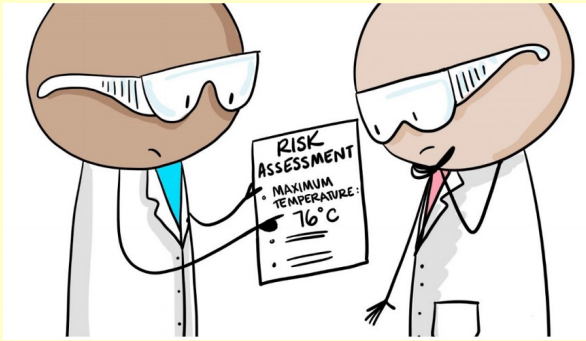
The University of Vermont

ACS Technical Division
Chemical Health & Safety (CHAS)

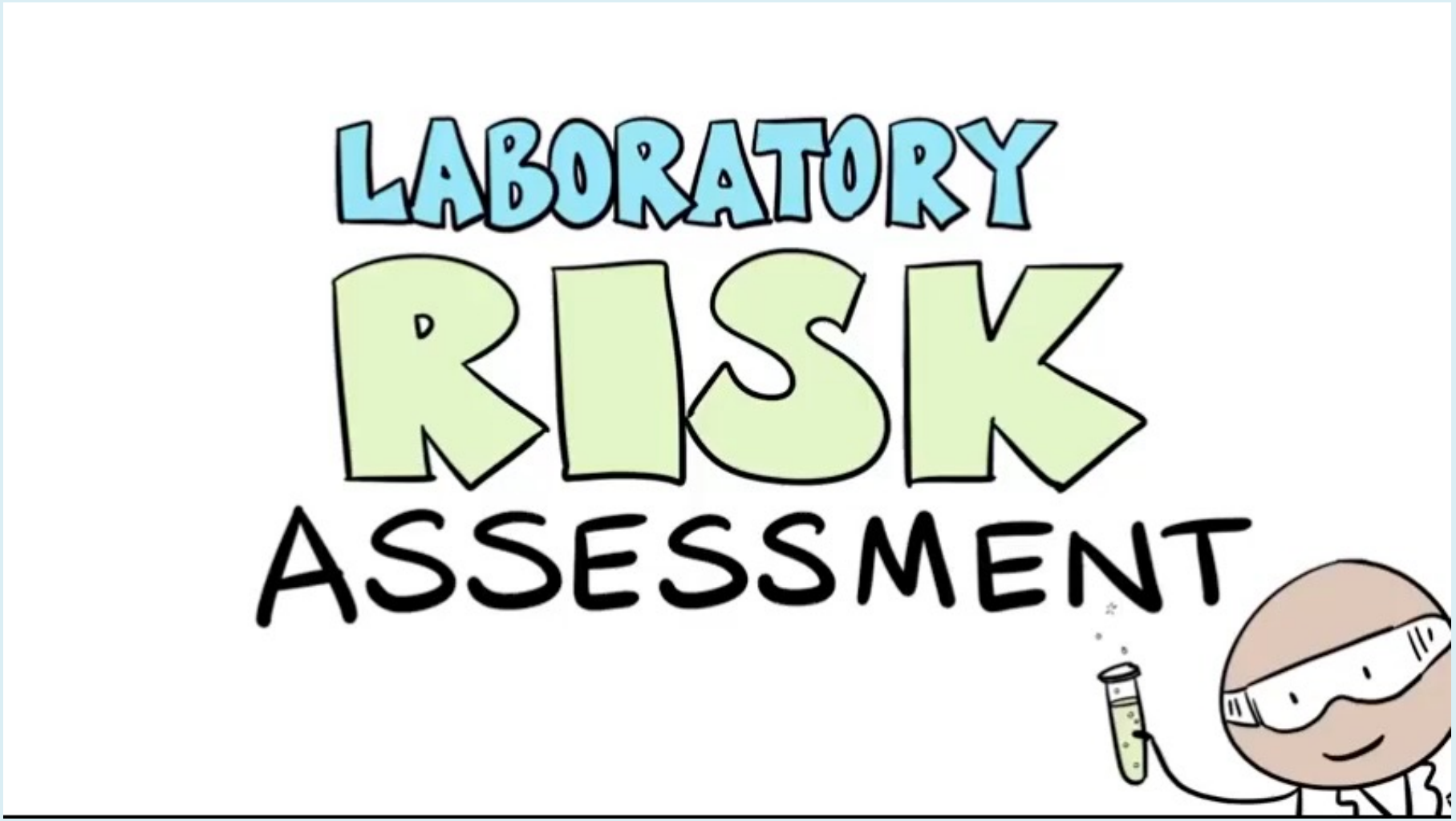
Audience Poll #1

Which of these best describes how often your review your lab's risks and safety practices:

1. We have regular (weekly or monthly) safety discussions as refreshers for all lab staff
2. We review our SOPs for safety concerns annually
3. We review safety as new people are hired or procedures change
4. We rely on consistent use of general best lab safety practices



The RAMP approach to Risk Assessment

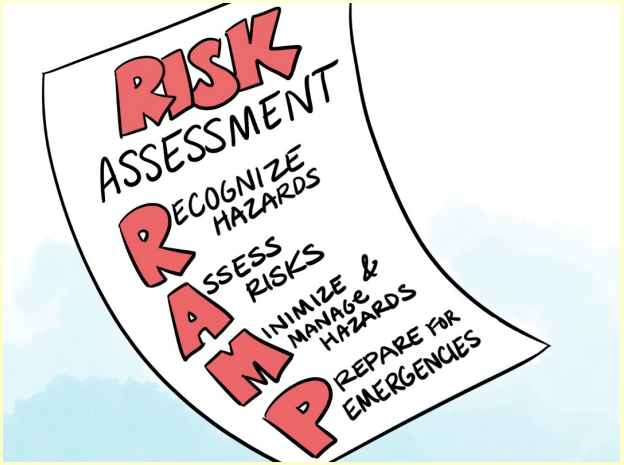


Video clip available to download at
https://dchas.org/wp-content/uploads/2022/06/RAMP-intro-video.mp4

Audience Poll #2

What part of your lab safety program do you find most challenging:

1. Recognizing Hazards
2. Assessing Risks
3. Managing Safety to Minimize Risks
4. Planning for Emergencies



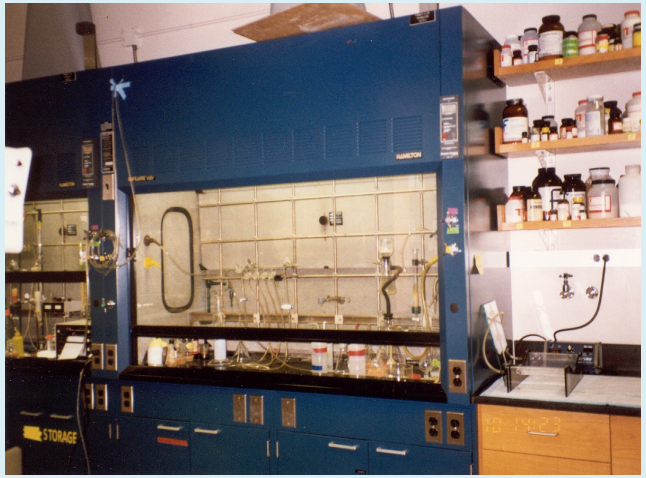
The Lab Safety Assessment Challenge

- Dr. Karen Wetterhahn was a Dartmouth University chemistry professor. Her lab explored the impact of heavy metals on the environment, focusing on mercury and cadmium.
- In August, 1996 she was exposed to *dimethylmercury*, when the chemical penetrated her latex gloves
- She died in June, 1997 of mercury poisoning
- OSHA investigated and determined that the glove selection criteria in Dartmouth's Chemical Hygiene Plan were inadequate
- Dartmouth was fined $9,000

Dr. Karen Wetterhahn
Picture from June 2022 CE&N cover:
25 years after Karen Wetterhahn died of dimethylmercury poisoning, her influence persists

The Investigation

The standard NMR reference for mercury was dimethylmercury (DMM). However, due to its toxicity, Dr. Wetterhahn's lab substituted mercury chloride to prepare their standards.



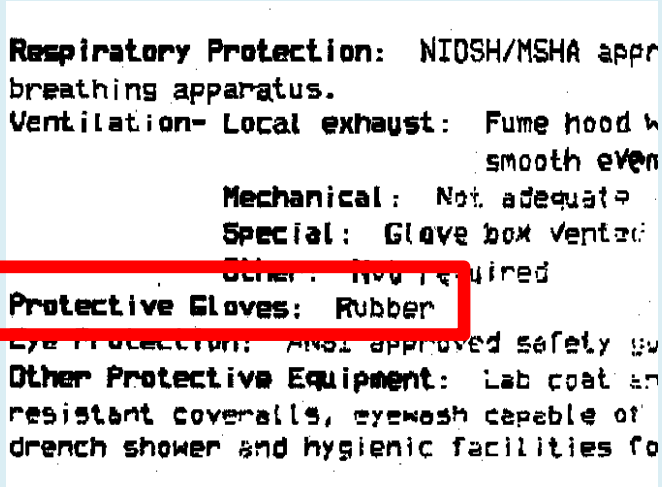
The lab returned to using DMM after the Hg levels found in their NMR samples were not what they thought they should be.

While Dr. Wetterhahn was transferring the liquid DMM, several drops spilled on the back of her gloved hand.

After several months, she noticed neurological symptoms of Hg exposure and 22 days after initial symptoms she became comatose. She died 298 days following exposure.

Follow Up Work by Dartmouth on Glove Suitability

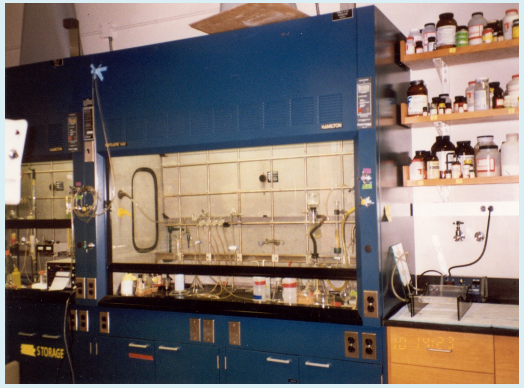
- The best practices at the time was to use "rubber" gloves when handling DMM.
- Dartmouth hired a lab to test gloves for DMM permeation rates.
- The results were:
  - PVC/latex <15 sec
  - Nitrile 15 sec
  - Neoprene <10 min
  - Butyl < 15 min
  - Viton < 15 min
  - Silver Shield > 240 min



DMM MSDS

An Informal RAMP review of this Incident

Recognize:
The lab understood the toxicity of DMM. DMM has a flashpoint of 5 degrees C, so it is also a flammability hazard.

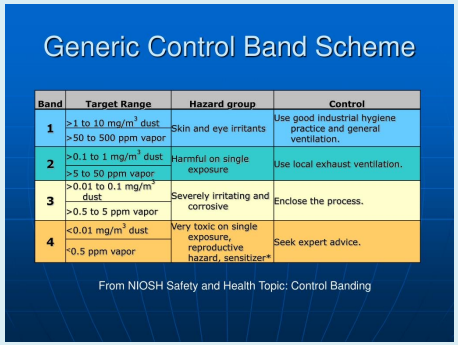


Assess:
Because of high risk of toxicity with DMM, Dr. Wetterhahn did the benchwork herself. According to the C&EN story *"this was a task she didn't want anyone else doing"*

Manage:
A fume hood controls the fire hazard, but does not provide protection against skin exposure to DMM. The *control banding* scheme for glove selection did not include information about DMM penetration time of latex gloves

Plan for Emergencies:
Dr. Wetterhahn did not recognize the emergency when it occurred. If she had sought medical attention immediately, chelation therapy could have potentially helped control the progression of her symptoms

A Quick Side Note on Control Bands

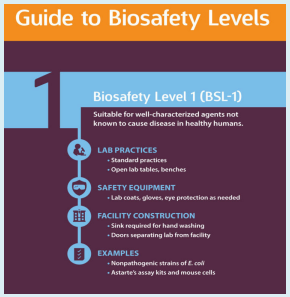


Band	Target Range	Hazard group	Control
1	>1 to 10 mg/m³ dust >50 to 500 ppm vapor	Skin and eye irritants	Use good industrial hygiene practice and general ventilation.
2	>0.1 to 1 mg/m³ dust >5 to 50 ppm vapor	Harmful on single exposure	Use local exhaust ventilation.
3	>0.01 to 0.1 mg/m³ dust >0.5 to 5 ppm vapor	Severely irritating and corrosive	Enclose the process.
4	<0.01 mg/m³ dust <0.5 ppm vapor	Very toxic on single exposure, reproductive hazard, sensitizer*	Seek expert advice.

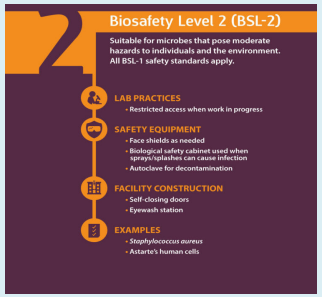
Control bands are used to guide the management of workplace risks by suggesting control measures based on a "band" of hazard levels (such as toxicity) and expected exposures (e.g. small, medium, or large exposure). The NIOSH web site discusses the opportunities and challenges CB presents in detail.

Biosafety Levels are examples of Control Bands

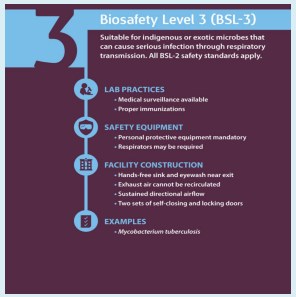
Biosafety controls include work practices, personal protective equipment, facility construction and operations, and emergency plans



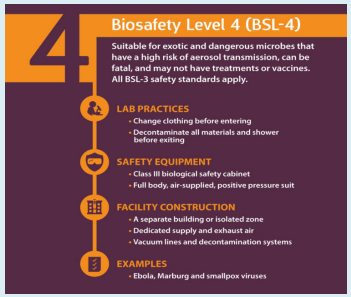
Benign microbes



Surface contact



Airborne transmission



Bioweapons

Control banding requires on going attention to be successful.

For example, the biosafety system worked reasonably well in biolabs from the 1980's until 2020. However, the Covid experience has shown how delicate control bands can be when an exotic hazard arises or when you leave the clinical or lab settings.

Would We Handle DMM More Safely Today? A RAMP review

Recognize:

GHS information available for DMM varies depending on the source used

Assess:

How would we rate the risk of working with DMM today? Has the way it is used now reduced its risk?

Manage:

PPE selection: today, nitrile gloves are the most common laboratory hand protection; however, they provide similar protection against DMM as latex gloves

Plan for Emergencies:

Do lab workers understand the signs and symptoms of exposures to the chemicals they work with?

GHS Information on DMM from PubChem

European Chemicals Agency (ECHA)

NITE-CMC (Japan)

A risk assessment considers:
1) the likelihood of a risk scenario
2) magnitude of the risk
3) the people who suffer the harm
4) the benefit of the work to be performed

Audience Poll #3

What is your primary approach to communicating your lab safety practices to people in your lab?

1. We put alerts in an Electronic Lab Notebook system
2. We rely on paper Standard Operating Procedures and Lab Guidance
3. We place notices and signs pointing out specific hazards in the lab
4. We focus on word of mouth and chemical intuition

RAMP Information Tools in 2022

Recognize Hazards:

- The Globally Harmonized System: Chemical labels and Safety Data Sheets
- Identify process and human hazards

Assess Risks:

Data that can help rank hazards

- Chemical literature
- Informal communications
- Crowd sourced information

Minimize Risks / Manage Safety:

- Control bands for ventilation, training and PPE
- Maintain worker's situational awareness

Plan for Emergencies / Protect the Environment:

- Connect with institutional emergency planning services
- Understand lab waste disposal services

Lessons Learned are how we continue to improve our safety practices

The Good News: 21st Century RAMP Tools

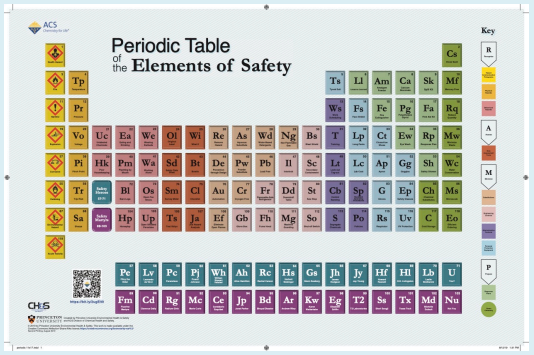
1. Educational resources:

Free ACS Safety materials based on RAMP

- On line courses
- Youtube videos
- Best Practice workshops

2. Brainstorming: A Periodic table of safety elements for brainstorming during risk assessments

3. Documenting: Draft "*What if*" discussion template from the Committee on Chemical Safety



Organizing and Sharing Your Lab Safety Information

Video is available to view on [ACS Chemical Safety Youtube Channel]

Managing Your Safety Information FAIRly

- Good lab risk assessments are:
  - Collaborative
  - Inclusive
  - Documented
  - Shared FAIRly

FAIR Data is:

- Findable
- Accessible
- Interoperable
- Reusable

Audience Poll #4

Who is involved in developing and reviewing your laboratory risk assessments?

1. The person who writes the SOP for procedure
2. Everyone who handles the chemicals involved in a SOP
3. Everyone in the lab, because they could be impacted by a safety incident even though they aren't conducting the procedure involved
4. Our emergency responders who are expected to provide assistance in can of an incident

4 Apparatus

4.1 Pipettes and other equipment

4.1.1 PCR plate cooling block or ice
4.1.2 Complete 'clean set' (1000 µl, 200 µl, 100 µl, 20 µl and 10 µl) of single-channel pipettes for PCR master mix set-up
4.1.3 10 µl single-channel pipette for transferring DNA to PCR tubes
4.1.4 Optional, 8-channel pipettes (200 µl and 10 µl) for dilution of PCR products and preparation of samples for fragment analysis, respectively
4.1.5 Sterile pipette tips

4.2 Apparatus

4.2.1 Incubator, capable of operating at 37 °C ± 1 °C
4.2.2 Thermocycler with heated lid option
4.2.3 Heat block or thermocycler, capable of operating at 95 °C ± 1 °C and 100 °C ± 1 °C
4.2.4 Centrifuge, accommodating 0.2 ml PCR tubes, 1.5 ml microcentrifuge tubes, and 96-well v-bottom PCR plates
4.2.5 Whirl mixer
4.2.6 CE genetic analyser set-up for fragment analysis in the range 140 to 615 bp. Applied Biosystems Filter set D/G5 dyes

For ABI CE genetic analysers, the standard fragment analysis protocols should work well.

5 Procedure

Note: Preparation of primer mix solution can be performed in advance.

5.1 Inoculation and incubation

5.1.1 Streak an isolated colony from test cultures to a 5% blood agar plate or comparable media. Incubate cultures overnight (14-20 hrs.) at 37 °C.

5.2 DNA isolation (optional)

5.2.1 Check that the plate contains pure cultures of *Salmonella* Enteritidis.
5.2.2 For each isolate to be typed, aliquot 100 µl of sterile water into a 1.5 ml microcentrifuge tube.
5.2.3 Use a sterile, disposable 1 µl loop to pick 1 colony (about ¼ of a loopful); rotate the loop in the tube to release the bacteria into the water.
5.2.4 Cap and vortex for 10–15 seconds to disperse any clumps.
5.2.5 Place the tubes in a 100 °C water bath or heat block for 10 minutes.
5.2.6 Cool briefly on ice or in a thermal cycler at 4 °C for 1 minute and centrifuge for 10 minutes at 10 000 rpm.
5.2.7 Transfer the supernatant containing the DNA into a new 1.5 ml microcentrifuge tube and discard the tube containing the pellet.
5.2.8 The DNA lysate can be stored at -4 °C if PCR is performed no later than the next day.
5.2.9 For longer term, the DNA lysates can be stored at -20 °C or -80 °C.

5.3 Preparation of primer mix solutions

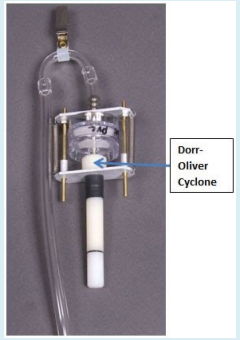
Note: This should be prepared in a clean laminar flow hood where no DNA is handled. Keep primers protected from excessive exposure to light.

Note: Avoid repetitive freeze-thaw cycles of concentrated primer stocks. It is advised to prepare a batch of new primer mix solutions every second month and also if a significant drop in fluorescence level is observed.

5.3.1 Use the 'primer mix scheme' in Annex 2. RNase-free water is provided with the Qiagen Multiplex PCR kit.
5.3.2 Thaw all reagents and place on ice.

Back to the Question: Do Lab Workers Recognize Problems when They Occur?

- I worked as a lab tech in an industrial hygiene lab in the early 1980's.
- I collected airborne dust in the Barre, Vermont granite industry to assess worker exposure to granite dust
- We used dimethyl formamide to dissolve the filters that the dust collected on to prepare them for X-ray diffraction analysis.



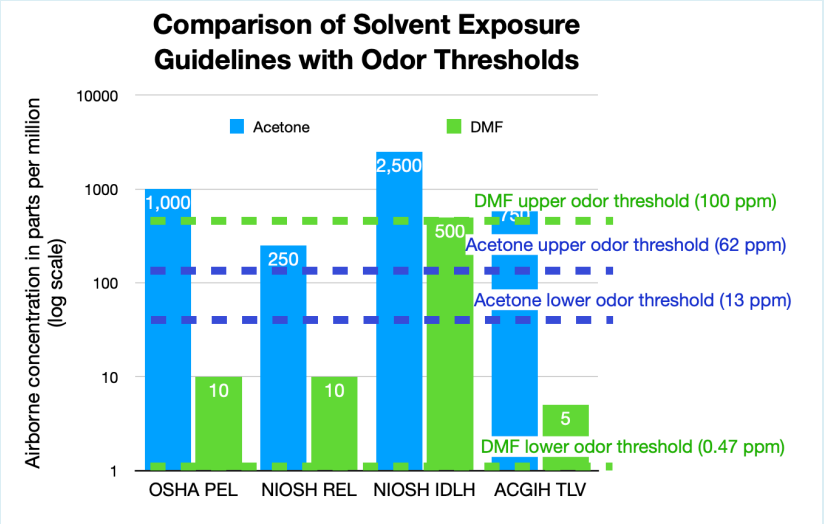
My Experience

- One day, when I went to dissolve about 50 filters, the hood was crowded with other materials.
- I decided to set the ultra-sonicator up at the front of the hood, which meant that I couldn't close the sash
- After about 20 minutes, I got dizzy, so I went and got a respirator; I completed the work with no further symptoms
- However, when I went out to play softball that evening and started exercising, I suddenly got very dizzy, felt faint and had to lie down. I didn't seek medical attention.

My Investigation into How I was Exposed

- Based on my literature review, my exposure could have come from inhalation or skin exposure (I was wearing latex gloves), or both
- Working at the edge of the hood meant that hood containment was compromised, but I did not notice any odor.
- However, DMF has poor odor warning properties, so it's not clear what level of exposure was respiratory; my guess is that the exposure was primarily inhalation, although it could have been both skin and respiratory

Property	DMF	Acetone
Lower odor detection	0.47 ppm	13 ppm
Upper odor detection	100 ppm	62 ppm
OSHA PEL (8 hours)	10 ppm	1000 ppm
NIOSH STEL (15 minutes)	250 ppm (skin)	1250 ppm



The Outcome

- Fortunately, I was a heathy male in my 20's and noticed no symptoms after that day.
- Pubchem now notes that "*There is limited evidence that dimethylformamide is carcinogenic for human beings."* I have no reason to believe that my short term exposure had this health impact.
- In 2022, GHS data on DMF is more easily available, but still requires interpretation.

DMF PubChem Entry

Pictogram(s)	 Flammable Irritant Health Hazard
Signal	Danger
GHS Hazard Statements	H226 (28.35%): Flammable liquid and vapor [Warning Flammable liquids] H312 (99.78%): Harmful in contact with skin [Warning Acute toxicity, dermal] H319 (99.85%): Causes serious eye irritation [Warning Serious eye damage/eye irritation] H332 (98.53%): Harmful if inhaled [Warning Acute toxicity, inhalation] H360 (100%): May damage fertility or the unborn child [Danger Reproductive toxicity]

https://pubchem.ncbi.nlm.nih.gov/compound/6228#datasheet=LCSS

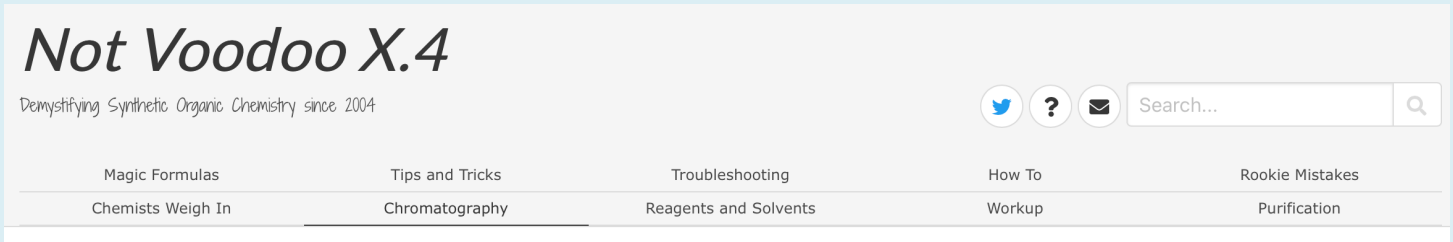
Flashpoint = 136 degrees F

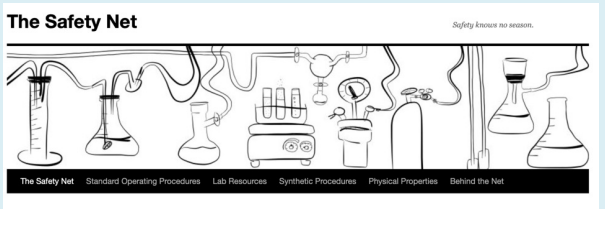
My Lessons Learned

1. Fume hood containment is delicate – make room in the hood before relying on it for protection.
2. Similarly, review glove protection information for the chemicals you work with
3. Investigate signs and symptoms of exposure of the chemicals before beginning work.
4. Shared lab equipment leads to housekeeping and safety concerns – coordinate with others to help everyone get their work done

Sharing Your Lessons Learned

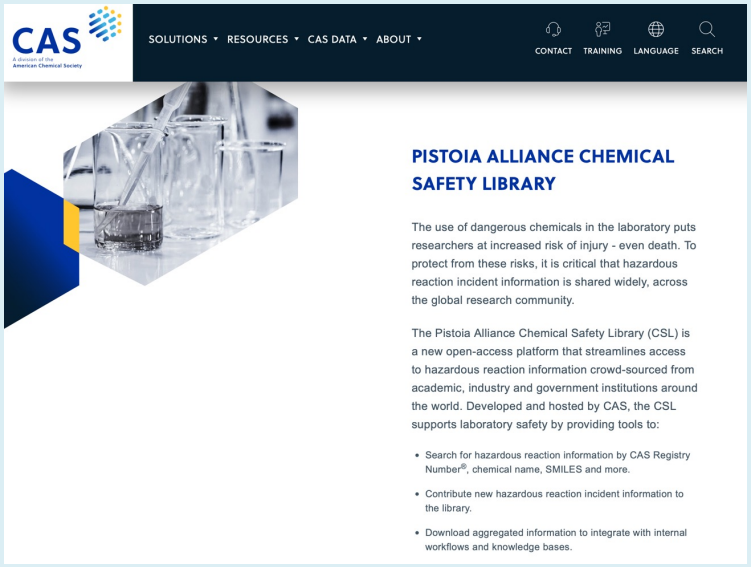
- For incidents involving two or more chemicals: *CAS Chemical Safety Library (CSL)* https://safescience.cas.org/
- Standard Operating Procedures in *The Safety Net* https://safetynet.web.unc.edu/ from Univ of North Carolina
- Not Voodoo X identifies human risks in the *"Rookie Mistakes"* section http://www.chem.rochester.edu/notvoodoo/index.php from Univ of Rochester





Your Opportunity

- The *ACS Division of Chemical Health and Safety*, in partnership with *CAS*, *the Division of Chemical Information* and *CSHEMA* to explore how to improve sharing of incident information and Lessons Learned among laboratories.
- If you are interested in joining us in a focus group to discuss the lab manager's perspective on this opportunity, please contact me at Ralph Stuart at membership@dchas.org

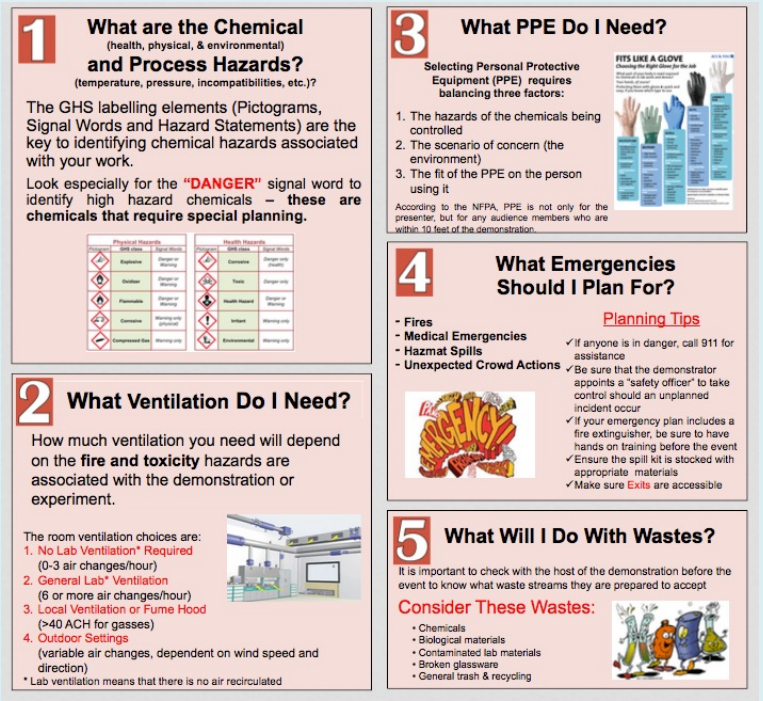


Closing Thought:
The Advantages of RAMP

The RAMP approach has several specific advantages over traditional rule based safety:

- It separates identification, prioritization and management considerations (too often, the control banding *tail* wags the assessment *dog*)
- RAMP includes emergency planning (*because there are humans in the lab, so there will be errors*)
- The RAMP approach organizes the lab safety discussion among diverse stakeholders

RAMP helps everyone answer these 5 questions about their labwork



Thank You!

- My question for you: Can you put one thing you learned today in the chat?
- What questions do you have?