LABORATORY RISK ASSESSMENT TOOL (Lab R.A.T.)

The Laboratory Risk Assessment Tool (Lab RAT) provides a framework for risk assessment complimenting the process researchers already use to answer scientific questions.

This tool provides a format for researchers to systematically identify and control hazards to reduce risk of injuries and incidents. Conduct a risk assessment prior to conducting an experiment for the first time and review the Lab R.A.T. Guidelines document for further details.

The risk assessment process involves rating the risk of the experiment from "low" to "unacceptable" risk. Consult with your PI/supervisor and EH&S if your risk rating is "high" or "unacceptable" to redesign the experiment and/or implement additional controls to reduce risk.

Procedure: Phospholipid synthesis using cyanamide and imidazole	
PI / Lab Group: Keller	
Department: Chemistry	**Building / Location:** Bagley 023 and Bagley 005
Form Completed By: Zachary Cohen	**Start Date:** 08-2022

PHASE 1: EXPLORE

Identify your research question and approach. What question are you trying to answer? What are you trying to measure or learn? What is your hypothesis? What approach or method will you use to answer your question? Are there alternative approaches?

Research Question(s)
Starting with fatty acids and glycerol phosphate, is the rate of phospholipid synthesis faster if the fatty acids are assembled into vesicles?
Approach(s) or Method
Phospholipid synthesis: 1) Dehydration at high temperature = by removing water, condensation reactions (i.e. formation of ester linkages between free fatty acids and glycerol) become more favorable. There are no alternative techniques to favor condensation reactions that are also prebiotically plausible (could have occurred on the early Earth). 2) Cyanamide and imidazole as condensing agents = with dehydration alone, I have observed that phospholipids are not synthesized from fatty acids and glycerol phosphate. Epps et al. 1978 do a similar procedure to what I've tried, but they include 0.1M cyanamide and 0.02M imidazole, and they do observe phospholipid synthesis during dehydration. I will try to reproduce their work. Phospholipid detection via quantitative mass spec: 1) I have experience doing this analysis with Rich Johnson in Genome Sciences. There are no hazards associated with this procedure.

Identify the general hazards (check all that apply). Perform background research to identify known risks of the reagents, reactions, or processes. Review protocols, Safety Data Sheets (SDSs), and safety information for hazardous chemicals, agents, or processes. Review accident histories within your laboratory/department.

Hazardous Agents

Physical Hazards of Chemicals

- ☐ Compressed gases
- ☐ Cryogens
- ☐ Explosives
- ☐ Flammables
- ☐ Organic peroxides
- ☐ Oxidizers
- ☐ Peroxide formers
- ☐ Pyrophorics
- ☐ Self-heating substances
- ☐ Self-reactive substances
- ☐ Substances which, in contact with water, emit flammable or toxic gases

Health Hazards of Chemicals

- ☐ Acute toxicity
- ☐ Carcinogens
- ☐ Eye damage/ irritation
- ☐ Germ cell mutagens
- ☐ Nanomaterials
- ☐ Reproductive toxins
- ☐ Respiratory or skin sensitization
- ☐ Simple asphyxiant
- ☐ Skin corrosion/ irritation
- ☐ Specific target organ toxicity
- ☐ Hazards not otherwise classified

Ionizing Radiation

- ☐ Irradiator
- ☐ Radionuclide
- ☐ Radionuclide sealed source
- ☐ X-ray machine

Non-Ionizing Radiation

- ☐ Lasers, Class 3 or 4
- ☐ Lasers, Class 2
- ☐ Magnetic fields (e.g., NMR, MRI)
- ☐ RF/microwaves
- ☐ UV lamps

Biohazards

- ☐ BSL-2 Biological agents
- ☐ BSL-3 Biological agents
- ☐ Human cells/blood/ BBP
- ☐ NHPs/cells/blood
- ☐ Non-exempt rDNA
- ☐ Animal work
- ☐ High risk animals (RC1)
- ☐ Other (list):

Hazardous Conditions or Processes

Reaction Hazards

- ☐ Explosive
- ☐ Exothermic, with potential for fire, excessive heat, or runaway reaction
- ☐ Endothermic, with potential for freezing solvents decreased solubility or heterogeneous mixtures
- ☐ Gases produced
- ☐ Hazardous reaction intermediates/products
- ☐ Hazardous side reactions

Hazardous Processes

- ☐ Generation of air contaminants (gases, aerosols, or particulates)
- ☐ Heating chemicals
- ☐ Large mass or volume
- ☐ Pressure > atmospheric
- ☐ Pressure < atmospheric
- ☐ Scale-up of reaction

Other Hazards

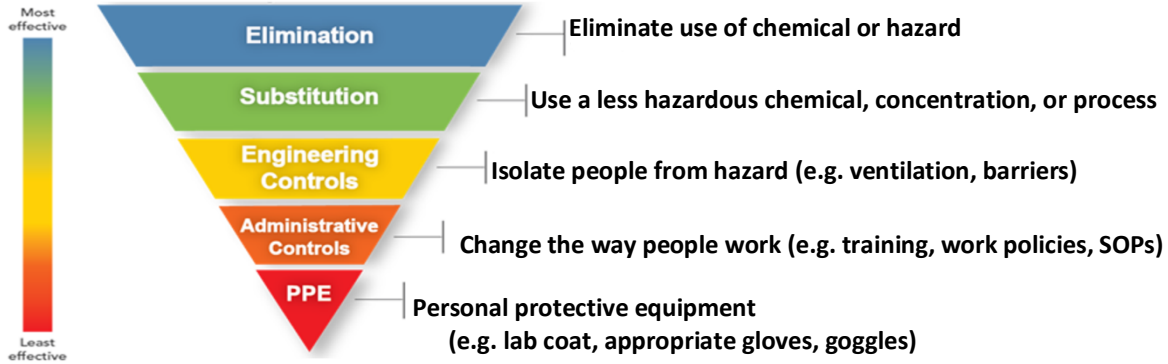
- ☐ Hand/power tools
- ☐ Moving equipment/parts
- ☐ Electrical
- ☐ Noise > 80 dBA
- ☐ Heat/hot surfaces
- ☐ Ergonomic hazards
- ☐ Needles/sharps
- ☐ Other (list):

PHASE 2: PLAN

Outline the Procedure. List the steps or tasks for your procedure and the hazard/potential consequences of each. Include set-up and clean-up steps or tasks. Define the hazard controls to minimize the risk of each step using the hierarchy of controls starting with the most effective (i.e., elimination, substitution, engineering controls, administrative controls, and personal protective equipment). List the hazard control measure you would use for each step or task (e.g., run at a micro scale, work in a fume hood, wear face shield and goggles).

Steps or Tasks	Hazard	Hazard Control Measure(s)
Prepare stock solutions of cyanamide and imidazole	Skin contact with solid powder	1) Deposit solid into glass vial in a fume hood. Seal the vial, then measure the mass of the solid powder within the sealed vial. 2) Wear gloves and lab coat.
Solution preparation	Skin contact with aqueous cyanamide or imidazole	1) Mix stock solutions in the fume hood. 2) Move the pH probe into Bagley 023 fume hood, and adjust pH in the fume hood.
Phospholipid synthesis reaction	Skin contact with aqueous cyanamide or imidazole	1) Move a heat block into Bagley 023 fume hood, and allow the reaction to occur in the hood overnight. 2) I have consulted with organic chemists who are experienced using these reagents. They agree that there are minimal hazards using this plan.

HIERARCHY OF CONTROLS



1 For guidance on selection of Personal Protective Equipment (PPE), use EH&S PPE Hazard Assessment Tool.
2 For guidance on selection of chemical-resistant gloves, see EH&S Website.

A hierarchy of controls should be applied starting with the most effective controls (i.e., elimination and substitution) at the top of the graphic and moving down. While personal protective equipment (PPE) should always be used, it should be considered the last line of defense from potential hazards.

Select the appropriate PPE and safety supplies for the procedure (check all that apply).

Laboratory PPE/Safety Supplies

☒ Appropriate street clothing (long pants, closed shoes)
☐ Gloves; indicate type: ___nitrile___
☐ Safety glasses
☐ Safety goggles
☐ Face shield and googles
☐ Lab coat
☐ Flame-resistant lab coat
☐ Fire extinguisher
☐ Eyewash/safety shower
☐ First aid kit
☐ Spill kit
☐ Specialized medical supplies (e.g. calcium gluconate for hydrofluoric acid and amyl nitrite for cyanides
☐ Other (list):

Identify the appropriate training (check all that apply). Identify the general safety and procedure based/specific training appropriate for your procedure.

General Safety Training

General/Chemical Safety
☐ Lab Safety Compliance & Practices
☒ Managing Lab Chemicals
☐ Compressed Gas Safety
☐ Fume Hood Training
☐ Hydrofluoric Acid Safety
☐ Formaldehyde Safety

Biosafety
☐ Biosafety Training
☐ Bloodborne Pathogens

Radiation Safety
☐ Radiation Safety
☐ Laser Safety

Field Safety
☐ First Aid & CPR
☐ SCUBA certification/diving safety
☐ Driving safety
☐ Other (list):

Job Specific Training

☒ Lab/job-specific training
☐ Lab SOP(s) to review (list):
☐ Emergency plans or field evacuation plans
☐ Equipment SOP(s) to review (list):
☐ Other (list):

PHASE 3: CHALLENGE

Question your methods. What have you missed and who can advise you? Challenge your hazard control measures by asking "What if…?" questions. "What if" questions should challenge you to find the gaps in your knowledge or logic. Include possible accident scenarios. Factors to consider are human error, equipment failures, and deviations from the planned/expected parameters (e.g., temperature, pressure, time, flow rate, and scale/concentration). Update your plan to include any new controls required to address these possibilities.

What If Analysis
What if…? I drop an aqueous solution of cyanamide.
Then… I will close the fume hood and allow the solution to evaporate before wiping up the solid cyanamide.
What if…? An aqueous solution of cyanamide or imidazole contacts my skin.
Then… The most likely site of skin contact would be my wrists (the region in between my gloves and lab coat). In this case, I will immediately wash the exposed area in the sink. In the unlikely case of contact to another area of my body, I will use the safety shower in Bagley 005 or in the hallway outside Bagley 023.
What if…?
Then…
What if…?
Then…
What if…?
Then…
What if…?
Then…
What if…?
Then…
What if…?
Then…

Assign a risk rating to the experiment. Based on your procedure outline and the what if analysis, determine the risk rating for the experiment or procedure.

Risk Rating: ____Low__

1The Risk Rating is subjective. The primary goal is for researchers to think about risk, and differentiate unacceptable and high-level risk steps from those with a lower level risk. This will help drive additional consultation and control measures where needed.

Likelihood of Incident Occurrence	Severity of Consequences – Personnel Safety			
	No injuries	Minor Injury	Significant Injury	Life threatening
Very Likely	Low	High *	Unacceptable **	Unacceptable **
Likely	Low	Medium	High *	Unacceptable **
Possible	Low	Medium	High *	High *
Rare	Low	Low	Medium	High *

Revise plan if the risk rating is too high. Are these risks acceptable? Use this table to determine the action to take based on the risk rating. What are the highest risk steps? What more can you do to control the risks? Return to planning and use the hierarchy of controls to design a safer experiment.

Hazard Risk Level	Action
Unacceptable **	**STOP!** Additional controls needed to reduce risk. **Consult with PI.**
High *	Additional controls recommended to reduce risk. **Consult with PI.**
Medium	Ensure you are following best practices. Consult with peers, PI, and EH&S as needed.
Low	Perform work within controls

PI/Supervisor Approval:

*Signature for **High** risk ratings. If needed, contact EH&S (206.221.2339) for recommendations.

NOTE: **Unacceptable risk-rated experiments **should not proceed**. Introduce further controls to reduce risk. Contact EH&S (206.221.2339) for recommendations and best practices.

PHASE 4: ASSESS

Perform a trial run. How you can test your experimental design? Can you do a dry run of the procedure without hazardous chemicals/reagents/gases to familiarize yourself with equipment and demonstrate your ability to manipulate the experimental apparatus? Can you run the procedure with a less hazardous material? Can you test your experimental design at a smaller scale? If your procedure requires multiple people, would a table top exercise be useful?

Trial Run
Trial Run Procedure / Date: 04-14-22
Did the trial go as expected? Yes ☐ No ☐
Experimental design changes needed (if any): None

Perform and evaluate. Run your procedure using the appropriate controls you've identified. Evaluate controls and hazards as you work. Critique the controls and process you used by answering the following questions. If changes to controls are needed, update your risk assessment tool and re-evaluate any time you revise your process (e.g. changes in scale, reagent, equipment, or conditions that might increase the hazard/risk). Share your assessment with your PI/colleagues for the next iteration of the experiment.

Evaluate Your Procedure
What went well? The experiment worked well and I collected good data.
Did the controls perform as expected? Yes, I saw that I could have observed phospholipid, if it had been synthesized.
Did anything unexpected occur? No
Did a hazard manifest itself that was not previously identified? No
Were there any close-calls or near misses that indicate areas of needed improvement? No
Did something go exceptionally well that others could learn from? No
I plan to evolve my procedure by... Including cyanamide and imidazole so that phospholipid synthesis occurs.

Procedure Risk Assessment is Complete	
Form Completed By: Zachary Cohen	
Signature: Zachary Cohen (e-signature)	**Date:** 08-04-22
PI / Supervisor Signature: Sarah Keller (e-signature)	