

Phenol-Chloroform Extraction^a

Revision Date: _____

Author (P.I., See Citation, Lab Investigator, Others):

Name: _____ Signature: _____

Name: _____ Signature: _____

Name: _____ Signature: _____

Reviewed by CHO/Safety Representative for Lab:

Name: _____ Signature: _____

SCOPE AND APPLICABILITY

- Department: _____
- Research Group: _____
- Lab Bldg., Room(s): _____
- Operation / Experiment: _____
- Material(s): _____
- Ventilation: General room Chemical fume hood Biosafety cabinet (A/B3, B2, B1) Snorkel Trunk

MATERIALS AND HAZARDS

Principal Materials Used	Corrosive	Irritant	Sensitizer	Reproductive toxin	Acutely Toxic	Carcinogen	Flammable	Combustible	Water-Reactive	Shock-Sensitive	Pyrophoric	Oxidizer	Biotoxin	Other Comments:
Phenol	X	X						X						See below 1 .
Chloroform		X		X		X								See below 2 . May affect CNS, liver
Isoamyl alcohol		X						X						
Ether		X					X			X				See below 3 .
Ethanol		X		X			X							May affect CNS
Tris-Cl														
Tris base		X												
HCl	X	X												See below 4 .
Hydroxyquinoline	X	X												
β -mercaptoethanol		X			X			X						

MSDS attached: Yes No If not, explain: (MSDSs are available at the following location: shelf in Room 1234, Building. XYZ; electronically via EHS Department's Web page)

Describe equipment/instrumentation used to monitor/control hazards:

- ♦ Perform all procedures in a laboratory hood (chemical fume hood).
- ♦ Use sealed safety cups when centrifuging phenol:chloroform, and wait at least 10 minutes before opening the centrifuge to avoid exposure to aerosols.

Other comments, Chemical hazards, Precautions:

1. Phenol is highly corrosive and can cause severe burns when contacted with skin. Rinse contacted areas of skin with a large volume of water and wash with soap and water. Do NOT use ethanol. Systemic

MATERIALS AND HAZARDS (CONT.)

effects include damage to the live and kidneys. Antidotes of glycerine, olive oil must be available for minor (droplet size) splashes. Apply polyethylene glycol to affected area promptly and repeatedly, or if possible immerse the part directly into the polyethylene glycol^b. (Here you place the citation, antidote, etc.)

2. Adding chloroform to phenol enhances the ability of phenol to be absorbed by the skin. Systemic effects include damage to the liver and the central nervous system.
3. Ether is extremely flammable and it forms explosive peroxides after prolonged exposure to light and air.
4. Handle with care as concentrated HCl is used. Hydrochloric acid is highly corrosive and causes severe burns on eye and skin contact and upon inhalation of gas.

Special PPE Required:

- Safety glasses
- Goggles (chemical-splash goggles)
- Face Shield
- Chemical Resistant Apron/Smock/Lab Coat
- Protective Clothing (e.g. sleeves, footwear, head cover)
- Gloves/Gauntlets
 - Single
 - Double
- Respirator (If yes, contact EH&S for additional assistance)

Special Protective Clothing Required:

Protective Clothing	Nitrile	PVC (Vinyl)	PVA	Latex	Neoprene	Other:	Additional Specifications
Aprons/Lab coat/Smock							<ul style="list-style-type: none"> ♦ PE, PVC ♦ Avoid traditional cotton-polyester white lab coat
Shoe Cover							
Full Body Cover							
Head Cover							
Gloves/Gauntlets							<ul style="list-style-type: none"> ♦ For splash protection; not for immersion protection. ♦ Double-gloved is recommended given the diversity of chemical hazards and highly corrosive chemicals. Double gloved with different colors and chemistry of gloves is recommended. ♦ Immediately replace with new gloves when splash occurs.
Phenol					X		
Chloroform			X				
Isoamyl alcohol	X	X			X		
Ethanol	X	X			X		
Ether	X	X			X		
Hydrochloric acid	X	X			X		

NOTE: If special PPE and/or protective clothing is not required, standard PPE and protective clothing required in Part II of the Harvard University CHP must be utilized.

ADDITIONAL PRECAUTIONS

- When centrifuging material:
 - Use plastic bottles and tubes instead of glass to reduce the risk of breakage.
 - **Note:** Chloroform and phenol are capable of degrading some types of plastics. Check the chemical resistance or compatibility of materials selected prior to use.
 - **Recommendation:** FEP/PTFE/PFA (Teflon fluorinated ethylene propylene/polytetrafluoroethylene/perfluoroalkoxy)
 - Use aerosol-proof rotors or safety caps during centrifugation to ensure aerosol containment, if a centrifuge tube were to break while spinning.
 - Wait at least 10 minutes before opening the centrifuge to allow aerosol(s) to settle.
- Keep liquid phenol tightly closed and away from heat and light. Store away from inorganic acid and oxidizer (such as chlorine, bromine, and calcium hypochlorite).
- Chloroform reacts violently with alkali metals, such as potassium and sodium, a mixture of acetone and base, strong base (such as potassium and sodium hydroxide, potassium *t*-butoxide, sodium methoxide, and sodium hydride. It reacts explosively with fluorine and dinitrogen tetroxide. In the presence of light, chloroform undergoes autoxidation to generate phosgene; this can be minimized by storing chloroform in the dark, under nitrogen. Do not store in aluminum containers.

MATERIAL SAFETY DATA SHEETS

MSDSs are available electronically via EHS Department's Web page:

http://www.uos.harvard.edu/ehs/onl_msd.shtml. An option, but consider collecting in a binder the MSDSs that arrive with each order.

WASTE

Refer to the *Harvard Longwood Laboratory Waste Guide* posted at

http://www.uos.harvard.edu/ehs/longwood/HarvardLongwoodLabWasteGuide_v1.0.pdf.

PROCEDURE

Attach the enumerated or bulleted steps to be followed in performing the procedure. The steps should be detailed and should include prohibited activities and cautionary statements, where applicable.

EMERGENCY PROCEDURES

Refer to the emergency flip chart titled “*EHS Procedures and Response Guidelines*,” posted in each laboratory and found on EHS’s webpage at http://www.uos.harvard.edu/ehs/lerg_main.shtml

Chemical Spill:

- For small spills, follow chemical spill response guidelines above. Don protective clothing, extinguish all ignition sources, and carefully apply vermiculite or other appropriate spill absorbent material to the spill. Place in durable containers for disposal.
- For spill clean-ups, use Barricade™ or Responder™ gloves (*chemical breakthrough time > 8 hrs*). Viton™ or Silver Shield™/4H™ gloves are acceptable, as well. Do not wear nitrile gloves, due to the risk of direct or prolonged contact when cleaning up a spill.
- Respiratory protection may be necessary in the event of a large spill or release in a confined area.
- For a large spill, vacate the lab, deny further entry, and call EHS for assistance.

Fire:

- In the event of fire, evacuate and bar further entry.

References:

- a. J. Sambrook, E. F. Fritsch, T. Maniatis, “Purification of Nucleic Acids,” *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, 1989, pE.3-4.
- b. G. D. Clayton, F. E. Clayton, *Patty’s Industrial Hygiene and Toxicology*, 3rd ed., Wiley-Interscience Publication, John Wiley & Sons, Inc., 1981, p2567-84.