



## 2. HAZARDOUS CHEMICAL(S)/CLASS OF HAZARDOUS CHEMICAL(S)

Reproductive Toxins are substances or agents that may have adverse effects on various aspects of reproduction in both women and men, including fertility, gestation/pregnancy, birth defects, lactation, genetic effects, and general reproductive performance. Many chemicals used in laboratory study and research, industrial processes, and daily activities pose reproductive hazards.

Materials that meet this criteria can be identified using the following Globally Harmonized System Hazard Codes, which should be included on current Safety Data Sheets:

1. H340 - May cause genetic effects;
2. H341 - Suspected of causing genetic effects;
3. H360 - May damage fertility or the unborn child;
4. H361 - Suspected of damaging fertility or the unborn child; and
5. H362 - May cause harm to breast-fed children.

A few examples of common Reproductive Toxins used at the UC Davis campus include, but are not limited to, the following:

1. Chloroform
2. Toluene
3. Benzene
4. Lead
5. Anesthetic gases (*e.g.*, halothane, isoflurane, etc.)

3,3'-Diaminobenzidine Tetrahydrochloride (DAB) is a widely used chromogen for immunohistochemical staining and immunoblotting. In the presence of peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol.

Chloroform is commonly used in DNA purification procedures in biology and biochemistry and as a solvent in organic synthesis. Chloroform is a carcinogen. It is harmful if swallowed. Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed.

Cobalt Chloride: Causes skin and eye irritation and may affect vision (corneal opacity and degeneration of optic nerve). Causes respiratory tract irritation and pulmonary edema. Harmful if swallowed. May cause gastrointestinal tract irritation with nausea and diarrhea. Chronic or repeated skin contact may cause dermatitis or skin sensitization. Chronic exposure via ingestion may affect behavior, blood and lungs, thyroid gland, pancreas, liver, heart. Chronic inhalation may cause respiratory hypersensitivity.

Ethidium bromide (EtBr) is commonly used as a non-radioactive DNA stain to identify and visualize nucleic acid bands in electrophoresis and perform other methods of nucleic acid separation. Solutions of EtBr fluoresce readily with a reddish-brown color when exposed to ultraviolet (UV) light. EtBr is a mutagen (may cause genetic damage) and is moderately toxic after an acute exposure. EtBr can be absorbed through skin, and will stain it purple. EtBr is an irritant to the skin, eyes, mouth, and upper respiratory tract.

### 3. ENGINEERING/VENTILATION CONTROLS

The following is a general plan for all Reproductive Toxins:

- A. Use containment devices (*e.g.*, chemical fume hoods, glove boxes, etc.) when:
  - i. Using volatile and/or semi-volatile substances;
  - ii. Manipulating substances that may generate aerosols; and
  - iii. Performing laboratory procedures that may result in an uncontrolled release.
- B. Use high-efficiency particulate air (HEPA) filters, carbon filters, or scrubber systems with containment devices to protect effluent and vacuum lines, pumps, and the environment whenever feasible.
- C. Ventilated containment should be used to weigh out solid chemicals (*e.g.*, certified laboratory chemical fume hood). Alternatively, the tare method can be used to prevent inhalation of the chemical. While working in a fume hood, the chemical is added to a pre-weighed container. The container is then sealed and can be re-weighed outside of the fume hood. If a chemical needs to be added or removed, this manipulation is carried out in the fume hood. In this manner, all open chemical handling is conducted in the fume hood.

If you must use Reproductive Toxins without engineering or ventilation controls, you must contact the Chemical Hygiene Officer or [healthandsafety@ucdavis.edu](mailto:healthandsafety@ucdavis.edu) for an exposure assessment.

- Work in a properly operating and certified chemical fume hood.
- Work at least 6" inside the hood, never place your head in the hood, set the sash at the lowest position possible (if using the horizontal sliding sashes do not open past the labeled positions).
- Safety shower and eye wash stations should be easily accessible .

### 4. ADMINISTRATIVE CONTROLS

The following elements are required:

1. Complete the [UC Laboratory Safety Fundamentals](#) (or approved equivalent) training prior to working in the laboratory;
2. Complete laboratory-specific safety orientation and training on laboratory-specific safety equipment, procedures, and techniques to be used, including any applicable laboratory-specific Laboratory Safety Plan(s), prior to receiving unescorted access to the laboratory;
3. Demonstrate competency to perform the procedures to the Principal Investigator (PI), Laboratory Supervisor, laboratory-specific Safety Officer, and/or trainer;
4. Be familiar with the location and content of any applicable Safety Data Sheets (SDSs) for the chemicals to be used (online SDSs can be accessed from [UC SDS](#));
5. Implement good laboratory practices, including good workspace hygiene;
6. Inspect all equipment and experimental setups prior to use;
7. Follow best practices for the movement, handling, and storage of hazardous chemicals (see Chapters 5 and 6 of [Prudent Practices in the Laboratory](#) for more detail). An appropriate spill cleanup kit must be located in the laboratory. Chemical and hazardous waste storage must

follow an appropriate segregation scheme and include appropriate labeling. Hazardous chemical waste must be properly labelled, stored in closed containers, in secondary containment, and in a designated location;

8. Do not deviate from the instructions described in this SOP without prior discussion and approval from the PI and/or Laboratory Supervisor;
9. Notify the PI and/or Laboratory Supervisor of any accidents, incidents, near-misses, or upset condition (*e.g.*, unexpected rise or drop in temperature, color or phase change, evolution of gas) involving the Reproductive Toxins described in this SOP; and
10. Abide by the laboratory-specific working alone SOP, if applicable.

For Reproductive Toxins, the following are also required:

11. Work surfaces should be protected (*e.g.*, disposable absorbent bench paper, aluminum foil, etc.) and must be decontaminated after each use.

Laboratory personnel considering pregnancy or who become pregnant may want to consult the additional information on the [Reproductive Health webpage](#).

All reproductive toxins should be purchased in the smallest practical amount.

DAB: Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 0°C (32°F). Freeze. Sensitive to light. Store in light-resistant containers. Reactive with oxidizing agents. Keep away from heat. Keep away from sources of ignition.

Cobalt Chloride: Keep container tightly closed in a cool, dry, and well-ventilated place away from incompatible materials and conditions. Avoid dust generation, moisture, and excess heat. Keep cool and protect from sunlight.

Chloroform: Do not breathe vapor. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure. Store in flammables cabinet. Keep containers tightly closed in a dry, cool, and well-ventilated place.

All work with ethidium bromide is to be done in designated areas in order to keep ethidium bromide contamination to a minimum. Personnel is required to wear personal protective equipment. Safety shower and eye wash stations should be easily accessible where ethidium bromide is used. Persons operating gel system are to take added caution when using ultraviolet light to visualize gels. Persons are to make sure the UV light is off before they open the UV box and that the UV light is turned off when they are finished. Avoid exposing unprotected skin and eyes to intense UV sources.

## 5. PERSONAL PROTECTIVE EQUIPMENT (PPE)

At a minimum, long pants (covered legs) and closed toe/closed heel shoes (covered feet) are required to enter a laboratory or technical area where hazardous chemicals are used or stored.

In addition to the minimum attire required upon entering a laboratory, the following PPE is required for work with Reproductive Toxins:

- A. Eye Protection: Eye protection is required for all work with Reproductive Toxins.
  - i. At a minimum ANSI Z87.1-compliant safety glasses are necessary.
  - ii. Splash goggles may be substituted for safety glasses, and are required for processes where splashes are foreseeable or when generating aerosols.

- iii. Ordinary prescription glasses will NOT provide adequate protection unless they also meet the Z87.1 standard and have compliant side shields.
- B. Body Protection: At a minimum a chemically-compatible laboratory coat that fully extends to the wrist is necessary.
  - i. If a risk of fire exists, a flame-resistant laboratory coat that is NFPA 2112-compliant should be worn.
  - ii. For chemicals that are corrosive and/or toxic by skin contact/absorption additional protective clothing (*e.g.*, face shield, chemically-resistant apron, disposable sleeves, etc.) are required where splashes or skin contact is foreseeable.
- C. Hand Protection: When hand protection is needed for the activities described in this SOP define the type of glove to be used based on: A) the chemical(s) being used, B) the anticipated chemical contact (*e.g.*, incidental, immersion, etc.), C) the manufacturers' permeation/compatibility data, and D) whether a combination of different gloves is needed for any specific procedural step or task.

When working with reproductive hazards all persons shall wear a lab coat, safety glasses, nitrile gloves and closed toe shoes.

## 6. SPILL AND EMERGENCY PROCEDURES

Follow the guidance for chemical spill cleanup from [SafetyNet #13](#) and/or the [UC Davis Laboratory Safety Manual](#), unless specialized cleanup procedures are described below. Emergency procedure instructions for the UC Davis campus and UCD Medical Center are contained in the [UC Davis Laboratory Safety Manual](#), [campus Emergency Response Guide \(ERG\)](#), and [UCD Health System ERG](#). The applicable ERG must be posted in the laboratory. All other locations must describe detailed emergency procedure instructions below.

Assess the extent of danger. Help contaminated or injured persons. Evacuate the spill area. Avoid breathing vapors. If possible, confine the spill to a small area using a spill kit or absorbent material. Keep others from entering contaminated area (*e.g.*, use caution tape, barriers, etc.).

## 7. WASTE MANAGEMENT AND DECONTAMINATION

Hazardous waste must be managed according to [Safety Net #8](#) using the appropriate [label](#). In general, hazardous waste must be removed from your laboratory within 9 months of the accumulation start date; refer to the [accumulation time for waste disposal](#). Hazardous waste pick up requests must be [completed online](#).

Store hazardous waste in closed containers that are properly labeled, and in a designated area. Contaminated pipet tips, eppendorf tubes, and gloves should be discarded as hazardous waste according to EH&S waste disposal procedures. Store liquid wastes in designated waste containers. Dispose of according to the EH&S hazardous waste guidelines.

Decontamination procedures vary depending on the material being handled. The toxicity of some materials can be neutralized with other reagents. All surfaces and equipment should be wiped with the appropriate cleaning agent following the dispensing or handling of reproductive hazards to prevent accumulation. Decontaminate vacuum pumps or other contaminated equipment before removing them from the designated area or before resuming normal laboratory work in the area.

Carefully inspect work areas to make sure no hazardous materials remain. Clean contaminated work areas with an appropriate cleaning agent, and dispose of cleaning materials properly. Be sure all ignition sources are secured before beginning clean-up with flammable liquids.

See paragraph below.

Upon completion of work with Reproductive Toxins and/or decontamination of equipment, remove gloves and/or PPE to wash hands and arms with soap and water. Additionally, upon leaving a designated Reproductive Toxin work area remove all PPE and wash hands, forearms, face and neck as needed. Contaminated clothing or PPE should not be worn outside the lab. Soiled lab coats should be sent for professional laundering. Grossly contaminated clothing/PPE and disposable gloves must not be reused.

#### **8. DESIGNATED AREA**

Designated area(s) are required for use and storage of Reproductive Toxins. Such areas must be clearly marked with signs that identify the chemical hazard and include an appropriate warning; for example: DANGER! REPRODUCTIVE TOXIN WORK AREA!

Room 3337.

#### **9. DETAILED PROTOCOL**

EtBr:

1. Measure 0.8 g of agarose.
2. Mix agarose powder with 80 mL 1xTAE in a microwavable flask.
3. Microwave for 1-3 min until the agarose is completely dissolved (but do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.).

Note: Caution HOT! Be careful stirring, eruptive boiling can occur.

4. Let agarose solution cool down to about 50°C (about when you can comfortably keep your hand on the flask), about 5 mins.
5. Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 µg/mL (usually about 2.5 µl of lab stock solution per 80 mL gel). Note: Caution EtBr is a known mutagen. Wear a lab coat, eye protection and gloves when working with this chemical.
6. Pour the agarose into a gel tray with the well comb in place.
7. Let sit at room temperature for 20-30 mins, until it has completely solidified.

Chlorophorm: see "Trizol RNA purification for small amounts of tissue" on Kopp Lab website.



