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Carcinogens

STANDARD OPERATING PROCEDURE (SOP)

Type of SOP: ☐ Process ☐ Hazardous Chemical ☒ Hazardous Class

All personnel who are subject to these SOP requirements must review a completed SOP and sign the associated training record. Completed SOPs must be kept with the UC Davis Laboratory Safety Manual or be otherwise readily accessible to laboratory personnel. Electronic access is acceptable. SOPs must be reviewed, and revised where needed, as described in the UC Davis Laboratory Safety Manual. Note that not all hazardous chemicals are appropriately addressed in a single control-banded SOP, and some chemicals are subject to several control-banded SOPs. Unique properties of each chemical must be considered before including it into a control band. This SOP is not appropriate for the "Listed" Carcinogens, as described in 8 CCR §5209 and controlled by the Listed Carcinogen SOP template.

Date SOP Written: **11/10/16** Approval Date: **11/11/16**

SOP Prepared by: **Olga Barmina**, **CLSC SOP Task Force**

SOP Reviewed and Approved by (name/signature): **Artyom Kopp**

Department: **EVE**

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Location(s) covered by SOP: Building: **Storer Hall** Lab Room #(s): **3337** Phone: **530-752-8328**

1. HAZARD OVERVIEW

Carcinogens are chemicals that are known to cause cancer in humans and/or animals, or are suspected of causing cancer. Some of the chemicals used in academic laboratory research, industrial processes, and daily activities are carcinogenic. Recognition of the hazards associated

with the transportation, use, storage, and disposal of these materials is essential. Precautions must be taken to minimize any potential chemical exposure to Carcinogens.

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

Carcinogens are chemicals that are capable of causing cancer or tumor development, typically after repeated or chronic exposure. Their effects may only become evident after a long latency period and may cause no immediate harmful effects.

Carcinogens regulated by the California Occupational Safety and Health Administration (Cal/OSHA) can be found in Title 8 of California Code of Regulations (8 CCR), Article 110, §5200-5220. Additionally, Cal/OSHA defines Carcinogens in 8 CCR §5191 as materials that meet any of the following:

1. Is a regulated Cal/OSHA Carcinogen;
2. Is listed as "known to be carcinogens" in the National Toxicology Program (NTP) Annual Report on Carcinogens;
3. Is listed as Group 1 ("carcinogenic to humans") by the International Agency for Research on Cancer (IARC) Monographs; or
4. Is listed in either Group 2A ("probably carcinogenic to humans") or 2B ("possibly carcinogenic to humans") by IARC or under the category, "reasonably anticipated to be carcinogens" by NTP, and causes statistically significant tumor incidence in experimental animals under defined conditions (see 8 CCR §5191 for more details).

Carcinogens can be identified in the Globally Harmonized System by the Hazard Codes H350 (May cause cancer) and H351 (Suspected of causing cancer). Some common examples of UC Davis laboratory Carcinogens include:

1. Arsenic and Arsenic compounds (inorganic)
2. Benzene
3. Cadmium and Cadmium compounds
4. Chromium (VI) compounds
5. Cobalt and Cobalt compounds
6. Dichloromethane
7. Formaldehyde
8. Lead and Lead compounds (inorganic)
9. Nickel compounds
10. Polycyclic Aromatic Hydrocarbons (PAHs)

Note, many Carcinogens have additional chemical hazards. Review a current Safety Data Sheet for each Carcinogen prior to use.

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

Chloramphenicol is an antibiotic commonly used in the laboratory in agar plates and growth medium to select for the growth of bacterial strains with a gene conferring chloramphenicol resistance.

- Chloramphenicol exposure can occur through ingestion, inhalation, skin and/or eye contact.
- Chloramphenicol is an irritant to the eyes and respiratory tract. It is only a minor irritant to skin.
- Acute exposure of the digestive tract can result in gastrointestinal irritation with nausea, vomiting and diarrhea. It may cause liver damage or hemorrhaging of the digestive tract. Exposure may also cause anemia and other blood abnormalities.

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.

3. ENGINEERING/VENTILATION CONTROLS

Use available engineering/ventilation controls to keep exposure to Carcinogens as low as possible. The following is a general plan for Carcinogens:

A. Use containment devices (*e.g.,* chemical fume hoods, glove boxes, localized exhaust ("snorkel"), 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.,* ventilated balance safety enclosure, etc.). 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 Carcinogens without/outside of engineering or ventilation controls, you must contact the Chemical Hygiene Officer or healthandsafety@ucdavis.edu for an exposure assessment. Formaldehyde use in anatomy, histology and pathology laboratories must be evaluated by EH&S to ensure airborne concentrations of formaldehyde are below the Action Level of 0.5 parts per million by volume.

- 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 Carcinogens described in this SOP; and
10. Abide by the laboratory-specific working alone SOP, if applicable.

For Carcinogens, 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;
12. All waste containing Carcinogen materials at greater than 0.001% wt., including preserved tissue samples, must be disposed as hazardous waste; and
13. This SOP is **not** meant to address 8 CCR §5209 "Listed" Carcinogens. If you are using one of these materials you must develop a separate Listed Carcinogens SOP.

All cancirogens 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. Sensitiveto light. Store in light-resistant containers. Reactive with oxidizing agents. Keep away from heat. Keep away from sources of ignition.

Chloramphenicol: Keep containers tightly closed in a dry, cool, and well-ventilated place.

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.

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 are required for work with Carcinogens:

A. Eye Protection: Eye protection is required for all work with Carcinogens.
   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: 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 cancirogens all persons shall wear a lab coat, safety glasses, nitrile gloves and closed toe shoes. When working with dilute solutions of chloramphenicol, in addition to the standard lab coat and closed toe shoes, gloves can be disposable latex gloves.

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.

For spills of solid materials, DO NOT dry sweep.

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.).

EH&S **must be notified immediately** for any uncontrolled release of Carcinogens; please call (530) 752-1493. Some examples of an uncontrolled release include, but are not limited to, equipment failure, rupture of containers, or failure of control equipment. EH&S must report this information to Cal/OSHA within 24 hours.

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 the EH&S hazardous waste guidelines.

Decontamination procedures vary depending on the material being handled. Carefully inspect work areas to make sure no hazardous materials remain. Following dispensing or handling, all surfaces and equipment should be wiped with the appropriate cleaning agent to prevent accumulation of Carcinogen chemical residue. Dispose of cleaning materials properly. Be sure all ignition sources are secured before beginning clean up with flammable liquids. Decontaminate vacuum pumps or other contaminated equipment before removing them from the regulated area or before resuming normal laboratory work in the area.

See paragraph below.

Upon completion of work with Carcinogens and/or decontamination of equipment, remove gloves and/or PPE to wash hands and arms with soap and water. Additionally, upon leaving a designated Carcinogen work area remove all PPE worn 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) for the use and storage of Carcinogens shall be established where limited access, special procedures, knowledge, and work skills are required. Signage indicating the materials being used and/or stored and the applicable hazards should be easily visible for the designated work space and/or storage area, for example: DANGER! CARCINOGEN WORK AREA!

Room 3337, especially fume hood and balances area.

9. DETAILED PROTOCOL

Chloramphenicol may be used as an antibiotic for agar plates and liquid cultures (sometimes along with ampicillin). The stock solutions may range from 30-50mg/ml. The working concentrations may range from 30-50µg/ml. When using the stock, it should always be added after autoclaving media,

once it is cool enough to touch. Care should be maintained when using aseptic techniques since chloramphenicol stock is flammable.

50mL (34mg/ml) stock solution

1. Wear appropriate Personal Protective Equipment (PPE) (goggles, lab coat, nitrile gloves and closed toe shoes) before working with chloramphenicol.

2. Weigh 1.7 grams Chloramphenicol powder (from 100g bottle) and put it into 50ml graduated cylinder.

3. Add 95% Ethanol to dissolve, and once dissolved bring up to 50ml. Alternately, bring up to 40mL, then rinse cylinder with 2x5ml of Ethanol and add to container (this method should remove residual chloramphenicol, cleaning the cylinder of hazards).

4. Label stock solution with Chemical Name and Concentration, as well all hazards: a) Reproductive Toxin b) Carcinogen c) Flammable - since it's dissolved in 95% Ethanol.

5. Store Stock solution in secondary containment labeled with hazards in a 'flammables' certified -20 freezer to prevent evaporation.

6. Rinse any still-contaminated glassware with 100% Ethanol and dispose of liquid in appropriate hazardous waste. Wipe small spills of liquid or powder in working area with small amount of 20% Ethanol, and dispose of towels and gloves into appropriate solid hazardous waste.

LB Miller agar plates or LB solution containing chloramphenicol

1. Dissolve 20g LB agar Miller and 12.5g LB Broth Miller powder in 500ml H_2O each;

2. Autoclave LB solution for 30-45min;

3. Cool down LB agar or the LB solution to room temperature, add 500µl of chloramphenicol stock (34mg/ml) to LB agar or the LB solution (final concentration 34µg/ml) and gently mix it.

4. Store the LB broth solution in the cold room for future use after it cools down.

5. Pour the LB agar solution onto plates.

6. After the agar solidifies, wrap the plates in a plastic bag and store at 4 C for future use.

Growing and collecting bacterial in LB solution containing chloramphenicol

1. Do transformation of requested construct in chloramphenicol-contained LB agarose plate and incubate overnight at 37 °C.

2. Choose one single colony to 50ml LB containing chloramphenicol (34µg/ml) shaking overnight at 37°C until OD600=1-2.

3. Add 40ml bacterial from above condition to 1L LB containing chloramphenicol (34µg /ml) with shaking at 37°C and keep checking bacterial OD at wavelength 600nm until the OD value is up to 0.6.

4. Add 1ml stocked 1M Isopropyl β-D-thiogalactoside (IPTG) to LB culture, keep shaking for 2~3 hours more.

5. Stop bacterial growing and put the culture on ice for 30min.

6. Pull down the culture to 750ml plastic centrifuge bottles and centrifuged at 4500rpm for 30min at 4°C.

7. Collect the bacterial pellet for continuing experiment and discard the culture medium to a big bottle labeled with hazard liquid chemical waste for disposal in 90 days.

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TEMPLATE REVISION HISTORY

Version	Date Approved	Author	Revision Notes:
1.0	4/14/2015	CLSC Task Force	New template
1.1	3/10/2016	Chris Jakober	Updated URLs following website redesign, added URL to UCDHS ERG, removed reference to Carcinogen Manual and SafetyNet #32

LAB-SPECIFIC REVISION HISTORY

Version	Date Approved	Author	Revision Notes:

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Documentation of Standard Operating Procedure Training

(Signature of all users is required)

- ✓ Prior to using **Carcinogens**, laboratory personnel must be trained on the hazards described in this SOP, how to protect themselves from these hazards, and emergency procedures.
- ✓ Ready access to this SOP and to a Safety Data Sheet for each hazardous material described in the SOP must be made available.
- ✓ The Principal Investigator (PI), or the Laboratory Supervisor if the activity does not involve a PI, must ensure that their laboratory personnel have attended appropriate laboratory safety training or refresher training within the last three years.
- ✓ Training must be repeated following **any** revision to the content of this SOP. Training must be documented. This training sheet is provided as one option; other forms of training documentation (including electronic) are acceptable but records must be accessible and immediately available upon request.

Designated Trainer: *(signature is required)*

I have read and acknowledge the contents, requirements, and responsibilities outlined in this SOP:

Name	Signature	Trainer Initials	Date