

# Safe Operating Procedure of using Carbon Tetrachloride for Animal Research

**NOTE: You must read this entire document and both you and the Principal Investigator must sign it before commencing any work.**

Project Number (e.g. Tick@Lab): \_\_\_\_ { University SOP} \_\_\_\_\_

Principal Investigator/Supervisor: \_\_\_\_ { University SOP} \_\_\_\_\_

Room and Building where SOP is used: Wet labs, LAF labs

## Summary of How the Material/Equipment/Process will be Used

Carbon tetrachloride is commonly used in experimental animals to induce chronic liver injury.

The processes associated with the use of carbon tetrachloride include:

- 1) Preparation of Carbon tetrachloride solution
- 2) Transport of Carbon tetrachloride from research lab to LAF for administration
- 3) Administration of Carbon tetrachloride into animals
- 4) Housing and handling of Carbon tetrachloride administered animals
- 5) Cage changing and washing of Carbon tetrachloride contaminated cages
- 6) Disposal of Carbon tetrachloride contaminated waste

## Potential Hazards

### Human Toxicity or Carcinogenic Exposure (Process 1-6)

- Toxic if swallowed, inhaled or in contact with skin (hazard statement H301, H331 and H311)
- May cause cancer (hazard statement H351)
- Damages the specific organ through prolonged or repeated exposure (hazard statement H372)

### Needle Stick Injury due to Injection (Process 3)

### Animal Bite (Process 3-5)

Carbon tetrachloride SDS weblink:

<https://www.sigmaaldrich.com/HK/en/sds/sial/289116?userType=anonymous>

## Safety Installations

### Engineering Control Measures

- Certified fume hood (FH) for Carbon tetrachloride preparation (e.g. open, weight, reconstitute and dilute)
- Certified Class II Biosafety cabinet (BSC) for injection, animal handling and cage changing
- Secondary container for transport
- Luer lock syringes for administration
- Animal restraint devices for administration (if applicable)
- Forceps for animal handling
- Animal cage changing station for cage changing
- Individually ventilated cage (IVC) and air handling unit for housing
- Cage liners for housing
- Cage washing machines for washing
- Carbon tetrachloride waste bin for disposal of contaminated waste
- Carbon tetrachloride sharp box for disposal of contaminated syringes
- Eye wash station for emergency use
- Availability of Carbon tetrachloride Safety Data Sheet (SDS) in handling areas

## Work Practices

### LAF Notification

- Inform LAF prior to using Carbon tetrachloride in LAF for better arrangement

### Medical Surveillance Program

- Enroll in medical surveillance program and indicate “toxic chemicals injected ”

### Training

- Complete Carbon tetrachloride-specific safety training provided by the PI
- Complete HSEO MC03 Chemical I, MC07 Chemical II, MC06 Biological Safety Training
- Complete general animal user training and animal handling training provided by LAF

### SOP

- Establish and follow in-house SOPs for specific process (e.g. Carbon tetrachloride administration, waste collection, cage washing, etc.)

### Labelling and Warning Sign

- Complete LAF blue cage card “Health Hazard Card” with administration information and place in each Carbon tetrachloride administered animal cage until 72 hours post-administration.
- Affix a Carbon tetrachloride warning sign with hazard information on each of the Carbon tetrachloride administered animal holding room
- Label all Carbon tetrachloride tubes, containers, waste bin, waste bag with appropriate hazard warnings and chemical identification.
- Post a “Carbon Tetrachloride Hazard” sign on the FH and BSC which is used for Carbon tetrachloride preparation and administration

### Proper Storage

- Store Carbon tetrachloride in a lockable secondary container, and in a well-ventilated place
- Use zipper bags and secondary containers during transport to avoid spillage

### Good Hygiene Practice

- Minimize exposure by implementing control measures and wear adequate PPE
- Clean work surfaces with detergent and water, followed by 10% sodium hypochlorite and rinse thoroughly
- Wash hands immediately after handling Carbon tetrachloride and Carbon tetrachloride-contaminated waste

## **Specific Experimental Procedures**

### Preparation, Transportation and Administration of Carbon Tetrachloride (process 1-3)

- Purchase only the necessary quantity of Carbon tetrachloride
- Wear adequate PPE when handling Carbon tetrachloride
- Inspect the outer package of Carbon tetrachloride to make sure it is intact
- Open, weigh and prepare Carbon tetrachloride solution inside a certified FH
- A plastic-backed absorbent pad should be placed under the work surface during preparation and administration process to avoid contamination
- Place a waste bag on the work surface for the collection of contaminated wastes
- Aliquot the Carbon tetrachloride solution into leak-proof, screw cap tubes and place inside a secondary container labelled with chemical name and hazard warning labels. Store the container in a fridge.
- During transportation, Carbon tetrachloride solution tubes must be stored in a zipper bag inside a secondary container (only bring the required amount to LAF)
- Conduct the administration inside a certified BSC
- Do not recap the syringes. Dispose of syringes used into Carbon tetrachloride sharp box inside BSC
- Dispose of all contaminated wastes (e.g. tubes, tips, absorbent pad, syringes, etc.) into a waste bag inside FH/BSC and then discard into designated Carbon tetrachloride chemical waste bin
- Clean work surfaces with detergent and water, followed by 10% sodium hypochlorite and rinse thoroughly.

### Housing and Handling of Carbon Tetrachloride-Administered Animals (process 4)

- Complete LAF blue cage card “Health Hazard Card” with administration information and place in each Carbon tetrachloride administered animal cage
- Conduct cage changes within a BSC or Cage Changing Station (CCS)
- Each Carbon tetrachloride-administered animal should return to a cage with the cage liner and “Health Hazard Card”, then housed in a designated animal holding room

### Cage Changing and Washing of Carbon Tetrachloride-Contaminated Cages (process 5)

- After 72 hours of last administration, the contaminated cage/water/feed can be changed inside a BSC or CCS
- Dispose all contaminated disposable waste into the cage liner inside BSC/CCS, and bag the cage liners into a chemical waste bag (refer to waste disposal for more details)
- After disposing wastes, the “Health Hazard Card” can be removed and considered free of Carbon tetrachloride
- Bag the non-disposable cage accessories’ (e.g. lid, cage base, wire bar, etc.) in a bag for LAF collection
- LAF staff collect the bagged cage accessories to cage washing area for normal washing process

## **Personal Protective Equipment**

### Skin Protection, Eye Protection, Face Protection and Respiratory Protection

- Wear double-layer Neoprene gloves. One glove should be placed under the coat / coveralls and one over.
- Lab coat or coveralls
- Sleeve covers if wrist is exposed
- Safety glasses or chemical goggles or face shield
- N95 mask or PAPR if work is not conducted in BSC or fume hood

## Waste Disposal

### Carbon Tetrachloride-Contaminated Waste Disposal (process 6)

- Discard all contaminated wastes during preparation and administration into waste bags.

#### **Double bags** all wastes.

- Tie up the waste bags and place in designated Carbon tetrachloride chemical waste bin for HSEO collection
- The waste bin should be kept closed at all times
- For contaminated sharps waste box, place the sharp box in designated Carbon tetrachloride chemical waste bin for HSEO collection
- For Carbon tetrachloride-administered animal carcasses, **double bags** before placing in the clinical waste fridge for contractor collection

### Bedding and Cage Liner Disposal (process 6)

- Place the bedding and cage liners in a chemical waste bag, and tie up the bag
- Place the waste bag in designated Carbon tetrachloride chemical waste bin for HSEO collection

## Spills and Incidents

### Minor Spill of Carbon Tetrachloride

- Absorb the spill with absorbent pad or paper towel with adequate PPE
- Cover, Clean work surfaces with detergent and water, followed by 10% sodium hypochlorite and rinse thoroughly
- Collect the wastes in a plastic bag and dispose in a Carbon tetrachloride-contaminated waste bin

### Major Spill of Carbon Tetrachloride

- Avoid inhalation and generating dust
- Remove contaminated clothing with gloved hands, remove gloves and place near the spill
- Remove all ignitable sources. If a fire is visible, DO NOT turn on the emergency ventilation.
- Evacuate other workers within the laboratory.
- Call Security Control Center Ext 8999 for assistance and do not allow anyone to enter the affected area
- HSEO will be notified by Security Control Center to clean up the spill

### Bites by Animals Treated with Carbon Tetrachloride

- Wash bite area with running water for 15 minutes
- Seek medical attention. Bring SDS to the clinic / doctor.

## Emergency Procedures

### Exposure

- If Carbon tetrachloride comes into contact with the eye, face and skin, wash the area with running water for 15 mins using the nearest emergency facilities and seek medical advice immediately.
- Notify HSEO of any exposures.

### Emergency Response

- Notify the Security Control Center by dialing ext. 8999 and provide information on the incident, including the chemical involved, the location, and any injuries.

## References

- Benson, J. M., & Springer, D. L. (1999). *Improved risk estimates for carbon tetrachloride* (No. EMSP-54940-). Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico; Pacific Northwest National Lab.(PNNL), Richland, WA (United States).
- Cho KJ, Jang JJ. Effects of carbon tetrachloride, ethanol and acetaldehyde on diethylnitrosamine-induced hepatocarcinogenesis in rats. *Cancer Lett.* 1993 Jun 15;70(1-2):33-9. doi: 10.1016/0304-3835(93)90071-g. PMID: 8330298.
- National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 5943, Carbon Tetrachloride. Retrieved November 5, 2025 from <https://pubchem.ncbi.nlm.nih.gov/compound/Carbon-Tetrachloride>.

Prepared by: \_\_\_\_\_ Date: \_\_\_\_ November 12<sup>th</sup>, 2025 \_\_\_\_

Reviewed by: \_\_\_\_\_ Date: \_\_\_\_\_