

Standard Operating Procedures

Laboratory Specific
Chemical: **Osmium Tetroxide**

Please fill out the form completely. Print a copy and insert into your
Laboratory Safety Manual and Chemical Hygiene Plan.
Refer to instructions for assistance.

Department:	Chemistry
Date SOP was written:	5/25/2017
Date SOP was approved by PI/lab supervisor:	5/25/2017
Principal Investigator:	Rongbiao Tong
Internal Lab Safety Coordinator/Lab Manager:	Jingxun Yu
Lab Phone:	23587393
Office Phone:	23587357
Emergency Contact:	Rongbiao Tong 53484541 (Name and Phone Number)
Location(s) covered by this SOP:	CYT/6014 (Building/Room Number)

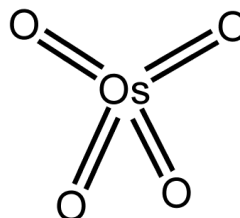
Type of SOP: ☐ Process ☒ Hazardous Chemical ☐ Hazardous Class

Purpose

Osmium tetroxide is a volatile, highly toxic solid that is used as a stain for electron microscopy, and as a fixative for biological samples.

Physical & Chemical Properties/Definition of Chemical Group

Synonyms: Osmium tetroxide
Osmic acid
Perosmic oxide
Osmium(IV) oxide



CAS#: 20816-12-0

Molecular formula: OsO4
Molecular weight: 254.23 g
Form: pale yellow solid

Potential Hazards/Toxicity

Toxic Effects: Oxidizing material. Highly toxic. Contact with combustible materials may cause fire. May cause sensitization by inhalation or skin contact.

Acute Effects: The acute toxicity of osmium tetroxide is high, and it is a severe irritant of the eyes and respiratory tract.

Exposure to osmium tetroxide vapor can damage the cornea of the eye. Irritation is generally the initial symptom of exposure to low concentrations of osmium tetroxide vapor, and lacrimation, a gritty feeling in the eyes, the eyes can temporarily cloud, and the appearance of rings around lights may also be noted. In most cases, recovery occurs in a few days. Concentrations of vapor that do not cause immediate irritation can have an insidious cumulative effect; symptoms may not be noted until several hours after exposure. Contact of the eyes with concentrated solutions of this substance can cause severe damage and possible blindness.

Inhalation can cause headache, coughing, dizziness, lung damage, difficult breathing and may be fatal.

Contact of the vapor with skin can cause dermatitis, and direct contact with the solid can lead to severe irritation and burns. Exposure to osmium tetroxide via inhalation, skin contact, or ingestion can lead to systemic toxic effects involving liver and kidney damage. Osmium tetroxide is regarded as a substance with poor warning properties.

Chronic Effects: Chronic phenol poisoning is characterized by vomiting, difficult swallowing, excessive salivation, diarrhea, anorexia, headache, fainting, vertigo, mental disturbances, and possibly skin eruptions. Prolonged cutaneous exposure may result in deposition of dark pigment in the skin.

Exposure Limits: Cal-OSHA PEL= 0.0002 ppm (0.002 mg/m³)

Personal Protective Equipment (PPE)

- Chemical goggles (safety glasses alone are not adequate protection because of osmium tetroxide's severe effects on the eyes).
- Disposable nitrile gloves (NOT latex). Double-gloving is recommended when working with pure osmium tetroxide or concentrated solutions. Change gloves frequently and when contaminated, punctured or torn. Wash hands immediately after removing gloves.
- A standard or disposable laboratory coat or disposable coveralls. A standard laboratory coat may be reused before laundering if it has not been contaminated with osmium tetroxide. If a garment is contaminated, remove, place in chemical hood, and decontaminate with corn oil or aqueous solutions of sodium sulfide or sodium sulfite before disposing of in hazardous waste or laundering.
- Long pants and closed-toed shoes must be worn.

- Wash hands thoroughly immediately after working with any concentration of osmium tetroxide.

Engineering Controls

- Osmium tetroxide solutions must be prepared and handled in a certified chemical hood.
- Choose a hood with minimal equipment or obstructions to ensure good containment of vapors.
- Working surfaces should be protected with plastic backed absorbent pads to insure containment of any spills.
- Post the hood with a warning sign to alert others to the hazards, see warning sign at the end of this SOP.
- Ensure that the safety shower and eyewash are operational and access is unblocked.

First Aid Procedures

Eye Contact:	Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Do not use an eye ointment. Seek medical attention.
Skin Contact:	After contact with skin, wash immediately with plenty of water. Gently and thoroughly wash the contaminated skin with running water and non-abrasive soap. Be particularly careful to clean folds, crevices, creases and groin. Cold water may be used. Cover the irritated skin with an emollient. If irritation persists, seek medical attention.
Serious Skin Contact:	Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.
Inhalation:	Allow the victim to rest in a well ventilated area. Seek immediate medical attention.
Serious Inhalation:	Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.
Ingestion:	Do not induce vomiting. Examine the lips and mouth to ascertain whether the tissues are damaged, a possible indication that the toxic material was ingested; the absence of such signs, however, is not conclusive. Loosen tight clothing such as a collar, tie, belt or waistband. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek immediate medical attention.

Special Handling and Storage Requirements

Precautions:	Keep locked up Keep away from heat. Keep away from sources of ignition. Keep away from combustible material. Do not ingest. Do not breathe dust. Wear suitable protective clothing In case of insufficient ventilation, wear suitable respiratory
--------------	---

equipment If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes.

Storage: Keep container tightly closed. Keep in a cool, well-ventilated place. Highly toxic or infectious materials should be stored in a separate locked safety storage cabinet or room.

Because of osmium tetroxide's high acute toxicity and powerful oxidizing ability, osmium tetroxide must be handled in the laboratory using prudent practices. In particular, all work with osmium tetroxide must be conducted in a fume hood to prevent exposure by inhalation, and personal protective equipment (see section) must be worn at all times to prevent eye and skin contact.

Osmium tetroxide should be purchased as a liquid to avoid particulate exposure from the powdered form. The solutions should be stored in labeled tightly sealed containers, and these should be placed in secondary containment. Secondary containment should be used anytime the material is transported to another lab location.

When osmium tetroxide is freshly prepared and active, it is colorless to pale yellow in color. When the material reacts and causes oxidation, it turns black. This is helpful to know especially in the event of a splash or spill (see below) or inadvertent dermal exposure (black dots on skin).

- Pure osmium tetroxide and concentrated solutions should be stored in a location that is secure to unauthorized access.
- A refrigerator containing osmium tetroxide must be labeled with a caution sign noting the presence of osmium tetroxide and its hazards.
- Store pure osmium tetroxide and its concentrated solutions in appropriate, sealed glass containers within unbreakable secondary containment (i.e., a bottle or vial within a sealed compatible plastic jar or metal can with lid). Label all containers, including secondary containment, with the chemical name and hazard warning.

Handling and Solution Preparation:

- When moving pure osmium tetroxide to a chemical hood, do not remove it from the secondary containment until it is in the hood.
- Prepare the smallest amount of solution necessary for the procedure, typically 50 mL or less. Prepare the solution volumetrically rather than gravimetrically. If a balance must be used, weighing must take place in the chemical hood.
- Pure osmium tetroxide or its concentrated solutions must be opened only in a chemical hood that has been certified within the last 12 months. Just before use, the operation of the chemical hood must be verified by the use of an installed chemical hood monitoring device, a smoke test using a smoke generating tube, or a mechanical or electronic device that indicates air flow. During use, the sash must be lowered to operating height.
- All lab ware that has contacted osmium tetroxide must be decontaminated by rinsing or dipping in corn oil or aqueous solutions of sodium sulfide or sodium sulfite before removing from the hood.
- Immediately after work with osmium tetroxide, decontaminate any spills with kitty litter soaked with corn oil. Discard kitty litter as hazardous waste. Or use aqueous solutions of sodium sulfide or sodium sulfite.

Spill and Accident Procedure

Chemical Spill Dial 911 and x59797

Spill – Help contaminated or injured persons. Evacuate the spill area. Avoid breathing vapors. Eliminate sources of ignition if the chemical is flammable. 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.).

Small (<1 L) – If you have training, you may assist in the clean-up effort. Use appropriate personal protective equipment and clean-up material for chemical spilled. Double bag spill waste in clear plastic bags, label and take to the next chemical waste pick-up.

Large (>1 L) – Dial **911** (or 310-825-1491 from cell phone) and EH&S at x59797 for assistance.

Chemical Spill on Body or Clothes – Remove clothing and rinse body thoroughly in emergency shower for at least 15 minutes. Seek medical attention. *Notify supervisor and EH&S at x59797 immediately.*

Chemical Splash Into Eyes – Immediately rinse eyeball and inner surface of eyelid with water for 15 minutes by forcibly holding the eye open. Seek medical attention. *Notify supervisor and EH&S at x59797 immediately.*

Medical Emergency Dial **911 or x52111**

Life Threatening Emergency, After Hours, Weekends And Holidays – Dial **911** (or 310-825-1491 from cell phone) or contact the Ronald Reagan UCLA Medical Center (emergency room) directly at **x52111** (located at 757 Westwood Plaza, enter from Gayley Avenue). *Note: All serious injuries must be reported to EH&S at x59797 within 8 hours.*

Non-Life Threatening Emergency– Go to the Occupational Health Facility (OHF), **x56771**, CHS room 67-120 (This is on the 6th floor, 7th corridor, room 120. Enter through the School of Dentistry on Tiverton Drive and proceed to the “O” elevator to the 6th floor.)Hours: M - F, 7:30 a.m. to 4:30 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All serious injuries must be reported to EH&S at x59797 within 8 hours.*

Needle stick/puncture exposure (as applicable to chemical handling procedure)– Wash the affected area with antiseptic soap and warm water for 15 minutes. For mucous membrane exposure, flush the affected area for 15 minutes using an eyewash station. Page the needle stick nurse by dialing **231** from a campus phone, enter **93333** when prompted and then enter your extension. Hours: M – F, 8:00 a.m. to 4:00 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All needle stick/puncture exposures must be reported to EH&S at x59797 within 8 hours.*

Decontamination/Waste Disposal Procedure

- To reduce hazards involved in discarding osmium tetroxide, the following neutralization procedure should be employed:
 1. Perform neutralization in a chemical hood.
 2. A 2% solution of osmium tetroxide can be fully neutralized by twice its volume of vegetable oil (corn oil is preferred because of its high percentage of unsaturated bonds). For every 10 mL of 2% osmium tetroxide solution, 20 mL of corn oil is required. Pour the corn oil into the osmium tetroxide solution.
 3. Wait for the oil to completely turn black.

4. To test if osmium tetroxide is fully neutralized, hold a piece of filter paper soaked in corn oil over the solution. Blackening indicates that osmium tetroxide is still present and more corn oil should be added.
5. Aqueous solutions contaminated with osmium tetroxide can be fully neutralized by adding sodium sulfide or sodium sulfite to reduce osmium tetroxide to less hazardous forms.
6. Dispose of neutralized solutions as hazardous waste.

- To dispose of waste:

SOLUTIONS: DO NOT dispose of leftover or spent solutions of osmium tetroxide by emptying into the laboratory sink. Collect solutions and solid osmium tetroxide in a labeled leak proof waste container for pickup and proper disposal by EH&S Hazardous Waste Pickup service. A completed waste label must be attached to the container.

SOLIDS: Collect pipette tips, gloves, ampoules, etc in a rigid leak proof container. Affix a completed waste label to the container. Due to the toxicity of osmium tetroxide, empty containers must also be collected and disposed as hazardous waste.

Material Safety Data Sheet (MSDS) Location

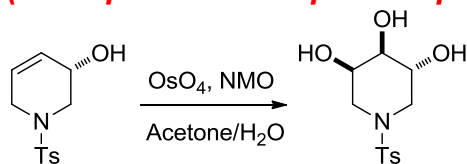
(State the location of MSDS)

Hardcopy or electronic copy must be available.

Online MSDS can be accessed at <http://msds.ehs.ucla.edu>.

Protocol/Procedure

(Add specific description of procedure)



In a flame-dried roundbottom flask of 10 mL was added (±)-1-tosyl-1,2,3,6-tetrahydropyridin-3-ol (1.0 equiv, 0.059 mmol, 15 mg) dissolved in a mixture of water/acetone (2.1 mL; 9:1). To this mixture was added NMO (2.0 equiv, 0.118 mmol, 13.9 mg) and an aqueous solution of OsO₄ (19.8 μL, 5 mol %, 2.96 μmol). The reaction was stirred at 25 °C for 48 h and then quenched with 270 μL of a saturated solution of sodium bisulfide. Next, the reaction was extracted with ethyl acetate (3 × 10 mL) and the solvent dried over sodium sulfate and removed by reduce pressure. The crude product was purified by flash column chromatography to furnish (±)-1-tosylpiperidine-3, 4, 5-triol (10.5 mg, 0.040 mmol, 70% yield).

II. Use of Osmium Tetroxide and Ruthenium Tetroxide (general guidelines from University of Minnesota)

Osmium Tetroxide and Ruthenium tetroxide are highly toxic chemicals, known to directly impact human health in a very negative manner. Ailments include skin disease, damage of the respiratory tract, blindness and death. The following safety procedures must be followed whenever you use these chemicals:

- A. Always use disposable elbow length gloves while handling these materials inside a fume hood. Make sure the fume hood is operating correctly! Wear a lab coat.
- B. Wear safety glasses to protect your eyes.
- C. Carefully transfer the chemical from the refrigerator to the hood.
- D. Work with the hood sash lowered for additional protection.
- E. Be careful when breaking an ampule of these solutions so that no spill occurs in the hood.
- F. Cover the dish containing the chemicals and your sample while in use.
- G. Pour excess chemical from the ampule into a flask and transfer back to the refrigerator for future use.
- H. In case of a spill on your gloves, dispose of gloves in a plastic disposal bag and seal them inside a labeled container.
- I. Dispose of all glassware (broken ampules, pipettes, etc.) and waste inside a sealed and labeled container.
- J. In case of a small spill (<10 ml) inside the hood, neutralize with corn oil (for osmium tetroxide) or sodium bisulfite solution for either chemical. Use about 1 teaspoon of sodium bisulfite in 25 ml of water.
- K. ~~For larger spills, evacuate the room and contact Environmental Health and Safety 6-6002 or 911.~~

Handling (The following general guidelines from Drexel University)

1. The Material Safety Data Sheet and this SOP must be reviewed before use of osmium tetroxide in the laboratory.
2. The laboratory's principal investigator must develop specific written experimental procedures for the use of osmium tetroxide in the laboratory before any work can be permitted to begin.
3. As this material may sublime and is a significant inhalation hazard, all manipulations with osmium tetroxide must be done in a properly working chemical fume hood, glove box, local exhaust system or other suitable containment device that exhaust directly outside. Even aqueous solutions. Use a NIOSH/MSHA approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced. If a respirator is to be used, the lab personnel must be admitted into HSEO of the university.
4. Designate an area in the laboratory chemical fume hood (or glove box, etc.) for only osmium tetroxide manipulations. This area must be labeled with the appropriate hazard communication labels (i.e. carcinogen in use area). All equipment and PPE must remain in this designated area. Never remove contaminated equipment or PPE from designated area.

5. Proper personal protection equipment (PPE) must be worn at all times to prevent eye and skin contact. Consult the MSDS for PPE recommendations. The minimum requirements are lab coats, chemical safety goggles, and rubber or neoprene gloves.
6. Be sure to inspect all PPE prior to and after use.
7. Any amount of osmium tetroxide spilled must be immediately reported as a major spill event. Keep container tightly closed at all times unless actively removing some material.
8. The laboratory must be equipped with a working eyewash station and safety shower.
9. Always practice good laboratory hygiene. Wash hands, face, neck and forearms frequently. Wash hands before eating and do not eat, drink, or smoke in the laboratory.
10. Keep good housekeeping procedures. All disposable materials contaminated with osmium tetroxide must be disposed as hazardous waste.
11. Containers of this product may be hazardous when empty as they may contain product residues (vapors, liquids).

Storage

1. The osmium tetroxide must be stored in tightly closed secondary containment that are opened only in the chemical fume hood.
2. The secondary containment container must be labeled according to University guidelines (i.e. full chemical name; hazard warning words – toxic; oxidizer).
3. The lid of both the primary and secondary containers must remain tightly closed at all times. The container should not be left open for any more time than what is necessary to measure out the required material, as this material sublimates.
4. Store away from combustible materials, reducing agents, finely powdered metals and heat. Contact with hydrochloric acid will cause the formation of poisonous chlorine gas.
5. Due to the hazardous nature of the material only minimal quantities of material should be purchased and stored.

Disposal

1. All waste must be collected in a sealable compatible container and disposed as hazardous waste.

Note: Any deviation from this SOP requires written approval from PI.

Documentation of Training *(signature of all users is required)*

- ✓ Prior to conducting any work with Osmium Tetroxide, designated personnel must provide training to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.
- ✓ The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the Osmium Tetroxide MSDS provided by the manufacturer.
- ✓ The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate laboratory safety training or refresher training within the last two years.

I have read and understand the content of this SOP:

[illegible][illegible][illegible]