



Saint Mary's
University

Electron Microscopy Centre

Title: SAFELY USE OF OSMIUM TETROXIDE

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NOTE: Osmium tetroxide use is restricted to the specific EM users who have had prior training in proper use, handling and storage. Each user who will be working on Osmium Tetroxide in EMC shall read this document. Signature is required to indicate that he/she has read and understands this safety precaution.

Introduction

Osmium Tetroxide, also known as osmic acid, perosmic oxide, and osmium (IV) oxide. (CAS # 20816-12-0).

Osmium tetroxide is acutely toxic and can cause blindness and skin burns. It is rapidly absorbed through the skin and its relatively high volatility makes it especially hazardous to work with outside of a chemical hood. The rat intraperitoneal LD₅₀ for osmium tetroxide is 14 mg/kg and the mouse oral LD₅₀ is 162 mg/kg.

All staff engaged in the use or handling of osmium tetroxide, or working within a laboratory using osmium tetroxide, are responsible for understanding all hazards associated with its use, and for using appropriate personal protective equipment (PPE).

Training

Prior to conducting any work with osmium tetroxide, supervisors are responsible to provide training to his/her students specific to the hazards involved in working with Osmium Tetroxide, work area decontamination, and emergency procedures. Supervisor must also provide students with a copy of this document and a copy of the osmium tetroxide MSDS provided by the manufacturer.

Signage

When osmium tetroxide is in use, warning signs must be posted on the chemical hood and laboratory door until the osmium tetroxide has been returned to storage.

Storage

Pure osmium tetroxide and concentrated solutions must be stored in a location that is secure to unauthorized access. Examples are a locked drawer or cabinet, or a refrigerator within a laboratory that is locked when authorized personnel are not present. A refrigerator containing osmium tetroxide must be labeled with a caution sign noting the presence of osmium tetroxide and its hazards (toxic, corrosive). **Plastic is permeable to Osmium - use glass!**

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). **Store away from organic materials.** Label all containers, including secondary containment, with the chemical name and hazard warning.

Personal Protective Equipment

The following minimum Personal Protective Equipment must be worn during operations with pure osmium tetroxide and concentrated solutions:

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 before disposing of in hazardous waste or laundering.

Closed-toed, leather shoes (not fabric or mesh).

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

Handling and Solution Preparation

When moving pure osmium tetroxide to a chemical hood, place the vial in a padded metal can and cover. Place on a lab cart with a supply of a neutralizing absorbent consisting of kitty litter soaked in corn oil.

Prepare the smallest amount of solution necessary for the procedure, typically 50mL 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 come in contact with osmium tetroxide must be decontaminated by rinsing or dipping in corn oil before removing from the chemical hood.

Immediately after work with osmium tetroxide, decontaminate any spills with kitty litter that has been soaked with corn oil. Discard kitty litter as hazardous waste.

Neutralizing Osmium Tetroxide.

To reduce the hazards involved in discarding osmium tetroxide, the following neutralization procedure should be employed:

Perform neutralization in a chemical hood. 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 10ml of 2% osmium tetroxide solution, 20ml of corn oil is required. Pour the corn oil into the osmium tetroxide solution.

Wait for the oil to completely turn black.

To confirm that the 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.

Dispose of the neutralized solution as hazardous waste. Please contact Jason Roch at Biology Department to safely dispose the neutralized waste.

Emergency Plans

1) Spill of Osmium Tetroxide Outside of the Chemical Hood:

DO NOT attempt to clean up a spill if you are not trained to handle the spill. Phone the Campus Emergency number (-5000) or public poison control (428-8191) and (496-8778) immediately.

Isolate the area to prevent the spread of contamination (e.g., close doors to affected area and post a warning sign.

Alert personnel in the immediate area to evacuate.

Attend to any injured personnel.

NOTE: Personnel must not attempt to clean up a spill of pure osmium tetroxide or its concentrated solution that occurs outside of a chemical hood.

2) Spill of Osmium Tetroxide Inside the Chemical Hood:

Cover the spill with corn oil-soaked kitty litter.

Use the test to ensure that the spill has been 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)

Scoop up the kitty litter and dispose of as hazardous waste. Clean the area with detergent solution.

3) Accidental Exposure to Osmium Tetroxide

i) If osmium tetroxide vapor has been inhaled from a spill, move the victim to fresh air immediately.

- ii) If osmium tetroxide has been spilled on skin or clothing, rinse the affected area with water, using a safety shower or eyewash, as appropriate, for a minimum of 15 minutes. During washing, remove contaminated clothing. A disposable laboratory coat or jumpsuit should be available for the exposed individual to wear after using a safety shower.
- iii) Notify the victim's supervisor, if immediately available. The supervisor, a co-worker, or the victim must contact the campus Employee/Occupational Health Program to determine what additional steps should be taken.
- iv) If the incident occurs off-hours, or an ambulance is needed because of injury, contact Public Safety to advise them of the medical emergency.

SIGNATURES

EM Users: the EM user's signature indicates that the user has read this document and understands the hazards and safe work practices as detailed in this therein.

Supervisor: a student supervisor's signature indicates that the supervisor understands the hazards of Osmium Tetroxide and will train his/her student safely handle osmium tetroxide according to this document.

Name of EM User: (Print): _____

Signature: _____ Date: _____

Name of Supervisor: (Print): _____

Signature: _____ Date: _____

Appendix:

1. Collections on how to store Osmium Tetroxide from Microscopy Listserv
2. Osmium Tetroxide: Storage Conditions (from EMS)

Appendix 1: Collections on how to store Osmium Tetroxide

Question:

Does someone have a method to store osmium in such a way as to avoid any escape of vapor? Also, is there something we could put into fridge that would trap osmium vapors as they leak out?

Answers:

1. You could try placing the bottle in a bath of oil, but that would get messy.
2. We store our osmium in a secondary container...a large poly pro bottle (capped) and then we bag that container in a plastic Ziploc. It helps a bit.
3. We use bottles with rubber septums to store the liquid, and keep the bottles within sealed falcon tubes, within a sealed bottle. This keeps the vapours from leaking and discolouring the fridge.
4. Use what you need, perhaps even batch the tissue samples, then throw out the unused in the waste.
5. We use Osmium in our lab and keep it in the refrigerator. We keep it in a glass bottle and cover the lid with parafilm. Then we put that bottle in another bottle with a screw top and wrap the bottle in foil. It works great, our refrigerator doesn't have and black residue, just the two bottles.
6. Osmium vapours are not stopped by simple bottle tops. We buy ours already made up. The bottle lids have a plastic (?) insert which is effective. Waste osmium is stored in any bottle which is then kept in a Kilner jar. It is made of glass, has a rubber seal and uses wire to clamp the lid very tightly. Osmium vapours do not seem to pass through rubber seals. Unfortunately new Kilner jars in my supermarket have plastic seals - so an experiment will be required.
7. I keep my working solution of Osmium in a glass Wheaton or Gibco bottle with a Teflon-lined cap and wrap the cap with Parafilm and put that into the metal can that's shipped with the ampoules of Osmium or a larger glass jar. I layer molecular sieves in the can/jar. Any escaping Osmium vapors are indicated by blackening Parafilm... or blackening refrigerator! Our frig. is clean.
8. We keep our osmium solutions in Schott Duran laboratory bottles, and keep the bottles in a paint can (a new one that never had paint in it). This is then kept in our fridge. This arrangement seems to keep the vapour contained (no marks in our fridge) and isn't a pain to open up. I also keep a bottle of whole milk powder in there for spills.
9. When OsO_4 came in metal cans, I found by placing the working solution in a Wheaton dropper bottle, wrap the bulb with Parafilm, place the bottle in the can, close the lid, wrap the lid with electrician tape, and place the can in a plastic Ziplock bag worked great. You could essentially use any clean glass container, not just a Wheaton bottle. After 20 years of working with OsO_4 , the inside of the refrigerator was pristine. It requires diligence to unwrap and wrap the container, but it worked. A short-cut to the above mentioned technique, was to use a clean pint paint, place the Wheaton bottle in the can and push the lid down tight, then place the entire can in a Ziplock bag.

10. My solution (which may not be practical for everyone) is that only sealed ampoules are stored in the fridge (in their original shipping can and packing materials). Vials are opened one at a time, the solutions are mixed and used, tissues are exposed, left-over supplies are stored, and waste is kept ONLY in the fume hood. In other words, opened supplies of osmium never leave the fume hood (until disposal as toxic waste). If a cold solution is needed, and ice bath can be used. We mix just about enough for each procedure, and the unused left-over OsO₄ working solution is kept in a small glass jar in the hood for the next procedure. So we have no blackened refrigerator surfaces and no OsO₄ fumes accumulating in the fridge (or escaping into the room). It seems to me that this approach could be expanded for a larger lab.

Appendix 2: Osmium Tetroxide: Storage Conditions

Don't take chances, this is the worst there is!

This is a subject that is hotly debated and there is probably no one "best" approach to storage of osmium tetroxide. **It is just about the strongest oxidizer known to mankind.** One should take very seriously the recommended safety precautions for use. And one should take very seriously the recommendations for storage.

We discriminate between osmium tetroxide in the "as received" and unopened ampoules vs. the aqueous stock solution that is mixed by dissolving the contents of an ampoule in water.

As-received ampoules

Although in theory, one could store the as-received ampoules at room temperature, we would recommend refrigerated storage just the same. We would recommend storage in the original SPI-Chem shipping vials and outer white packaging. While it is highly unlikely, with a possibility approaching zero (at least for SPI-Chem ampouled osmium tetroxide), that an ampoule will leak during these conditions, in the event that should happen, it would be immediately obvious as a result of a blackening of the outside of the white shipping box. Should this blackening be observed, the entire box should immediately be removed for further storage and disassembly to a properly operating fume/exhaust hood.

The outside of the refrigerator used for such storage should be clearly marked "No Food or Beverage" and "EM Lab Chemical Storage Only". Be sure to check on your institution's safety policies as to whether a more ordinary household refrigerator could be used for this purpose or whether a special made-for-laboratory-use refrigerator should be used.

Already mixed stock solution, such as 4% aqueous

Typically, the stock solution is made up by taking a 1 gram ampoule, scoring and then snapping it open, and then dropping the entire contents *and ampoule* into the bottom of an Erlandmeyer flask (or equivalent) containing 25 ml de-ionized and distilled water (to make a 4% stock solution). The crystalline osmium tetroxide is quite slow to dissolve in water at room temperature and patience is needed. We would recommend this be interpreted as meaning that an overnight dissolution time might be quite necessary. Before going away and leaving it to dissolve by itself, the following procedure should be followed:

The mixture of partially dissolved osmium tetroxide and water, are poured into some kind of a glass-stoppered reagent bottle (leaving the glass bits and pieces behind in the flask), the top is wrapped with Parafilm® which in turn itself is placed inside of a second bottle with a screw on cap. The larger second bottle should contain something to "cushion" the interior bottle from banging (with the risk of breaking) on the bottom of the outer bottle. While some laboratories have been using cotton for this purpose, we would recommend something that is not prone to lint, thereby becoming a possible source of contamination. Instead we would recommend (SPI-Wipers) lint free cotton wipers.

The lint-free cotton has the advantage of not only being lint free but also in the event of any drippage or spillage, it will act as an excellent absorbent, and since the osmium tetroxide will eventually react with the organic cotton matrix, it will also be reduced to the less hazardous osmium dioxide, OsO_2 . The second bottle top should also be wrapped with Parafilm® in order to provide yet a further seal against leakage into the environment.

We would recommend carrying the safety concern one step further by placing the just described outer glass bottle inside of a third bottle, this one being of opaque plastic construction. Lint free cotton wipers should also be placed on the bottom of this now third and outermost plastic bottle to act as a cushion.

The three container assembly should be left in the hood, at room temperature, for a minimum of twelve hours to dissolve.

And after the passing of twelve hours, and once it is clear that dissolution is complete, the entire three bottle assembly can be placed in refrigerated storage. And the stock solution is ready to use.

The outside of the refrigerator used for such storage should be clearly marked "No Food or Beverage" and "EM Lab Chemical Storage Only". Be sure to check on your institution's safety policies as to whether a more ordinary household refrigerator could be used for this purpose or whether a special made-for-laboratory-use refrigerator should be used.

Osmium tetroxide is very volatile and also reactive, and despite what you believe to have been perfect stoppering of the inner most bottle, you may find that by the next morning, the Parafilm has turned black. This is not all that unusual. You might even find that after some several days, the second bottle's Parafilm is starting to turn black. This too is not at

all unusual. When you switch to the refrigerated storage of the solution, and apply new Parafilm wrapping, you will still find that the Parafilm will eventually start turning black. But since everything is at a lower temperature, this process should be occurring much more slowly.

But by following these instructions, and by replacing the Parafilm wrappings on a regular basis, you should be able to be successful at keeping osmium tetroxide vapors from leaking out into the environment and turning the entire refrigerator black. Now this has not ever happened to us in our own laboratory, but we have heard of it happening to others. So apparently it can happen. Therefore it is important that you have set up, as part of your safety procedures, the constant checking of your stock solution bottle to make sure that nothing is leaking out into the refrigerator or the environment.

Be sure to consult the MSDS sheet for osmium tetroxide for further information about safety precautions and procedures.