Formalin and Paraformaldehyde

INTRODUCTION
Standard operating procedures (SOP) are intended to provide you with general guidance on how to safely work with a specific class of chemical or hazard. This SOP is generic in nature. It addresses the use and handling of substances by hazard class only. In some instances multiple SOPs may be applicable for a specific chemical (i.e., both the SOPs for flammable liquids and carcinogens would apply to benzene).
If compliance with all the requirements of this standard operating procedure is not possible, the principal investigator must develop a written procedure that will be used in its place. This alternate procedure must provide the same level of protection as the SOP it replaces.

FORMALDEHYDE AND PARAFORMALDEHYDE DESCRIPTION
Most commercial formaldehyde is prepared with paraformaldehyde (polymeric formaldehyde) dissolved in distilled/deionized water, with methanol added to stabilize the aqueous formaldehyde. Solution stabilization is important to prevent oxidation to formic acid and the eventual repolymerization to paraformaldehyde, and therefore commercial formaldehyde may contain up to 10% methanol. To avoid using methanol-contaminated formaldehyde for fixation, many protocols recommend making “fresh” formaldehyde from paraformaldehyde immediately before sample fixation.

FORMALIN AND FORMALDEHYDE
The terms “formalin” and “formaldehyde” are often used interchangeably, although the chemical composition of each fixative is different. Formalin is made with formaldehyde but the percentage denotes a different formaldehyde concentration than formaldehyde solutions. For example, 10% neutral-buffered formalin (NBF or simply formalin) is really a 4% formaldehyde solution; the basis for this difference is that historically, formalin was prepared with commercial-grade stock formaldehyde, which is 37 to 40% formaldehyde, by diluting it 1:10 in phosphate buffer.

POTENTIAL HAZARDS
Formalin and paraformaldehyde solutions can emit formaldehyde gas, a known human carcinogen, and can irritate the eyes and skin.
Working with paraformaldehyde powder (and, to a lesser extent, flakes or granules), can expose employees to paraformaldehyde dust, which is a strong irritant/sensitizer.
Contact with these solutions or paraformaldehyde solids may also cause drying of the skin and/or allergic dermatitis.
The OSHA Permissible Exposure Limit for formaldehyde is 0.75 ppm for 8 hours or 2 ppm for 15 minutes. There is a substance-specific OSHA standard for formaldehyde, and an action limit of 0.5 ppm.

EXPOSURE ISSUES
When working with formaldehyde based chemicals be aware of your physical conditions. The eyes, nose, and throat are irritated by formaldehyde vapors at levels as low as about 0.3 part formaldehyde per million parts of air (0.3 part per million, or 0.3 “ppm” — see “Legal Exposure Limits”). This exposure can cause red, teary, burning eyes, sneezing and coughing, and sore throat. Some people have irritant symptoms at these very low exposure levels, while others can tolerate levels as high as a few ppm with little or no reaction.
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Malaise, headache, sleeping disturbances, irritability, and impairment of dexterity, memory, and equilibrium may result from a single, high level, exposure or prolong exposure to formaldehyde.

Previously sensitized individuals can develop severe narrowing of the bronchi at very low concentrations (e.g., 0.3 ppm). Bronchial narrowing may begin immediately or can be delayed for 3 to 4 hours; effects may worsen for up to 20 hours after exposure and can persist for several days. Over time repeated exposures or very high doses can lead to Reactive Airway Dysfunction Syndrome (RADS), a chemically- or irritant-induced type of asthma. In persons who have been previously sensitized, inhalation and skin contact may cause various skin disorders, asthma-like symptoms, anaphylactic reactions and possibly death.

If you begin to experience these symptoms take precautions to limit your exposure or stop using the chemicals.

Engineering Controls
- Work with concentrated (>4% formaldehyde/paraformaldehyde) solutions only in a chemical fume hood.
- Handle paraformaldehyde powder (and, preferably, granules or flakes) only in a chemical fume hood.
- Dilute solutions (<4% formaldehyde) may be used on the bench top in small quantities.
- If there is any possibility that an employee's eyes may be splashed with solutions containing 0.1 percent or greater formaldehyde, an eyewash/drench hose must be available within the immediate work area for emergency use.
- If employees' skin may become splashed with solutions containing 1 percent or greater formaldehyde, for example, because of equipment failure or improper work practices, the OSHA formaldehyde standard requires a conveniently-located safety shower. Contact EH&S at extension 2338 to determine if a safety shower will be needed.

Work Practice Controls
Laboratory-specific written procedures are required for formalin and paraformaldehyde, including a designated work area. Designate an area for working with concentrated formalin, concentrated paraformaldehyde solutions, and paraformaldehyde solid, and label it as such.
- Keep containers closed as much as possible.
- Use in the smallest practical quantities for the experiment being performed.
- If you are weighing paraformaldehyde powder and the balance cannot be located in a fume hood or BSC, tare a container then add powder in the hood and cover before returning to the balance to weigh the powder.
- Labs handling moderate to large quantities of formaldehyde-containing solutions on a regular basis should contact EH&S at extension 2338 for assessment of exposure.
- Areas that handle only small (100 ml or less) pre-filled specimen containers, or that work with formaldehyde-containing solutions exclusively in a functioning chemical fume hood, would have low potential for overexposure, but should contact Risk management if there are concerns.

PERSONAL PROTECTIVE EQUIPMENT (PPE)
The level of skin and eye protection should be selected based on the potential for splashing and other forms of exposure.
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Minimum potential for splash & exposure:
- Chemical splash goggles
- Single pair of nitrile, neoprene, PVC (vinyl), butyl, or Viton™ gloves
- Immediately replace with new gloves when splash occurs.
- Protective clothing (e.g. non-porous lab coat, impervious sleeves; closed-toed impervious shoes)

When using or transferring large quantities:
- Chemical splash goggles
- Face shield (if not working in a fume hood or if hood’s sash is not in the down position)
- Double nitrile, neoprene, PVC (vinyl), butyl, or Viton™ gloves
- Immediately replace with new gloves when splash occurs.
- Chemical resistant apron/smock/lab coat (PE or PVC)
- Avoid using the traditional cotton-polyester white lab coat, which readily collects/absorbs compounds.
- Protective clothing (e.g. non-porous sleeves, closed-toed impervious footwear)

If you develop symptoms of exposure a respirator may be needed when working near these chemicals.

RESPIRATORY PROTECTION
NOTE: Employee Lab personnel intending to use/wear a respirator must be medically fit, trained and fit-tested by EH&S. This is a regulatory requirement. The department will supply the respirator. Students supplying their own respirators should have their doctor go through the respiratory questionnaire with them to confirm they are medical fit to wear a respirator. EH&S can provide them training and fit test them for proper respirator sizing after we receive the OK from their doctor. Students will not be part of the respirator program.


ADDITIONAL PRECAUTIONS
- Reacts violently with nitrogen dioxide, perchloric acid/aniline mixtures and nitromethane.
- Reacts with HCl to form the potent carcinogen, bis-chloromethyl ether.
- Keep away from heat, sparks, and flame.
- Keep separate from oxidizing agents, alkalis, inorganic acid, ammonia, phenol, isocyanates, peracids (non-chlorine bleaching agents such as H2O2), and anhydrides.
- Store containers of formaldehyde in secondary containers in areas separate from the incompatibles.

TRANSPORTATION AND STORAGE
- Transport formaldehyde solutions in secondary containment, preferably a polyethylene or other non-reactive acid/solvent bottle carrier.
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- Keep container in cool, well-ventilated area.
- Keep container tightly closed and sealed until ready for use.
- Store in secondary containment with flammables, away from oxidizers, reducing agents, metals, and acids.
- Keep containers of PFA solid away from water.
- Avoid storing on the floor

Waste Disposal

EXPOSURES/UNINTENDED CONTACT

- Flush exposed eyes or skin with water for at least 15 minutes, and then seek medical attention.
- If there is respiratory irritation associated with exposure, call 911.
- Follow-up medical attention should be sought.

SPILL PROCEDURES

Employees in the area should be prepared to clean up minor spills, including most spills confined to the chemical fume hood. Wearing double nitrile gloves, splash goggles, face shield and lab coat (and impermeable apron, if available); use absorbent pads to absorb spilled material. (For small spills of solid PFA, dampen the absorbent pad with methanol before placing over the spilled material and allowing to sit for a few minutes before wiping up.) Contaminated PPE and clean-up materials must be placed in a clear plastic bag or compatible container for pick-up by Risk Management.

NOTE: If there is respiratory irritation associated with exposure, remove all persons from the contaminated area and call 911.

Chemical Spill

For small spills, 25 ml/25 g or less, follow chemical spill response guidelines above. Don protective clothing, extinguish all ignition sources, and carefully apply vermiculite or other appropriate spill absorbent material to the spill. Place in appropriate containers for disposal. For a large spill, vacate the lab, deny further entry, and call EHS for assistance.

FIRE:

Toxic vapors, including irritating gaseous formaldehyde, may be given off in a fire. In the event of fire, evacuate and bar further entry.

Report all emergencies, suspicious activity, injuries, spills, and fires to the University Campus Police Department by calling 911.

TRAINING OF PERSONNEL

- All laboratory personnel are required to attend a general laboratory safety session that includes an introduction to general chemical safety.
- Training on lab- specific procedures is required all personnel working with these materials, and must be documented (topics covered, date, employee names and signatures).
- Furthermore, all employees working with formalin or paraformaldehyde solutions or PFA powder are required to take WISHA-required Formaldehyde training. Contact EH&S for training.
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Formaldehyde, Glutaraldehyde and Other Chemical Fixatives

**Formaldehyde**

The most widely used chemical fixative is formaldehyde, which shows broad specificity for most cellular targets. The water-soluble, colorless, toxic, and pungent gas reacts with primary amines on proteins and nucleic acids to form partially-reversible methylene bridges.

**Glutaraldehyde**

Glutaraldehyde is a dialdehyde that reacts with amino groups, sulfhydryl groups and possibly with aromatic ring structures. Fixatives containing glutaraldehyde are stronger protein crosslinkers than formaldehyde. They penetrate tissue more slowly, causing extraction of soluble antigens and modification of the structure. Tissues that have been fixed with a glutaraldehyde-based fixative must be treated with inert amine-containing molecules prior to the immunoassay because free, unsaturated aldehyde groups are available to covalently link amine-containing moieties such as antibodies. The most efficient aldehyde blockers are ethanolamine and lysine.

**Other Fixatives**

**Mercuric chloride-based fixatives** are used as an alternative to formaldehyde-based fixatives to overcome poor cytological preservation. These fixatives work by additive and coagulative properties. The major advantages of using these fixatives include good penetration resulting in more intense immunostaining and the preservation of cytological detail allowing for easier morphological interpretation. These fixatives often contain neutral salt to maintain tonicity and can be mixed with other fixatives to provide a balanced solution. Mercuric chloride-based fixatives include Helly and Zenker's Solution. Sections must be cleared of mercury deposits before immunostaining.

**Precipitating fixatives** include ethanol, methanol and acetone. They precipitate large protein molecules and are good for cytological preservation. These fixation reagents can also permeabilize cells, which may be critical depending on the sample. They are not good for electron microscopy, though, because they cause tissue shrinkage.

**Diimidoester fixation** using dimethyl suberimidate (DMS), an amine-reactive crosslinker, is an alternative to aldehyde-based fixation (Hassel, J. et al., 1974). DMS is a bifunctional reagent with the α and ε-amino groups of proteins. Diimidoesters are unique in that they carry an amido group next to the functional groups on the molecules. As a result, DMS does not affect the net charge of the protein. The advantages of using DMS as a fixative for both light and electron microscopy include retention of immunoreactivity of the antigen and the lack of aldehyde groups that require blocking.

FOR SPILL INFORMATION SEE
EWU EH&S Hazardous Chemical Spill Cleanup Guidelines

FOR WASTE DISPOSAL INFORMATION SEE
EWU EH&S Guidance; Hazardous Waste Management Program
EWU EH&S Guidance; Hazardous Waste Satellite Accumulation Manager Responsibilities
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RELATED DOCUMENTS:
   EWU EH&S SOP Carcinogens
   EWU EH&S Guidance; Disposal of Laboratory Containers
   EWU EH&S Guidance; Disposal of Laboratory Glassware
   EWU EH&S Procedure; Chemical Hazard Communication Program
   EWU EH&S Guidance: Standard Operating Procedures for Fume Hoods
   Specific department chemical hygiene plan and building emergency contingency plans

WASHINGTON STATE REGULATIONS
   WAC 296-856 Safety Standards for Formaldehyde