



Chemical  
And  
Radiation  
Protection

Lab Safety

Spectrum

August 2003

Volume 3 Issue 3

UW - Madison Safety Department Chemical and Radiation Protection  
30 N. Murray St. 262-8769 <http://www.fpm.wisc.edu/safety>

Help Line 265-5518

### Shipping Hazardous Materials

DOT requires **all** persons involved in shipping or receiving hazardous materials (i.e., candy-stripped form) be trained and certified every three years in proper handling of these materials. The Chemical & Radiation Protection Office provides the training and certification needed for shipping and receiving of hazardous materials. Topics include: how to recognize hazardous materials, safe receipt and handling of hazardous materials, preventing and preparing for a hazardous material spill and preparing hazardous materials for transport.

*All training sessions to be held at Union South from 8 AM to 12 Noon. Pre-registration is required. Call Jeff at 265-9080 to register.*

Class dates are: August 27, September 23, October 22, November 12, December 17.

The Office of Biological Safety (OBS) provides training and certification for shipping infectious substances and other biological materials with an emphasis on laboratories and research groups. Among the topics covered are: Regulatory Definitions of Infectious Substance, Diagnostic Specimen, and Biologic Product; use of the Hazardous Materials Table; requirements for shipping biologicals with dry ice or liquid nitrogen and correct shipping documentation.

Attendees who complete the quiz will receive a certificate showing compliance with the DOT training requirement. The next class will be offered October 2003. *Pre-registration is required. Contact Margy Lambert to register ( 263-9013 or [mlambert@fpm.wisc.edu](mailto:mlambert@fpm.wisc.edu)).*

### Radiation Regulation Changes

Wisconsin became an Agreement State on 11 August. That means that Wisconsin agrees to regulate byproduct (i.e., NRC) radioactive material as stringently as the NRC. Also, the UW will be regulated and inspected by the State Radiation Protection Section instead of the NRC. There will be several changes because of this.

The NRC form 3 will be replaced by the blue-colored **Notice to Employees**. Because we will be an Agreement State, the Radiation Protection Branch is your first point of contact.

Wisconsin revised all of its radiation protection regulations. That means they are regulating byproduct (i.e., NRC) material, accelerator produced material and ionizing radiation produced by machines (e.g., x-rays, accelerators, etc.). Prior to this the State only licensed these machine producing sources. To keep abreast of these revised rules, the Safety Department will begin a program of "authorizing" machine produced sources.



## Useful Facts About Common Fixatives\*

Many biological research laboratories on the University of Wisconsin –Madison campus use some kind of animal and/or cell culture model. Frequently, the researcher needs to obtain final data through fixing and staining organs, tissues or cells. Indeed, sometimes the most significant data is best represented through photographic images of fixed and stained cells. The fixatives and stains used are numerous and frequently present hazards of some kind. This article will present some information regarding commonly used fixatives and the hazards associated with them. Always use chemical fixatives in a certified fume hood. For a more complete discussion of chemical hazards, please refer to Chapter 2, Chemical Hazards in Laboratories and Chapter 7, Chemical Disposal Procedures in the UW Chemical Safety and Disposal Guide. See the summary table of toxicity information at the end of this issue.

The objective of fixation is to preserve cells and tissue constituents in as close to a life-like state as possible and to allow them to undergo further preparative procedures without change. Fixation should also provide for the preservation of tissue substances and proteins. No single substance or known combination of substances has the ability to preserve every tissue component. The selection of an appropriate fixative is usually based on considerations such as the structures and entities to be demonstrated.

**Formaldehyde**, as 4% buffered Formaldehyde (10% buffered Formalin), is the most widely employed universal fixative, particularly for routine paraffin embedded sections. Formaldehyde is a gas with a very pungent odor, soluble in water to a maximum extent of 40% by weight and is sold as such under the name of Formalin. Formaldehyde is also obtainable in a stable solid form composed of high molecular weight polymers known as **Paraformaldehyde** (frequently used on our campus to decontaminate equipment such as biosafety cabinets) which, when heated generates pure gaseous Formaldehyde. Formalin is thought to form cross-links between proteins preferentially with the basic amino acid lysine.

A concentrated solution of Formalin will become cloudy with storage due to the slow production of Paraformaldehyde (decreasing the strength of the fixative solution), but may be filtered and used. In storage, Formalin may also oxidize to Formic acid. *Cosmetics often contain formaldehyde or a formaldehyde-releasing substance. The formaldehyde acts as a preservative. In the United States many cosmetics contain Quaternium 15, which is a formaldehyde-releasing agent. Quaternium 15 and other formaldehyde-releasing materials are found in shampoos, conditioners, deodorant soap, hairspray, styling mousse, fluoride toothpastes, mouthwash, mascara, talcum powders, haircolors, makeup foundations, firming facial creams and fingernail polish. Even "Natural" cosmetics such as those marketed by Garden Botanika™ contain diazolidinyl urea, which is another formaldehyde-releasing preservative.*

**Glutaraldehyde**, like Formaldehyde, acts through the formation of cross-links between proteins and is also used extensively as a fixative. It does cause a loss of up to 30% of the alpha helix structure of protein, depending on the type of protein. An aqueous solution of Glutaraldehyde yields a complex mixture of free aldehyde, monohydrate, dihydrate and hemiacetal. When solutions are kept for long periods, there is a tendency for precipitates to form and for aldehyde levels to fall so that some method of purification may be required.

There are many "recipes" for the preparation of this fixative, including varying the percentage of Glutaraldehyde, additives and buffers. Other uses for Glutaraldehyde include the preparation of tissue xenografts, particularly cardiac valves. *From the early 1970s, glutaraldehyde has been used to treat skin disorders eg (warts, herpes simplex, and hyperhidrosis - excessive sweating of the hands and feet), and used as an adhesive in dentistry. It was also used as a fixative component in the manufacture of tissue transplants (from the late '80s), in washable wallpaper, sausage casings, cork gaskets, wet strength paper/cardboard (including food packaging) and paper towels, and in textile sizing mixtures made from PVA to waterproof materials.*



**Acrolein** (acrylic aldehyde) is mainly used in the tanning industry, but it has been employed as a fixative for enzyme cytochemistry as labile enzymes like glucose-6-phosphatase are retained in tissue fixed in 4% Acrolein. *Back in 1914, Thomas A. Edison wrote to Henry Ford regarding the dangers of cigarette smoking, specifically the brain impairment caused by acrolein, a constituent of tobacco smoke. Edison claimed he would not hire anyone who smoked. In World War I, Acrolein was used as a tear gas under the name Papite.* (<http://medicolegal.tripod.com/edison1914.htm>)

**Osmium Tetroxide** is the most commonly used metal compound in secondary fixation for electron microscopy. It is known to form cross-links with proteins and also reacts with, stains and stabilizes lipids. It is used for preservation of fine structures. *Osmic Acid (Osmium Tetroxide), first used in 1951, is still used for alleviation of pain and swelling associated with arthritis. It is injected into the joint (Rheum.2003;42:1-28).*

**Methyl and Ethyl Alcohol** are the only alcohols that have a role as fixatives. These alcohols alter the tertiary structure of proteins due to disruption of hydrophobic bonds. Absolute ethanol preserves glycogen, although it can cause distortion of nuclear detail and shrinkage of cytoplasm. If fixation is prolonged, the alcohols remove histones from the nuclei and later extract RNA and DNA. *Methanol may cause inebriation but by itself is almost completely non-toxic. The methanol is metabolized by alcohol dehydrogenase to formaldehyde and then to formic acid. Clinical findings correlate better with formic acid levels than with methanol levels. It is these two metabolites that cause toxicity with formic acid being more responsible. It is the formic acid that causes the profound metabolic acidosis and blindness that is typical of methanol poisoning. The overall mortality of methanol poisoning is approximately 20% and among survivors the rate of permanent visual impairment is 20-25%.*

**Picric Acid**, when used in combination with other ingredients, leaves tissue soft and penetrates well, precipitating proteins. It will continue to react with the tissue structures unless the specimen is thoroughly washed following fixation.

*Acids have long been used as homeopathic remedies for different ailments. Picric Acid has been suggested for the treatment of "the brain fog of businessmen who become depressed and wearied from slight fatigue. The great characteristic is that slight exertion brings on exhaustion and headache, incapacitating for work, and extinguishes that quality which we call grit. Sexual irritability may be a prominent symptom."* (<http://www.drvaishnav.com>)

**Microwave (MW) irradiation** is a form of non-ionizing radiation, which exposes dipolar molecules such as water, and polar side chains of proteins to rapidly alternating electromagnetic fields. This induces instantaneous heat which is proportional to the energy flux. This form of heat production can be regulated sufficiently well to produce fixation of a 2-3mm thick sample when immersed in saline and irradiated to an internal temperature of 50-68 °C. MW fixation does not have any deleterious effect on special stains. It has also been shown that tissue antigens are often better preserved in MW irradiated tissue than those fixed routinely in 10% Formalin and processed in the usual manner. The irradiation of experimental brain tissue, previously perfused with physiologic saline has produced excellent tissue sections without the morphological changes that result from autolysis and dehydration and impregnation for routine paraffin processing. MW irradiation can also be used to accelerate the fixing action of aldehydes or alcohols.

**\* Extracts from Woods and Ellis, Laboratory Histopathology, 1994**



### Radiation / Chemical Safety Training

Chemical and radiation safety training is available weekly. There are two types of classes. Chem AM classes have the chemical safety class beginning at 9:30 AM and the radiation safety class begins at 12:30 PM. Rad AM classes have the radiation safety classes beginning at 8:30 AM and the chemical safety class beginning at 1 PM.

All training classes are held at Union South. No sign up is needed; a quiz is used to document training. Booklets for either class can be picked up at our Annex, room 62, Biochemistry (11 AM - 2 PM, daily). A complete listing of classes is found at: <http://www.fpm.wisc.edu/safety>.

### Radiation and Chemical Safety Training Schedule

<b>Chemical AM</b>	<b>Start Time</b>	August 21; September 2, 10, 18, 24; October 2, 8, 14, 20; November 5, 13, 19; December 3, 11, 17
Chemical Safety	9:30 AM	
Radiation Safety	12:30 PM	
<b>Radiation AM</b>	<b>Start Time</b>	August 29; September 5, October 28; November 25
Radiation Safety	8:30 AM	
Chemical Safety	1 PM	

### Basic Biosafety Training Schedule

The Basic Biosafety Class provides an introduction to the following topics: roles and responsibilities of researchers, Institutional Biosafety Committee, etc., risk assessment fundamentals, good laboratory practices and precautions for Biosafety level 1 and 2, types of containment equipment, proper disposal of biological materials, including sharps and infectious agents, The biosafety protocol review process and Emergency preparedness. The class is intended primarily for students and staff who are new to this institution and/or new to working in a laboratory. Everyone is welcome to attend. The next class is scheduled for September 17 at Union South from 1:30 PM to 3:30 PM. *Pre-registration is required. Contact Margy Lambert to register ( 263-9013 or mlambert@fpm.wisc.edu).*

**UW-Safety Dept.**  
**30 N. Murray St. 53715-1227**

**(608) 262-8769**

**Help Line: (608) 265-5518**