

Uranium Is The Issue

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Uranium compounds, especially uranyl acetate, have been widely and routinely used as transmission electron microscopy contrast stains for biological materials since 1958^{1,2}. Those of us who do TEM of biologicals use small quantities of uranyl acetate, nitrate, formate, sulfate and perhaps other uranium compounds almost daily and therefore keep inventories of these salts and their solutions.

In the 1980's growing concerns about medical and research wastes entering regional dump sites prompted state radiation officials in Oregon to begin tightening the regulations for monitoring and controlling all radioactive substances including the uranium compounds commonly used in processing biological specimens for TEM. Oregon has come to be a state with high levels of awareness about environmental and safety issues and is often at the forefront of regulatory and management trends in these areas. What happens in Oregon may therefore frequently serve as both a harbinger of changing attitudes and a model for standards which are evolving elsewhere. Messages seen in electronic news groups and conversations with colleagues suggest that other states may have also addressed, or may plan to address, the purchase, storage, distribution, use, and disposal of uranium compounds used for staining biological preparations for TEM.

Radiation-producing machines and radioisotopes used or stored at locations under State of Oregon jurisdiction are subject to provisions of the Oregon Rules for Control of Radiation, set forth by the Radiation Protection Services, Health Division, Oregon State Department of Human Resources. In addition, radioactive material transport, storage, and disposal must comply with rules issued by the Oregon Department of Energy, Oregon Department of Environmental Quality, and applicable federal agencies such as the U.S.

Nuclear Regulatory Commission and U.S. Environmental Protection Agency. Regulations covering naturally occurring radioactive materials (NORM), a class of materials which includes the uranium compounds used as stains in EM, have been in place in Oregon for several years³. When these regulations were established, Oregon State University (OSU) undertook regulatory investigations to determine if use of small quantities of NORM could, or should, fall within the guidelines for exemptions allowed under these regulations. In 1992 the investigations were completed and a process to include uranium compounds used as stains for electron microscopy in the state's radiation regulatory and safety program was begun. Hence, it was at this time that our use of uranium based compounds was first called into question. There are many isotopes of uranium. Depending on what mix of these one may be dealing with, various levels of alpha, beta, and gamma radiation can result from their radioactive decay.

On the OSU campus, the radiation safety program is managed by a radiation safety officer responsible to University administrators and a University Radiation Safety Committee. The radiation safety officer supervises radiation safety training programs, use, storage, disposal and licensing matters, and coordinates functions of a faculty committee which establishes local policy and reviews safety and compliance matters.

In compliance with radiation safety regulations our facility periodically has its electron microscopes surveyed for potentially dangerous escaping x-radiation. However, prior to 1992 neither we nor our radiation safety officials felt that the small amount (less than 25 grams with activity less than 10 microcuries) of uranium compounds on hand in the facility, and their limited methods of use, posed a sufficient hazard to warrant regulatory action.

Our campus radiation safety personnel have always been understanding, knowledgeable, and non-adversarial in carrying out their responsibilities, but as the state mandates for stricter control were imposed, neither we nor our radiation safety officer were sure how best to proceed with minimal adverse impact toward compliance. The EM Facility at OSU is a service laboratory: several dozen students, technicians, and faculty use the facility and its supplies, and uranium-stained embedments and grids are typically dispersed into the possession of these clients. If materials that contained uranium were used by large numbers of people or removed from the "authorized" facility, could we allow this and still comply with the spirit and intent of the stricter regulations? The concerns went beyond possible health effects from radioactivity to include possible health effects related to more conventional chemical toxicity issues and the possibilities for contamination effects where inadvertent dispersal might result in inaccuracies in low level radiation monitoring activities.

We began by discussing and arriving at a mutual understanding of the regulatory needs and concerns and the complications that altered protocols and compliance. Management would entail on both the EM facility and the radiation safety program. We next undertook to quantify the hazard potential. Our small stock of uranium compounds in powder form were identified, inventoried, weighed and surveyed for radiation levels within their containers (bottles) and exterior to the sealed containers. Two dilute uranyl acetate solutions were likewise identified, inventoried and quantified. In addition, we processed an assortment of plant, animal, and microbial specimens through standard protocols and submitted for analysis samples of the tissues, pellets, and the used and unused processing solutions before and after uranyl compound staining. We also provided samples of the plastic embedded tissues and pellets as well as sections, on copper TEM grids, cut from these embedments.

One interesting complication in quantifying the hazard arose early in the process. Every other campus user of radioisotopes was using these materials as tracers. These users were concerned with specific activity. As a consequence their inventories were quantified and managed in protocols in terms of microcuries. For electron microscopy, we were concerned only with the electron scattering potential of the uranium atoms. We quantified our inventories and formulated our solutions on the basis of weights and volumes (grams, milliliters). A calculation of 0.33 microcurie/gram of ²³⁸U was made to convert uranium compound supplies from weight to specific activity units for inclusion under the hazard as-

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assessment required by the stricter regulations.

Radiation levels (millirem/hr) were measured from opened and closed containers of uranium compound powders and solutions. Specific activity values associated with these measurements were then calculated from the specific activity of depleted uranium and the weight percent of uranium in the compounds. Working strength uranyl acetate solutions, nominally 1% w/v, gave specific activity measurements below 1×10^{-3} microcurie.

A variation of neutron activation analysis, gamma-ray spectroscopy using a germanium/lithium (GeLi) detector, was required to detect uranium in processed tissues and pellets, where uranium levels in the 0.8 – 0.9 ppm range were reported. To assure a high level of accuracy, the analysis equipment was calibrated a number of times, and the results from samples submitted in December, 1993, were not obtained until late in May, 1994. The presence of uranium in stained materials on grids was below the detection limit of the analytical equipment and procedures used.

The final result of the quantitative and regulatory processes produced the development and implementation of a policy by which the Radiation Safety Office and EM Facility agreed to cooperate to enforce defined procedures. These are the central features of this policy:

Persons using uranium compounds in the EM facility take a four hour radiation safety training class from our campus radiation safety officer. They then receive authorization to use uranium compounds as stains in our facility. Individuals who want to purchase and maintain their own stocks of uranium-based staining compounds at other locations must obtain a radiation use authorization (RUA) permit for those locations from the campus Radiation Safety Office.

After authorization is granted, users may obtain and manage their own stocks of uranium compounds or may use uranium stain solutions available in the EM facility. Uranium salts and stain solutions provided for use by and in the EM facility may not be taken to other locations.

Persons using uranium-based stains in the facility are personally responsible for complying with all requirements for use, clean-up, in-lab disposal, and radiation monitoring. The EM facility provides radiation safety and monitoring, clean-up, and disposal materials for client use.

The EM facility manager maintains and documents the status of the uranium compound and radiation safety materials inventory, monitors for indications of radiation contamination or unsafe procedures, and attends to the safe and timely disposal of accumulating radioactive wastes.

Specimens which have been stained with any uranium compound(s) for EM and subsequently either solvent-rinsed, plastic embedded, or put on grids may be removed from the EM facility for archiving or further processing at other locations as long as uranium concentration is less than 0.05 microcurie/gram.

Our policy took effect 01 January 1994. Despite a small burden of additional managerial tasks and the minor inconveniences of more record keeping chores, the policy has to date worked well.

Two additional points should be made. Authorization of NORM use in Oregon, and presumably elsewhere, is rather complex. Under Oregon regulations there are still some exemptions allowed for specific NORM uses, materials, concentrations and amounts. Users of uranium compounds should investigate their specific situation, giving consideration to ALL uses of NORM at their organizations, not only the use of uranium for EM stains.

Finally, it should be noted that a particular problem is associated with the disposal of uranyl nitrate waste. This compound is a radioactive substance, a nitrate, and an oxidizer, and therefore is classified as a mixed radioactive and chemical hazard.

1. Watson, M.L. (1958). J. Biophys. Biochem. Cytol. 4, 475.
2. Swift, H., Rasch, E. (1958). Sci. Inst. News 3, 1.
3. Radiation Safety Manual, Oregon State University.

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