INTERNAL SAFETY RULES

Sample staining using uranium solutions for electron imaging

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1 Application

This document applies to activities related to electron imaging related activities using heavy radioactive ions as staining agents. Use of uranium, uranium powders and salts for other purpose is not covered by this document.

1.1 Acronyms

- SRPO: School Radioprotection Officer from the VPO-SE OHS.
- FRPO: Faculty Radioprotection Officer.
- RPR: Radiation protection referent.
- SBSO: School Biosafety officer

2 General information

Uranium-based staining uses many forms of uranium salts, both anhydrous and hydrous. The most common anhydrous salts are:

- Uranyl acetate $UO_2(CH_3COO)_2$, CAS 541-09-3.
- Uranyl nitrate $UO_2(NO_3)_2$, CAS 10102-06-4.
- Uranyl formate $UO_2(HCOOH)_2$, CAS 16984-59-1.

Due to the presence of the heavy uranium ion (mainly 99.9% of U-238), these salts expose humans as well as the environment to chemical and radioactive hazards (see Figure 1 and Figure 2).

Figure 1 The three GHS hazard pictograms and the symbol for ionizing radiation required on any container of uranyl salts in powder form.

Once diluted in water or other solvents, the following chemicals are usually referred as “uranium staining solutions” or “stock solutions”. Their dilution limits their acute toxicity, but the chronic toxicity and radioactivity hazards remain.

Figure 2 The two GHS hazard pictograms and the symbol for ionizing radiation required on a container of 2% uranyl stock solutions.

2.1 Purpose

Uranyl acetate is extensively used as a negative stain in electron microscopy, mostly related to biological samples due to their low content in metal ions. It is highly water soluble and once prepared in stock solution, it has a shelf life of more than a month when stored in a fridge.

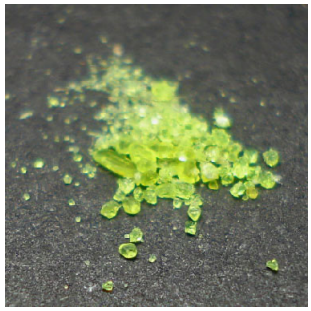

Figure 3 Uranyl acetate crystals.

Uranyl formate on the other hand, does not easily dissolve in solution and once dissolved, has a rather limited lifetime, typically a few days. It is also sensitive to light, especially ultraviolet light, and will precipitate if exposed to it.

2.2 Health

The Swiss exposure limit value (8 hours, VME) for uranium compounds is 0.2 mg/m^3. Altogether, the damage potential to the kidneys is the major effect involved in chronic exposures to uranium salts, independent of the route of exposure [1].
Uranium salts are considered radioactive compounds (given that they contain uranium), but its decay takes place by mainly alpha particle release (even if a less intense gamma ray and X-ray emission is present) which are easily stopped (e.g., by the skin). Therefore, the risk of exposure to radiation is low, unless there is an internal direct contact, for example due to inhalation or ingestion.
Uranium salts are not currently classified as carcinogenic or reprotoxic. However, there are animal studies that indicate that subcutaneous exposures could lead to such effects.
The main potential routes of exposure for uranyl acetate or uranyl nitrate dihydrate in the industrial/occupational context are mainly via the respiratory tract, but also via the digestive system or the skin. The kinetic behavior and the absorption of uranium compounds in the respiratory tract are essentially determined by their solubility and their particle size. Absorption of the particles deposited in the bronchial tubes and the bronchioles can be restricted since they are eliminated relatively rapidly to the digestive tract via mucociliary clearance. Smaller particles that reach the alveoli and are retained there must be expected to be rapidly and completely absorbed in the case of easily soluble compounds like uranyl acetate. Since in the case

of uranium compounds the share of particles capable of penetrating the alveoli is generally very low, because of the substance's high specific weight, absorption of easily soluble uranyl salts in the respiratory tract is also expected to be altogether low. About 5% of it remains in the lungs in the long term [2].

2.3 Regulations

Uranium compound manipulation is regulated by law and it should happen in controlled area, like C-laboratories [3] [2] [4]. It is allowed to work with uranium salts in a normal chemistry laboratory if the uranium activity is not higher than 900 Bq (Becquerel, disintegration per second) of uranium-related materials. This limit is called the authorization limit (LA) and applies for each authorization holder (usually a unit with a certified radioprotection expert, see [5]). The specific activity of pure depleted uranium (99.3% 238-U) is 1.887×10^4 Bq/g. The specific activity of the salt can then be calculated from their individual molecular weight:

- Uranyl acetate (dihydrate): 10.4 kBq/g,
- Uranyl formate (hydrate): 11.9 kBq/g,
- Uranyl nitrate: 11.4 kBq/g.

The maximal amount of raw salt authorized without an authorization [5] is (rounded to fifty):

- Uranyl acetate: 85 mg,
- Uranyl formate: 75 mg,
- Uranyl nitrate: 80 mg.

Suppliers do not sell powders in such small quantities. For these reasons, a valid authorization from the authorities and the SRPO is required to buy pure salts.

When diluted, the maximum amount of liquid stock solution for each unit allowed outside a C-laboratory is

Dilution (%)	0.5	1.0	1.5	2.0	4.0
Volume (ml)	15	8	5	4	2

Table 1 Maximum volume of stock UA solution stored and used outside C-laboratories for different dilutions and per research unit.

The clearance limit (LL) states the limit at which the risk for the human as well as the environment is sufficiently low to treat the waste as non-radioactive. For U-238, this limit is set at 1 Bq/g. An equivalent criterion for disposal as non-radioactive waste is that the absolute activity does not exceed the kgLL, meaning the absolute activity of 1 kg of material whose specific activity corresponds to the LL. This absolute activity is 1 kBq for Uranium.
Once the specific activity or absolute activity has been estimated, the waste can be disposed of if and only if the measured dose rate at 10 cm from the entire surface of the waste does not exceed 0.1 µSv/h after subtracting the background noise and if no contamination is present externally.

3 Operational rules

3.1 Registration in the EPFL radiation protection database

Before starting any activity with uranium salts solutions, all users must register in the EPFL radiation protection database. In the registration form, the isotope U-238 must be indicated in the dedicated section, and the box labeled "working with uranium salts for staining" must be checked. You will then need to follow the instruction provided by the system.

3.2 Uranium salts

3.2.1 Stocks

The possession of uranyl acetate, uranyl formate or uranyl nitrate salts for electron-imaging staining purpose is only allowed for the following units:

- SV PTECH PTBIOEM.
- SV SV-IN.

Other units are only allowed to possess these compounds diluted up to the limits shown in Table 1. In specific cases, the possession of small amounts of pure salts (below the limit mentioned in section 2.3) can be allowed if duly justified and validated by the SRPO.

3.2.2 Purchases

Purchase of uranium salts and staining solutions is limited following section 3.2.1 and must be reported in the EPFL radiation protection database. This task is either done by each unit's RPR or by the FRPO with his/her accord.

3.2.3 Storage

Storage of uranium salts is prohibited outside C-laboratories. The salts must be stored in their original container inside a ventilated cabinet and according to the safety data sheet (SDS).

3.2.4 Safeguard

The purchase of uranium salt must be reported within 15 days upon receipt to the Swiss Federal Office of Energy (OFEN in French).
The allowed producers of the diluted solutions keep a record of the preparation of the products. This register contains at least the date of production, the quantity of the prepared product and its dilution as well as the purchasing groups.

3.3 Stock solutions

3.3.1 Purchase

It is allowed to purchase stock solutions diluted up to the limits shown in Table 1. Any purchase can be don only through the EPFL radiation protection database, as described in the tutorial "Uranium salts for staining: buy and return".

3.3.2 Preparation of the stock solution

Preparation of stock solutions is limited following section 3.2.1. In specific cases, the preparation of stock solution by other groups than the ones listed in section 3.2.1. can be allowed if duly justified and validated by the SRPO. In such case the shared C-laboratory AI 0229 is the only lab allowed to be used for the preparation of the stock solution.
It is strongly encouraged to limit the preparation of the stock solution for one or two weeks of usage, thus reducing as much as possible the quantity of the stock solution outside C-laboratories.
All work implying the use of uranium precursor powders such as the preparation of the stock solutions must be performed inside a fume hood. The working area in the fume hood must be dedicated to the preparation of the stock solutions and clearly marked using radioactive material warning tape as show in Figure a. Any material or device used in the preparation procedure which is not used for handling the radioactive products may be used outside the fume hood (e.g., hot plate for warming up the dilution water).

Avoid any contamination of the outside of the stock solution bottle. Any bottle, flask or tube containing the stock solution must be labelled according to the standard good chemistry practices (see this webpage for chemical storage) and have an ionizing radiation warning symbol as well (e.g. as shown in Figure 4).
Examples of procedures for the preparation of the staining solution and the staining process itself are shown in the annex.

3.3.3 Storage of the staining solution

Stock solutions must be stored in retention trays inside fridges or ventilated cupboards. Below 100 µl volume, only the container and the container holder need to have the radioactive warning symbol. Above this limit, both radioactive and toxic warning symbols must be applied on the door of the ventilated cabinet as well (see Figure 4).

Figure 4 Ionizing radiation and toxic materials hazard symbols, ISO 7010.

3.4 Laboratory practices

General good laboratory practices should always be followed when manipulating uranium compounds, including wearing appropriate PPE (lab coat, safety glasses, gloves), avoiding the use of sharps, following good hand hygiene, performing frequent cleaning of the workplaces (established periodicity by the unit).

3.5 Waste management

The clearance limit for uranium is 1 kBq. Waste resulting from staining activities generally has a low level of activity. At EPFL, a monitoring system has been established in order to dispose of those wastes before the clearance limit is reached. The SRPO informs users and FRPOs when they are authorized to dispose of waste, based on the activity of their materials and the total amount of radioactive waste already disposed of at the school level.
Users are responsible for providing accurate information about their waste by completing the appropriate form related to a specific waste container in the laboratory.
A decision tree for the management of waste from uranium staining activity is available at the following link.
Examples of containers available in EPFL shops are shown in Figure 5.

Figure 5 Polyethylene (HDPE) containers with extra lid ;

3.5.1 Case of BSL 1 grids preparation

In laboratories where grids with BSL1 samples are prepared, the grids can be disposed of in the same container used for other items contaminated with uranium salts.
A log sheet associated with the container is available in the laboratory and must be filled out each time a manipulation is completed.
Each row of the sheet corresponds to the amount of waste produced during the manipulation and must include the following fields, in order:

- First and last name;
- Research group;
- Uranium salt used (UA or UF);
- Percentage of uranium salt in the solution (%);
- Volume (in mL) of solution that contaminated the discarded waste;
- Number of grids produced;
- Number of grids disposed;
- Date of the manipulation.

Once the disposal of the container has been requested by the SRPO, the FRPO or the RPR will disposed the container with the OMoD code 15 02 02.

3.5.2 Case of BSL 2 grids preparation

As for the BSL 1 grid case, a log sheet associated with the container is available in the laboratory and must be filled out each time a manipulation is completed.

Once the disposal of the container has been requested by the SRPO, the small container has to be placed in the non autoclavable BSL 2 container (blue container). Each blue container can contain only one small container with uranium salt contaminated wastes. The blu container has to be disposed of with the OMoD code 18 01 03.

3.5.3 OMoD label complementary information

Before the elimination, all radioactive pictograms must be removes from the waste and the only standard waste label containing the OMoD code must appear.
This label, along with other information about the contents, must include the following statement:

" Ce déchet contient de la contamination de solution de sels d'uranium et peut être éliminé comme non-radioactif conformément à l'Article 111 de l'Ordonnance sur la Radioprotection."

A pre-printed version of the label containing this sentence can be asked to the FRPO.

3.5.4 Leftover solution

If any of the initial solution remains, it must be returned to the PTBIOEM platform.
The return of the solution must be announced through the EPFL radiation protection database, as explained in the tutorial "Uranium salts for staining: buy and return".

3.6 Monitoring

3.6.1 Dosimetry

The ambient air monitoring at the workplace is not mandatory given the relative low usage of the uranium salts by the groups (≤ 200LA/year). The contamination limit (CA) is set at 1 Bq/m^3.

Incorporation measurements are solely done in case of incorporation following accident at the workplace. The measurement is done by liquid scintillation counting of a urine sample, in Bq/l [2].

3.6.2 Control

Surface contamination checks must be done after each preparation of the stock solutions using a portable alpha/beta contamination monitor (see Figure 6). The monitor must be calibrated for U-238. The contamination threshold (CS) is set at 1 Bq/cm^2. This value is converted in count per second (CPS) and is given by the mandatory calibration of each device.

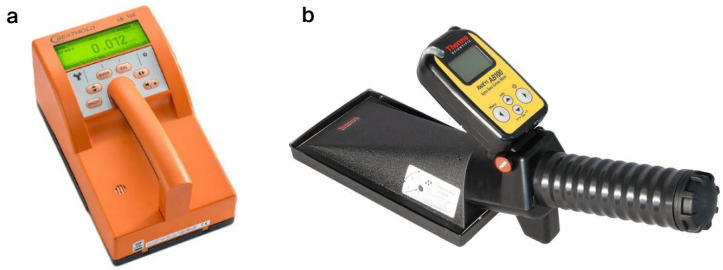

Figure 6 Portable contamination monitor for alpha and beta/gamma measurement **(a)** Berthold LB124 SCINT. **(b)** Thermo Fisher RadEye™ AB100.

Periodic tests of the workplace contamination are performed by the faculty radioprotection expert as well as by the EPFL radioprotection officers. The contamination can either be verified using a surface counter or by means of wipe tests (liquid scintillation counting).

3.7 Workplace

3.7.1 Labelling

Any material and equipment used for uranyl related activities needs to be labelled with a radioactive material warning tape (Figure 7a) or, for small objects or vials, with an ionizing radiation warning symbol (Figure 7b). This equipment must not be used for other activities. If necessary, only after a verification by a radiation protection expert the equipment can be reassigned to other tasks.

Figure 7 Radioactive warning tape. **(a)** With text and **(b)** with only the radioactive symbol.

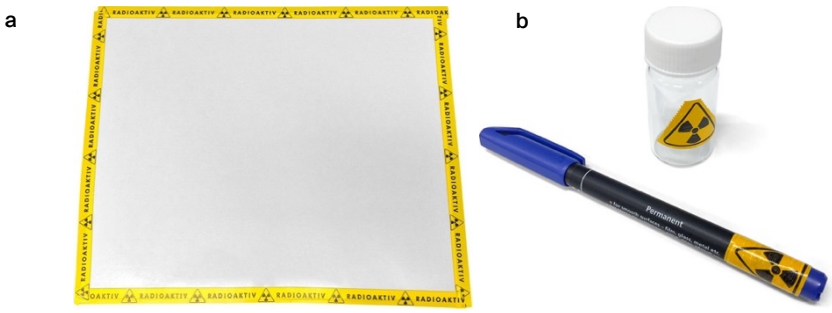

Figure 8 Labelling the workplace and equipment. **(a)** The workplace and protective foils are clearly marked using the radioactive warning tape. **(b)** Small equipment is clearly labeled and must be kept at the workplace.

To protect the benches from spills and contamination, absorbent paper with a plastic bottom layer must be used to cover the workplace during the whole activity (see the annex for few references). An example is shown in Figure 8. This absorbent paper exists both as a roll or as individual foils and the protective surface must be changed at least once per month or immediately in case of a spill.

3.8 Spillage and emergency

In case of spillage, use a moistened tissue. Discard the tissue into the designated plastic container for solid waste. Monitor hands first, then yourself and the working area with a surface contamination counter (see Figure 6). In case of a large spill (more than 5 ml) call the emergency service of EPFL (115 using a local phone, or 021 693 30 00 for any external line or cellphone).

In case of incorporation (inhalation or ingestion) or suspected or confirmed external contamination, immediately call the emergency number.

4 References

[1] Institute for Occupational Safety and Health of the Germand Social Accident Insurance, "GESTIS Substance database," 2020. [Online].

[2] Département fédéral de l'intérieur (DFI), *Ordonnance sur la dosimétrie (ODosim),* 2018.

[3] Conseil fédéral suisse, *Ordonnance sur la radioprotection (ORaP),* 2019.

[4] Département fédéral de l'intérieur suisse (DFI), *Ordonnance sur l'utilisation des matières radioactives (OUMR),* 2018.

[5] OFSP, "Procédures d'autorisation et formulaires de demande pour l'utilisation de rayonnements ionisants," 2020. [Online].