

# 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 hydrated. 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 “uranyl 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  $0.2 \text{ mg/m}^3$ . Altogether, the damage potential to the kidneys is considered to be 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  $1.887 \times 10^4$  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: 850 mg,
- uranyl formate: 750 mg,
- uranyl nitrate: 800 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  $\mu$ Sv/h after subtracting the background noise and if no contamination is present externally.

## 3 Rules

### 3.1 Uranium salts

#### 3.1.1 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.

#### 3.1.2 Purchases

Purchase of uranium salts and staining solutions is limited following section 3.1.1 and has to be reported in the School radiation protection database (weight, radioactivity). This task is either done by each unit's RPR or by the FRPO with his/her accord.

#### 3.1.3 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).

#### 3.1.4 Safeguards

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.

### 3.2 Stock solutions

#### 3.2.1 Purchase

It is allowed to purchase stock solutions diluted up to the limits shown in Table 1. Any purchase has to be reported into the School radiation protection database (weight, dilution, radioactivity). Stock solutions prepared by the unit listed in section 3.1.1 also must be reported using their name as the manufacturer.

#### 3.2.2 Preparation of the stock solution

Preparation of stock solutions is limited following section 3.1.1. In specific cases, the preparation of stock solution by other groups than the ones listed in section 3.1.1. 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 has to 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 10a. 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 10b).

Examples of procedures for the preparation of the staining solution and the staining process itself are shown in the annex.

### 3.2.3 Storage of the staining solution

Stock solutions have to be stored in retention trays inside fridges or ventilated cupboards. Below 100  $\mu$ l 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.

## 3.3 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).

## 3.4 Waste management

Any material or equipment in contact with the uranium salts or the stock solution as to be considered as radioactive, unless it has a specific activity below the clearance limit. The waste management for each group is discussed with and validated by the SRPO. If you intend to dispose of stained grids, keep in mind that: grids that have been stained with the stock solution roughly have 0.2 Bq of radioactivity<sup>1</sup>. A single container, bag, can, is considered as a single waste item and each waste item has its own activity.

A decision tree for the management of waste from uranium staining activity is available at the following [link](#). Any group that does not hold an authorization issued by the Federal Office of Public Health and prepares grids

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<sup>1</sup> Considering 10% of a 1% 15  $\mu$ l drop of uranyl acetate solution remain on the sample after staining the total amount of radioactivity per grid is roughly 0.2 Bq.

with uranium salts may dispose of up to 1 kBq per week as regular wastes. This value is the cumulative total of the activities of all waste items produced. However, it is strongly recommended to organize waste management in such a way as to remain significantly below this threshold.



Figure 5 Yellow leaflet with unique ID. Information required are : name, unit, isotope, activity in Bq (if possible), date, form (solid/liquid), pH (if known).

The waste container must contain both the OMoD sticker and the radioactive yellow leaflet with all information dully completed as shown in Figure 5. The total activity of each waste has to be estimated and indicated on the label/leaflet, either in Bq or in Ci. The waste operational management is then done by the FRPO In accordance with this person, the waste is retrieved from the unit on demand or at a fixed frequency. Waste that is not properly labeled will not be accepted by the FRPO.

The waste storage container size needs to be chosen according to the amount of waste generated. Liquid and solid waste must be stored in separate container. It is advised to separate as much as possible contaminated from non-contaminated waste. The smallest containers adapted to the expected waste production should be used. Wide-mouth HDPE and PP boxes are recommended for dry waste. Small-opening containers have to be used for liquid waste as they ensure a lower risk of spill. Examples of containers available in EPFL shops are shown in Figure 6 and Figure 7.



Figure 6 Boxes for storing the dry waste of uranyl acetate activities. (a) Polyethylene (HDPE) black cap; (b) Polyethylene (HDPE) colored cap with extra lid ; (c) Quick-Box® Waste-boxes for sharps, Polypropylene (PP).

Once retrieved by the FRPO, the waste is stored in the radioactivity waste facility of each Faculty. Each year, the total uranium-related waste is sent to a treatment facility, typically the Paul Scherer Institute (PSI); depending the amount of waste, the cost of this procedure can be transferred to each unit.



**Figure 7** Wide mouth fluorinated chemical bottles for liquid waste. Three different sizes available: 250 ml, 500 ml and 1000 ml (HDPE).

## 3.5 Monitoring

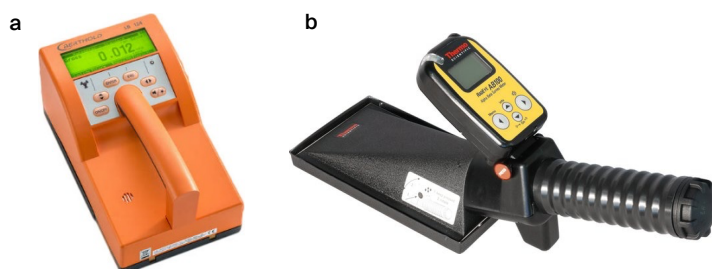
### 3.5.1 Dosimetry

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

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  $\text{Bq/l}$  [2].

### 3.5.2 Control

Surface contamination checks must be done after each preparation of the stock solutions using a portable alpha/beta contamination monitor (see Figure 8). The monitor must be calibrated for U-238. The contamination threshold (CS) is set at  $1\text{ Bq/cm}^2$ . This value is converted in count per second (CPS) and is given by the mandatory calibration of each device.



**Figure 8** 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).

### 3.6 Workplace

#### 3.6.1 Labelling

Any material and equipment used for uranyl related activities needs to be labelled with a radioactive material warning tape (Figure 9a) or, for small objects or vials, with an ionizing radiation warning symbol (Figure 9b). 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 9 Radioactive warning tape. (a) With text and (b) with only the radioactive symbol.

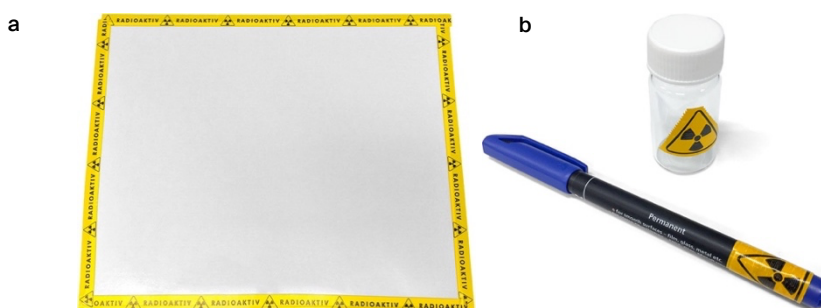


Figure 10 Labelling the workplace and equipment. (a) The workplace and protective foils are clearly marked using the radioactive warning tape. (b) Small equipment is clearly labeled and must be kept at the workplace.

In order 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 10. This absorbent paper exists both as a roll or as individual foils and the protective surface has to be changed at least once per month or immediately in case of a spill.

### 3.7 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 8). 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 German 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].