University of Oxford open radiation source risk assessment form.

Use of Uranium compounds in electron microscopy

Ref: OUSO/Risk1/EM-U-Th	Date: August 2017	
Department: *WTCHG* **Location of work:** *EM Room 673.00.97, Fume Hood (Supervised Area) in 673.00.95*	**Persons involved** *Users of U compounds for microscopy*	**Others at risk** *Laboratory access restricted to names See the list of Authorized STRUBI users*

Description of procedure: *Use of prepared uranium compounds for electron microscopy*

Substance used	Quantities used (mg)	Frequency of use	Hazards identified	Exposure route	Dose per procedure (μSv)
Uranyl acetate (U nat)	*Stock: Typically 50 g bottle* *Per assay: 5g for 5% stain 2g for 2% stain*	*1 x per year*	*α, β, γ* *External dose rate from U/Th stocks.* *Internal hazard from contamination by open sources & intakes.* *Very toxic, carcinogenic and mildly radioactive.*	whole body Y /~~N~~ skin Y /~~N~~ eyes ~~Y~~ /N inhalation Y /~~N~~ ingestion Y /~~N~~ absorption Y /~~N~~	*Whole body: < 1 μSv (exposures on intake may be considerably higher)* *Extremities: < 5 μSv*
Uranyl formate (U dep)	*Stock: Typically 50 g bottle* *Per assay: 5g for 5% stain 2g for 2% stain*	*3 x per year*	*α, β, γ* *External dose rate from U/Th stocks.* *Internal hazard from contamination by open sources & intakes.* *Very toxic, carcinogenic and mildly radioactive.*	whole body Y /~~N~~ skin Y /~~N~~ eyes ~~Y~~ /N inhalation Y /~~N~~ ingestion Y /~~N~~ absorption Y /~~N~~	*Whole body: < 1 μSv (exposures on intake may be considerably higher)* *Extremities: < 5 μSv*
Uranyl Mg acetate (U nat)	*Stock: Typically 2 g bottle* *Per assay: 5g for 5% stain 2g for 2% stain*	*1 x per year*	*α, β, γ* *External dose rate from U/Th stocks.* *Internal hazard from contamination by open sources & intakes.* *Very toxic, carcinogenic and mildly radioactive.*	whole body Y /~~N~~ skin Y /~~N~~ eyes ~~Y~~ /N inhalation Y /~~N~~ ingestion Y /~~N~~ absorption Y /~~N~~	*Whole body: < 1 μSv (exposures on intake may be considerably higher)* *Extremities: < 5 μSv*

Could a less hazardous substance (or form of the substance) be used instead? ~~Yes /~~ No

Justify not using it: *Alternative non-active substances, such as Pt-Blue, OTE, $KMnO_4$ and PTA, are available but studies such as that found at http://www.ncbi.nlm.nih.gov/pubmed/19767626 have concluded that none of the reagents examined showed staining results of the same quality or better than the conventional method with uranium.*

Could a lower activity be used? ~~Yes~~ / No

Justify quantity of material in use: *Mass of material used determines quantity of stain that can be prepared at the required percentage concentration. Prepared stain solutions are aliquots, retained and used over extended periods after preparation*

What measures have you taken to control risk?

Engineering controls & safety equipment: Main consideration is control of contamination; particularly airborne contamination by powder stocks of alpha- emitting isotopes. Work in drip trays. Dispensing from powder stock bottles performed within fume hood/contained workstation. Staining grids by pipette from sub-stocks performed within drip tray over absorbent material. No sharps required. EP15 contamination monitor used throughout.

PPE: Lab coat fastened to neck with elastic cuffs. Nitrile gloves. Safety eyewear.

Procedural & management controls: Designation of fume hood as supervised area during stock and aliquoting operations. No other work in hood. Restrict non-essential access to lab during work with radioactive stocks. Work in accordance with written arrangements in departmental local rules. Good laboratory practice prohibits eating, drinking, chewing etc in laboratory. Contamination monitoring with EP15 before, during & after procedure. Immediate decontamination of spills to B/G (wipe up powder spills with moist cloth and dispose; absorb liquid spills with paper towel and dispose). Fixed contamination noted and advice sought from RPS. Monitoring recorded. Stock, use & solid/aqueous waste records kept. Stock & waste limits assigned in local rules.

Checks on control measures:

RPS supervises laboratory. SRPS approval required for all orders of stocks in accordance with document SOP1. Contamination monitoring of work areas throughout work. Results recorded. Fume hood checked before use. Faults reported. Annual fume hood check. Work practices reviewed during departmental safety inspections. Work practices reviewed during periodic radiation safety inspections by the University Safety Office.

Radiation monitor:	*Mini 900 EP15 contamination monitor*	**Training requirements:**	
Is dosimetry required?	~~Yes /~~ No	*URPO document "Radiation safety in electron microscopy" Laboratory induction* *At-bench training by experienced user / RPS Mentoring until*	
Type of dosimetry:	~~Extremity / Whole body / Biological~~		

Emergency procedures:

Reasonably foreseeable incidents identified in departmental laboratory local rules are relevant to work with open sources of U compounds. In the event of spill of powder stock, close hood and contact SRPS. Contingency plans in local rules are appropriate.

Decontamination procedures:

See contingency plans in departmental local rules and appendix 16 of S8/05

Waste disposal:

Solid (%): *100 %* (must not exceed 5g per day)

Aqueous (%): *0 %* (must not exceed 5g per week) Organic liquid (%): 0

Gaseous (%): 0

Are overall risk control measures adequate?	**Yes ~~/ No~~**	**Signature:**
Name & position of assessor:	**Zuzana Bencokova (SRPS)**	**Signature**:
Name of radiation protection supervisor:	**Geoff Sutton (RPS for EM activities)**	**Signature**:

Date of routine review	Date:	/ / 2017	/ / 2018	/ / 2019	/ / 2020	/ / 2021
	By:					

Dose estimates:

External doses from Uranium stocks: Dose rates in contact with Uranium compound stock vessels may be of the order 50 microsieverts per hour but they are typically significantly lower. If it is pessimistically assumed that preparation of the stain requires handling the stock vessel for 5 minutes, this will result in an extremity dose of less than 5 microsieverts. Dose rates in contact with the vessel containing the prepared stain solution are negligible and subsequent external exposures can reasonably be ignored. If the stipulated PPE is worn, skin contamination can reasonably be discounted. However, significant skin doses from personal contamination are not foreseeable, particularly if contamination is promptly identified by the vigilant use of contamination monitoring equipment. The accessible radiation dose rate at 30cm from the stock vessel is not significant and whole body exposures can therefore be discounted.

Internal exposures from intakes of uranium compounds: Assuming the preparation requires the weighing of 5g of Uranyl acetate (i.e. approx 3g ^{238}U atoms), then according to Delacroix data:

- Inhalation of approx 10mg of Uranyl Acetate (or 2% of the dispensed quantity of powder assumed for the purposes of this assessment) would result in a committed effective dose of 1 mSv, which is equivalent to the University's investigation level for open source work. The requirement that work with powder stocks is carried out inside a functioning fume hood should prevent any inhalation.
- Ingestion of approx 3g of Uranyl Acetate (60% of the dispensed quantity of powder assumed for the purposes of this assessment) would result in a committed effective dose of 1 mSv. The adoption of good laboratory practice and vigilant use of suitable PPE and contamination monitoring equipment should minimise the risk of intakes through ingestion. Nonetheless, an intake of 3g of the radioactive stock is not considered reasonably foreseeable throughout the year.

Procedures to minimise intakes of radioactivity or personal contamination are contained in the departmental *written arrangements for work with uranium salts in electron microscopy*.

Potential foetal exposures: The IRR99 require that the exposure of a foetus must not exceed 1 mSv during the term of pregnancy between declaration and birth. It is not reasonably foreseeable that external exposures during use of Uranium compounds in electron microscopy could give rise to significant exposure of the maternal abdomen.

As explained above, significant intakes of radioactivity or personal contamination likely to give rise to a maternal exposure of 1 mSv are not considered reasonably foreseeable if departmental arrangements are followed. Since uranium is not preferentially taken up by the foetus in comparison to maternal uptake[□], the foetal dose would not therefore exceed the 1 mSv exposure restriction level specified in IRR99.

It is not considered necessary on the basis of radiation risks to the foetus to alter the working conditions of a pregnant/breastfeeding worker. However, if the work (in particular the preparation of stains) can be carried out by another worker during pregnancy/breastfeeding it would be prudent to do so on the basis of ALARP and to ensure complete reassurance.

Investigation level: The University has set an investigation level of 1 millisievert effective dose. Any exposures above this level will be investigated by the department and URPO to determine whether everything is being done that is reasonably practicable to restrict exposures or whether more can be done. In practice, the academic supervisor/Radiation Protection Supervisor should be informed of any incidents that may lead to significant personal exposures; for example, a significant spill/release; widespread contamination or personal contamination. Such incidents will be investigated.

Ref: HSE Contract research report 397/2001: *Doses to the embryo/fetus and neonate from intakes of radionuclides by th*

Uranyl Formate negative stain recipe and protocol
Makes ~10 mls of 0.75% w/w Uranyl Formate (UF) at ~pH 7

NB: Uranyl salts are very toxic, carcinogenic and mildly radioactive.
To use them you must have completed the radioactivity safety course and had your registration card for radiation workers signed off by Geoff and supervisor.

A lab coat, safety glasses and gloves are required at all times when dealing with powders. Only perform work in designated poisonous heavy metal handling areas.

Uranyl Formate aliquot from heavy metals cabinet - for key see Alistair or Geoff.
Designated UF small stir-bar and stirrer plus tube rack
10ml plastic syringe
Disposable 0.2µm syringe filter
dH_2O
Parafilm
Designated UF p100 and p20 pipettes
Whatman filter paper
Access to lab microwave

1) Use a preweighed aliquot of uranyl formate. Details of the aliquot are on the log sheet including quantity of water to add.
2) Heat 10ml dH_2O in microwave until water is just under 100°C.
3) Carefully decant appropriate quantity of water onto UF powder. Add stir-bar, close lid and stir for 5 minutes (keep covered from now on with large aluminium foil-wrapped beaker to protect from light).
4) Add 5 + 5ul 5M NaOH & watch for a slight colour change.
5) Cover and stir for a further 5 minutes.
6) Filter through disposable 0.2µm filter into 15ml Falcon tube.
7) Wrap Falcon tube with foil, label "TOXIC - 0.75% w/v Uranyl Formate" and refrigerate. The stain should last 2-3 days, re-filter or make afresh if precipitant is observed.
8) Dispose of UF contaminated 0.2µm filter in UF disposal bin.
9) Thoroughly flush (in designated radioactive sink) all other plasticware that has been in contact with UF solutions and dispose of in the regular lab waste stream.
9) Chronicle UF usage on logsheet (on wall next to safe box).
10 Working area with EP15 Geiger counter to assess spillage. Seek assistance if significant counts detected.
11) Stain solutions should be stored in the designated area in the cold-room.

Staining protocol:
Only to be performed in designated staining areas using designated uranyl solution pipettes.

1) Place two 50µl drops of deionized water and two 50µl drops of fresh UF stain on a piece of parafilm.
2) Apply 3-5µl of sample to a freshly glow-discharged EM grid covered with a continuous carbon film and let the sample adsorb for 30 sec.
3) Blot the grid from the side with a piece of filter paper, briefly touch the first drop of water with the grid, blot with filter paper, briefly touch the second drop of water, blot with filter paper, briefly touch the first drop of UF, blot with filter paper, touch the second drop of UF for 20 seconds, and blot with filter paper (avoid complete drying of the grid in between the steps).
4) Completely blot dry the grid by touching only the rim. Incubate grid for >5mins to air-dry prior to loading into EM.
5) Dispose of filter paper in the UF disposal bin and all UF contaminated plastics as detailed above. Aqueous UF can only be disposed of in the designated sluice/sink.
6) Tweezers that have been in contact with uranyl salts should be cleaned in water and ethanol.

Disposal:

1) Excess liquid down the sink. Record quantity (mg) disposed.
2) Sign off the log sheet detailing quantity to sink.

Aliquots (100µl) of prepared uranyl formate have been frozen in a freezer in the T12 prep room. Each should be sufficient for an experiment and there is no need to refreeze any which remains unused.

Please do not take aliquots and squirrel them away.

When the low volume screw cap tubes are available similar aliquots of uranyl acetate will be prepared.

There is no need to sign out the aliquots. However, it is up to the users, collectively, to let myself or Cornelia know when we are running out.

Since the aliquots are not radioactive there is no need to label them or the freezer.

Tests have shown that the quantities of uranium in the volumes used for staining grids (e.g. 100µl aliquot) are essentially undetectable by Geiger counter, hence **NONE of the WASTE from a grid staining experiment needs treating as if it were radioactive** (since it is not).

Gloves, tissues etc can go into an autoclave bag. Please empty the bag so it does not overfill.

Tips, filter paper, parafilm go into the labelled dispo jar. Note this will then stream by a different route to autoclave waste. Leave full jars on the marked shelf in the room.

The only item which shows any detectable radiation is the stock of uranyl acetate; hence if we get this into aliquots we completely minimise our risk.

The uranyl solutions remain a heavy atom risk so please don appropriate PPE.

Also it is still advisable to work in the designated area in the T12 prep room. You can still use the pipettes since they are uncontaminated.

Uranyl Formate work flow

Aliquots may be weighed out by Geoff – please note, this is not a service.

Details of the aliquots are posted next to the heavy metals cabinet.

Sign out the aliquot – user, signature, date.

Monitor the uranyl work area and record the counts.

Ensure that the tube is correctly labelled and stored.

Disposal

Do not leave your aliquots to fester – when you have finished dispose as detailed below and fill in the records.

Excess liquid uranyl formate can be put down the designated sink in the T12 room – flush with water, monitor the area with a Geiger counter and record the counts.

Complete the Green sheet by the sink; cross-reference the date (left column) with the UF or UA column (start one if required) and put the quantity disposed (in mg).

Monitor the uranyl work area and record the counts.

Sign off on the aliquot sheet with the date and the quantity sent to sink.