### Description of Procedure:

**14C-creatinine uptake by cells** – see attached protocols.

<table>
<thead>
<tr>
<th>Substances used</th>
<th>Quantities used</th>
<th>Frequency of use</th>
<th>Hazards identified</th>
<th>Exposure route</th>
<th>Dose per procedure (µSv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14C-creatinine hydrate</td>
<td>Between 37 and 74 kBq (1-2 µCi) per experiment</td>
<td>2-3 times per week</td>
<td>Low energy β-emitter (157 keV). Skin dose rate from 1MBq point source at 30cm is 0 mSv/h. Bremsstrahlung radiation may be significant. [Delacroix et al. Radiat. Prot. Dosim. 98, 2002]</td>
<td>Skin (mostly fingers)</td>
<td>Whole body: Effectively zero Extremities: Effectively zero</td>
</tr>
</tbody>
</table>

**Could a less hazardous substance (or form of the substance) be used instead?** Yes

**Could a lower activity be used?** No

**Justify Quantity of Material in Use:** Quantities used are already very low and cannot be further reduced due to sensitivity levels required in assay.

**What Measures Have you Taken to Control Risk?**

**Engineering Controls:**
- Work carried out in designated areas on radioactivity spill trays.
- Reduce dosage by control of distance, time and shielding.

**PPE:**
- Lab coat, nitrile gloves and safety eyewear

**Management Measures:**
- Follow the As Low As Reasonably Practicable (ALARP) rule
- Adhere to local rules, EPR2010 and IRR99
- Monitor work area before, during and after use. Wipe tests taken after each set of experiments.
- Lone working prohibited
- Work only in designated Supervised areas
- Radioactivity stock pot stored in locked refrigerator in Room 10/088
- Adhere to limits of designated sink and bins
- Adhere to storage time limits for bins [12 months for both ‘solids’ green/yellow band bin & ‘organic liquids’ orange bin]

**Checks on Control Measures:**
- Regular monitoring (including wipe tests by users and RPSs) and supervision
- Checks on documentation – control of Monthly returns

**Radiation Monitor:** EP15

**Is dosimetry required?** No

**Training Requirements:** URPO lecture “Working with unsealed radioactive sources” and in-house training.

**Emergency Procedures:**
- Refer to Contingency Plans in Local Rules & University Policy Statement S8/05: Appendix 16.
- Decontamination where necessary with Count-Off and/or Deco90 decontaminants as recommended. Dispose of contaminated materials in designated bins and sinks. For any incident beyond minor contamination of radioactive workspaces alert RPS (and SRPS/URPO if appropriate).

**Waste Disposal:** Approximations – actual amounts determined from scintillation counting
- Aqueous 99% to designated sink
- Solid 1% to green-yellow band bin
- Scintillant 10% to orange bin
<table>
<thead>
<tr>
<th>Are overall risk control measures adequate?</th>
<th>Yes</th>
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</table>

**NAME OF RADIATION PROTECTION SUPERVISOR:** Dr James Brown

**SIGNATURE:**

<table>
<thead>
<tr>
<th>Date of routine review</th>
<th>Date:</th>
<th>By:</th>
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</table>
Title: \textsuperscript{14}C-creatine uptake by cells

The undersigned have read the above radiation risk assessment and understand the safety arrangements required and their own obligations in ensuring compliance.

<table>
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<tr>
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<th>TRAINED BY (NAME &amp; SIGNATURE)</th>
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</table>
Carbon - 14

**Half life:** 5730 years

**Specific activity:** $1.66\times10^{11}$ Bq.g$^{-1}$

**Risk group:** 4

**Risk colour:** Green

### Main emissions (keV)

<table>
<thead>
<tr>
<th>E</th>
<th>Gamma or X</th>
<th>Beta (E_{max})</th>
<th>Electrons</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>E %</td>
<td>E %</td>
<td>E %</td>
<td>E %</td>
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<tr>
<td></td>
<td>157</td>
<td>100</td>
<td>0</td>
<td></td>
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<tr>
<td>E2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E3</td>
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#### Exemption levels

- **Quantity** (Bq): $1E - 07$
- **Concentration** (Bq.g$^{-1}$): $1E - 04$

#### Transport (TBq)

- IAEA ST1 A$_1$ value: $4E + 1$
- IAEA ST1 A$_2$ value: $3E + 0$

### EXTERNAL EXPOSURE (mSv.h$^{-1}$) for an activity of 1 MBq or 1 MBq.m$^{-2}$ (as appropriate)

- **Point source** (30 cm)
- **Infinite plane source**
- **10 ml glass vial**
- **Contact with 50 ml glass beaker**
- **Contact with 5 ml plastic syringe**

#### Contamination skin dose (mSv.h$^{-1}$)

- **Uniform deposit (1 kBq cm$^{-2}$)**: $3.24E-1$
- **0.05 ml droplet (1 kBq)**: $2.76E-3$

### Detection

#### Recommended probes$^*$

- **Alpha**
- **Beta**
- **Gamma**
- **X rays**

### Derived limits (Bq.cm$^{-2}$)

- **Removable contamination**: $4E - 2$
- **Fixed contamination**: $4E + 4$

### Shielding (mm)

- **Glass and electrons** (Total absorption): Plastic, 0.3
- **Gamma and X rays** (half thickness): Lead, 1/10

### Internal Exposure for Workers

**Committed effective dose per unit intake (Sv.Bq$^{-1}$)

| Ingestion          | $t_i$ | Inhaled (for soluble and reactive gases and vapours) | Vapour | $5.8E - 10$
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</thead>
<tbody>
<tr>
<td>Labelled organic compounds</td>
<td>1.00</td>
<td>5.8E-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest dose organ</td>
<td>Whole body</td>
<td>20 mSv AL$_{ingest}$</td>
<td>$3.4E + 07$ (Bq)</td>
<td>20 mSv AL$_{inhal}$</td>
</tr>
</tbody>
</table>

### Maximum Recommended Activities in Low Level or Intermediate Level Laboratories (Bq)

<table>
<thead>
<tr>
<th>Physicochemical State</th>
<th>Supervised area</th>
<th>Controlled area</th>
</tr>
</thead>
<tbody>
<tr>
<td>All compounds except below</td>
<td>Volatility factor (k)</td>
<td>Bench</td>
</tr>
<tr>
<td>Oxide &amp; anhydride</td>
<td>0.01</td>
<td>1E + 07</td>
</tr>
</tbody>
</table>

*If no probes are indicated the recommended technique is to use a wipe test in association with a probe or liquid scintillation technique.*
**14C-creatine uptake by cells – Dose estimation**

 Almost all work will be carried out either behind a Perspex shield or using Perspex tube racks so the dose should be limited to fingers; whole body dose will be insignificant.

 Bremsstrahlung radiation is unlikely to be significant due to the low energy of the radiation and the use of Perspex shielding.

 The external dose will be effectively zero since Perspex shielding, assay tubes, gloves and skin will all stop low energy beta-particles arising from 14C decay.

 The maximum dose likely to be encountered arises from handling the stock material which is in screw-capped microcentrifuge tubes at a maximum activity of 4.6MBq (1250μl @ 3.7MBq/ml). This is handled for a matter of seconds and estimation of the dose is impractical based on the lack of dose values from Delacroix et al.
1. Plate cells into 6 well plates in preparation for protocol, including a duplicate plate which will not be spiked with isotope, but will be used for the determination of protein levels in each well.

2. Check hot lab for contamination by previous user prior to beginning work with the isotope. Can use Geiger counter, but using cotton buds dipped in Decon 90 to wipe the area, then placing them in scintillation vials containing scintillation fluid is essential. These can be counted later together with everything else.

3. Prepare 13ml creatine-containing complete media (creatine concentration 500 µM) in a 50ml Falcon tube. To this, add 10 µl (37 kBq) $^{14}$C-creatine.

4. Record the amount of isotope taken from the pot on the appropriate sheet.

5. Transport plate of cells to hot lab from the incubator, aspirate media and discard.

6. Add hot media containing creatine (2ml per well). Keep a volume of hot media (100µl) in order to measure later, to confirm the initial amount of isotope added to the cells.

7. Transport the 6 well plate containing the radiolabel-spiked media to the designated CO$_2$ incubator in 10/079 in a suitable Tupperware (or similar) container with a sealed lid.

8. Incubate the plate containing the radiolabelled creatine for 60 minutes alongside the duplicate (non $^{14}$C spiked) plate of cells, which has standard complete media containing 500µM creatine, but no isotope. The hot plate should be placed on a suitable (ie lipped) tray in the incubator, in order that it would contain any spills if they were to occur. This plate should be clearly labelled as radioactive, and if possible, incubated on the bottom shelf of the incubator in order that spills cannot contaminate anything below it.

9. Return the plate to the hot suite in the sealed container. The duplicate plate can be dealt with on the bench and should not be taken to the hot suite.

10. Aspirate the hot media from the 6 wells. To do this, use disposable 3ml plastettes (disposable plastic Pasteur pipettes). Pipette the waste hot media into a 50ml Falcon tube.

11. Wash each of the 6 wells 3 times with 2ml PBS /well. This PBS and the media previously aspirated can also be placed in a 50ml Falcon tube, ready for disposal.

12. Solubilise cells with the addition of 0.5ml of 0.5% Triton-X.

13. Scrape cells free from the base of the wells using a cell scraper. This should be performed with the plate behind the Perspex shield, in order to eliminate the risk of any potential splash reaching you.

14. Aspirate the contents of each well using a P1000 micropipette with filter tips to a scintillation vial, then add a further 0.5ml of 0.5% Triton-X in PBS to the wells to rinse them, and finally aspirate this and add this also to the scintillation vials. Then, add 9ml FluoranSafe XE scintillation buffer to the vials, bringing the total volume in each one to 10ml. Also prepare 1 vial with 10ml of the scintillation buffer alone, and 1 with 9.9ml of the buffer and 100µl of the hot media which was saved at the beginning of the experiment.

15. Monitor the hot area using cotton buds or small pieces of filter paper dipped in Decon 90 to wipe the appropriate surfaces. Include pipette, Tupperware container etc as well as the area itself. Place buds in scintillation vials containing scintillation buffer, labelled with the area wiped.
16. Discard liquid waste collected in Falcon tubes down the designated sink, after first pouring a small volume of Decon 90 down the sink in order to aid the removal of the isotope. Record this on the sink waste sheet, with initials and details of isotope.

17. Discard the solid waste, such as tips and falcon tubes in the green solid waste bin, and mark the assumed amount of isotope on these items (eg 1% of total starting amount for tips).

18. Transport the vials to the scintillation counter in an appropriate sealed container, and count using carbon-14 program.

19. Discard scintillation waste in orange scintillation bin by the counter, and record it on the appropriate scintillation waste sheet (orange).

20. Finally, document the fate of all the waste on the original stock sheet where the amount of isotope taken was first recorded.

* - determined from scintillation counting