RDM Division of Cardiovascular Medicine
University of Oxford

Radiation Risk Assessment

		DATE:	21st Aug 2017
DEPARTMENT: Cardiovascular Medicine Wellcome Trust Centre for Human Genetics **LOCATION OF WORK:** Room 10/088 (Lab 3)		**PERSONS INVOLVED:** See attached training record	**OTHERS AT RISK:** None

DESCRIPTION OF PROCEDURE:

^{14}C-creatine uptake by cells – see attached protocols.

SUBSTANCES USED	QUANTITIES USED	FREQUENCY OF USE	HAZARDS IDENTIFIED	EXPOSURE ROUTE	DOSE PER PROCEDURE (µSv)
^{14}C-creatine hydrate	Between 37 and 74 kBq (1-2 µCi) per experiment	2-3 times per week	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]	Skin (mostly fingers)	*Whole body:* Effectively zero *Extremities:* Effectively zero

COULD A LESS HAZARDOUS SUBSTANCE (OR FORM OF THE SUBSTANCE) BE USED INSTEAD? ~~Yes~~/No

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**	**TRAINING REQUIREMENTS:**	URPO lecture "Working with unsealed radioactive sources" and in-house training.
Is dosimetry required?	No		

EMERGENCY PROCEDURES:

Refer to Contingency Plans in Local Rules & University Policy Statement S8/05: Appendix 16.
Decontamination where necessary with Count-Off and/or Decon90 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	89% to designated sink
Solid	1% to green-yellow band bin
Scintillant	10% to orange bin

Are overall risk control measures adequate? Yes	
NAME OF RADIATION PROTECTION SUPERVISOR: Dr James Brown	**SIGNATURE**: 

Date of routine review	**DATE:**	/ / 2018	/ / 2019	/ / 2020	/ / 2021	/ / 2022
	BY:					

RDM Division of Cardiovascular Medicine
University of Oxford

Radiation Risk Assessment Training Record

Title: ^{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.

DATE	NAME & SIGNATURE	TRAINED BY (NAME & SIGNATURE)

Carbon - 14

$^{14}C_6$

Half life: 5730 years
Specific activity: 1.65E+11 Bq.g^{-1}

Risk group: 4
Risk colour: Green

Main emissions (keV)							
	Gamma or X		Beta (Emax)		Electrons		Alpha	
	E	%	E	%	E	%	E	%
E1			157	100				
E2								
E3								
% omitted				0				

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)

Betas, electrons (skin dose): 0.00E+0

Gammas, X rays (deep tissue dose): 0.00E+0

Infinite plane source

Betas, electrons (skin)

10 cm	0.0E+00
1 m	0.0E+00

Photons (skin)

10 cm	0.0E+00
1 m	0.0E+00

Photons (deep dose)

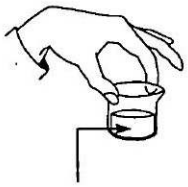
10 cm	0.0E+00
1 m	0.0E+00

10 ml glass vial

100 cm

Brem. Rad.

Contact with 50 ml glass beaker



Brem. Rad.

Contact with 5 ml plastic syringe

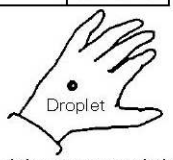
Brem. Rad.

The values above do not include Bremsstrahlung radiation. Brem. Rad. indicates that it may be significant.

CONTAMINATION

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

Uniform deposit (1kBq.cm^{-2})	3.24E-1
0.05 ml droplet (1 kBq)	2.70E-3



Detection

Recommended probes*	
Alpha	
Beta	+ +
Gamma	
X rays	

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

Removable contamination: 4E+2

Fixed contamination: 4E+4

* If no probes are indicated the recommended technique is to use a wipe test in association with a probe or liquid scintillation technique

SHIELDING (mm)

Betas and electrons (Total absorption)

Glass	0.2
Plastic	0.3

Gamma and X rays (half and tenth value thickness)

	½	1/10
Lead	-	-
Steel	-	-

INTERNAL EXPOSURE FOR WORKERS

COMMITTED EFFECTIVE DOSE PER UNIT INTAKE (Sv.Bq^{-1})

Ingestion	f_1	
Labelled organic compounds	1.000	5.8E-10

Inhalation (for soluble and reactive gases and vapours)	
Vapour	5.8E-10
Dioxide	6.2E-12
Monoxide	8.0E-13

Highest dose organ Whole body **20 mSv ALI$_{ingestion}$** 3.4E+07 (Bq) **20 mSv ALI$_{inhalation}$** 3.4E+07 (Bq)

MAXIMUM RECOMMENDED ACTIVITIES IN LOW LEVEL OR INTERMEDIATE LEVEL LABORATORIES (Bq)

PHYSICOCHEMICAL STATE	*Subject to external exposure requirements which may be more restrictive*					
	Volatility factor (k)	Supervised area		Controlled area		
		Bench	Fume hood	Bench	Fume hood	Glove box
All compounds except below	0.01	1E+07	1E+08	3E+07	3E+08	5E+09
Oxide & anhydride	1	Forbidden	1E+06	Forbidden	3E+06	3E+08

^{14}C-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 ^{14}C 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*.

Radiolabelled Creatine Uptake Experiment Protocol

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.

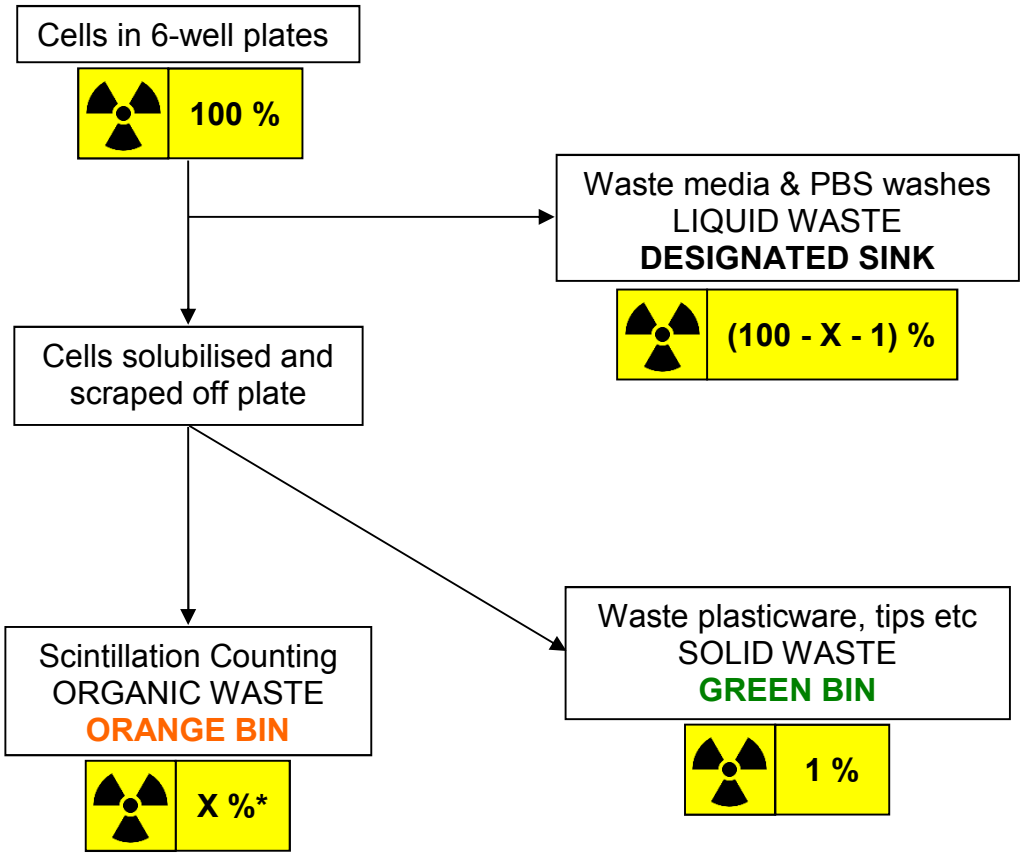
16. Discard liquid waste collected in Falcon tubes down the designated sink, after first pouring a small volume of Decon 90 down the sink in order to aid the removal of the isotope. Record this on the sink waste sheet, with initials and details of isotope.

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