DURHAM UNIVERSITY
Board of Studies in Chemistry

THE USE OF UNSEALED SOURCES OF RADIOACTIVE MATERIALS
These notes form **Special Code of Practice S-COP I** of the current Chemistry Safety Policy

Background

This document is intended to cover use of radioisotopes in the Department of Chemistry in laboratory 217A. These laboratories are communal laboratories principally intended for experiments involving "high" energy emitters such as ^{32}P and low energy emitters, notably ^{14}C, ^{35}S and ^{3}H. These are used for procedures such as labelling, hybridisation, purification of radiolabelled chemicals/biomolecules and blot washing. Room 217A is equipped with two types of workstation, one with Perspex shielding for β-emitters and the other unshielded station for low energy emitters. The room also contains a scintillation counter, fume cupboard and a disposal sink with a waste disposal unit, both designated for use with radioactive material. This laboratory also contains the Departmental Radioisotope Store. Further related information is available from the Radiation Safety Information Page which can be found on the Chemistry Sharepoint website (http://baron/sites/chemistry) in the Safety Information folder listed under Documents and Information. A link to the University Health and Safety Manual Ionising Radiation Guidance can be also consulted.
(https://www.dur.ac.uk/healthandsafety/local/office/guidance/r/radiation/guidance/)

Use of Room 217A

1. Room 217A is under the control of the Departmental Radiation Protection Supervisor (DRPS; currently Dr Ehmke Pohl).

2. Persons intending to use laboratory 217A to undertake unsupervised work with radioactive materials must become "Approved Workers". An "Approved Worker" will be registered with the University Radiation Protection Officer (URPO), must have received instruction on the use of radioisotopes, and should be familiar with Departmental and University regulations concerning the storage, use and disposal of radiochemicals. Their exposure to ionising radiation will be monitored by TLD dosimeters and with finger monitors as deemed appropriate by the URPA. Responsibility for ensuring training of research staff in the use of radioisotopes lies with the supervisor of the individual or laboratory in which the individual is working. Application and training forms are available from the DRPS/URPO. Unsupervised work must not be undertaken until you have received a clearance letter from the URPO and your name is listed on the radiation safety page on the Departmental SharePoint site under Safety Information.

3. Access to Rooms 217A is restricted to "Approved Workers". Other personnel will only be allowed in the laboratory if accompanied **at all times** by an "Approved Worker", who will be responsible for their safety and actions.

4. Before commencing an experiment involving radioisotopes, the worker must either familiarise themselves with the "approved scheme of work" governing the experiment, or must have submitted a 'Risk Assessment for use of Radioisotopes' (Appendix 2) to the DRPS and had it approved. Particular attention should be paid to problems with waste and contamination (see below).

5. Use of laboratory 217A is regulated. Intending users MUST book a workstation in the laboratory, using the book provided. The booking sheet acts as a log for laboratory usage. It is anticipated that most work will be carried out within the workstations, but even if the perspex shielding is not required, a workstation must be booked. Outside normal working hours, workers must ensure that their presence in the laboratory is known to at least one other person, who is available to assist in the event of accident or emergency.

6. Room 217A should be kept locked at all times, as far as is practical and consonant with its safe use.

Monitoring of Radiochemical/isotope Purchase Use and Disposal

1. A record of all items entering the department will be kept online in ISIS (Isotope Stock and Inventory System, https://isis.ncl.ac.uk).

2. All radiochemicals must be ordered using the designated order book, which must be countersigned by the DRPS (currently Dr Ehmke Pohl) or his named representative and new stocks must be added to ISIS.

3. The DRPS must also approve the external transfer of radiochemicals into the department which must also be added to ISIS.

4. For internal transfer of radiochemicals within the department, the individual transferring must update ISIS to indicate new recipient and new location.

5. Radioactive sources are kept in the approved stores; the -20°C freezer or the 4°C fridge in room 217A.

6. The reference number generated by ISIS will be used as the control number. For example, this has the form 2012*** for a sample registered in 2012 and is unique.

7. No samples should be purchased or brought into the department without completion of a COSHH assessment and updated to ISIS.

8. Upon arrival all radiochemicals must be logged into ISIS and be labelled with the control number and stored in the fridge or freezer in room 217A. These rooms are locked when not in use.

9. All recorded usage must be updated on ISIS. Failure to record usage or disposal is a serious breach of regulations, and will be treated as such.

10. When stock is exhausted, the vial or stock is disposed and the "vial disposed" entry is completed on ISIS.

11. Any secondary stocks or retained samples are issued a control number by ISIS and must be labelled with this.

12. An audit of isotope use must be carried out on a monthly basis by checking stores stocks against the record on ISIS.

13. Periodic inspections of record keeping will be performed by the DRPS and/or as part of normal departmental safety inspections.

Contamination Monitoring

1. Contamination monitoring of room 217A will adhere to the guidance set out under the Radiation Monitoring General Guidance (https://www.dur.ac.uk/resources/healthandsafety/local/RG7RadiologicalMonitoringV1.2.pdf). A copy of this guidance is available in room 217A.

2. Monitoring plans of room 217A (Appendix 3) with designated 'hot' and 'warm' areas will be available in the room at all times and updated as necessary.

3. Hot area monitoring will be recorded on the form in Appendix 4 after each experiment has been completed ("experiments" will normally last no longer than a day).

4. Laboratory contamination checks will be recorded using the form in Appendix 5 at the end of each calendar month. Any readings over those specified under the Radiation Monitoring General Guidance will be reported to the DRPS and decontamination procedures followed.

5. Any decontamination required will be recorded using the form in Appendix 6. Following decontamination, re-monitor and record the result.

Safe working

1. Protocols covering the routine manipulation of radioactive material will be held in the SOP manual in room 217A. Any new usage or application which differs significantly from approved SOPs should be described in written form and approved by the supervisor of the designated worker and the DRPS.

2. Workers should make sure they are wearing the correct protective clothing; the minimum acceptable is laboratory coat (done up), disposable gloves and safety spectacles. Dosimeters should be worn in a position where they will give a reasonable assessment of exposure to radiation; breast pocket is as good as any. Finger monitors should be worn when handling high energy emitters or as advised by the URPA and demonstrated in the Risk Assessment, or working with such isotopes using unfamiliar protocols.

3. Do not use any of the equipment in room 217A until you are sure you know how to use it properly; consult documentation available, or experienced workers. Please do not damage the perspex workstations. In particular, take care to avoid getting phenol/chloroform on perspex. Do not attempt to clean perspex with scouring liquids or green scouring pads; the surface is irreversibly damaged. If you damage an item of equipment (it happens), or notice that equipment is damaged, please report it to the DRPS; damaged items cannot be replaced unless notification is given.

4. Before starting work, check your assigned workstation and area for radioactive contamination. If it is not clean then either (a) clean it yourself or (b) get the person who used it last to clean it. If necessary, inform the DRPS.

5. It is the worker's duty to protect themselves and others from ionising radiation. Use shielding as necessary, and make sure high level sources are kept properly shielded whenever possible. Keep isotopes within the shielding provided. Warn other workers before removing sources from the shielding.

6. Carry out work within the perspex workstations if possible; if not, on the disposable plastic trays. Clean up spillages immediately, using kitchen roll, which can be disposed via the waste disposal unit. Spillages that cannot be cleaned must be notified to the DRPS.

7. The following procedures must be carried out in the fume cupboard, with the extractor switched on:
   - denaturation of DNA probes using the probe boiler;
   - dispensing scintillation fluid into vials using a pump dispenser;
   - opening of radioactive stocks which may be under pressure or vacuum.

8. Work involving activities of high energy β emitters of >5 MBq must be undertaken in the fume cupboard or enclosed perspex glovebox.

9. In the case of accident:
   - **DO NOT PANIC**;
   - don't try to cover it up, or carry on as if nothing has happened;
   - don't spread contamination any further than it has spread already;
   - act to minimise the danger to others first, then to yourself;
   - get assistance as soon as possible;
   - report it to the DRPS and the URPA.

Disposal of radioactive waste

1. If you have to store radioactive solutions (e.g. probes, etc.) use glass bottles with tightly fitting plastic screw caps. The solutions can be stored in the fridge or freezer if labelled with your name, date and ISIS ID number. Unlabelled material will be disposed of.

2. All liquid waste should be disposed down the radioactive sink, washed down with copious amounts of water. This includes Ecoscint scintillation fluid, and gels. Only Ecoscint fluid should be used; if you want to use any non-biodegradable formulation, you must consult the DRPS before use. **Make sure you record all your disposals on ISIS**.

3. The radioactive sink is fitted with a waste disposal unit, which will allow gels and paper to be disposed of as liquid waste. Only use the unit with copious quantities of water flowing through it. Please use kitchen roll in the radiochem laboratory, and not the "barrel roll" paper found in other labs; this tends to jam the waste disposal unit. Do **NOT** attempt to put any of the following into the waste disposal unit: plastic items, including tips and tubes; polythene bags, or pieces of polythene; heavy paper or card. **Please treat the waste disposal unit with respect**; if it jams, switch off and attempt to free it off manually; if this does not work, notify the DRPS. Contaminated solids must be washed until non-radioactive, disposing of the washings as liquid waste. Use Decon detergent. Plastic items decontaminate very successfully. If items have to be soaked to remove contamination, make sure they are not abandoned - the next user of your workstation can make you dispose of them. Use the Geiger counter to ensure that washed solids are free of contamination; readings for an item held at 0.5 cm from the counter should be <10 cps from all angles. Dispose of such items as non-radioactive waste. **Under no circumstances should radioactive waste be placed in the ordinary waste bin** - this is a serious breach of regulations. Solids that cannot be decontaminated should be date labelled and stored as solid waste until the isotope decays. This can only be done by prior arrangement with the DRPS. Due to limited storage facilities, the DRPS will insist that good reason be given for storing solid waste. For further details refer to Disposal of Radioactive Waste procedures (https://www.dur.ac.uk/resources/healthandsafety/local/RG14DisposalV1.2.pdf).

4. Material disposal via the very low level waste (VLLW) route should follow procedures and be documented as described by the URPO.

Finishing work in room 217A

Whenever you leave laboratory 217A you should:

1. Check your gloves, laboratory coat and shoes for contamination.

When you have completed an experiment, or a session of work in laboratory 217A, you should:

1. Check the area you have been using for contamination, and clean up any spillages.
2. Record contamination checks.
3. Make sure you have recorded isotope usage or disposal correctly on ISIS.
4. Remove your gloves after decontamination, dispose, and wash your hands in the hand wash basin.

Breaches of good working practice

1. The cleanliness and tidiness of room 217A is the responsibility of its users. A rota system for laboratory cleaning has been in force among radiochemistry laboratory users, and all workers are expected to cooperate with this. It is not the job of the DRPS to clean up mess made by workers in the laboratory. If the condition of the laboratory deteriorates, it will be necessary to close it until it is cleaned. Stringent standards of laboratory cleanliness and discipline are expected by the Environmental Agency, and we are obliged to comply with these standards.

2. Laboratory 217A are provided as a radiochemical facility by the Department, and usage of the laboratory is conditional on the worker following the above regulations, and any further instructions given by the DRPS. The DRPS retains the right to exclude persons who act in a dangerous or irresponsible manner from this facility.

Emergency contacts

Departmental Open Source Radiation Protection Supervisor (Lab 217, Biosciences): Dr Gary Sharples (ext: 43986, telephone: 07813 350583; e-mail: gary.sharples@durham.ac.uk)

Departmental Radiation Protection Supervisor (DRPS in Chemistry): Dr Ehmke Pohl (ext: 43619; out of hours please ask Security to contact Dr Pohl on your behalf on 0191 334 2222: e-mail: ehmke.pohl@durham.ac.uk)

Departmental Radiation Protection Supervisor (DRPS in Biosciences): Dr Martin Schröder (ext: 41316; e-mail: martin.schroeder@durham.ac.uk)

University Radiation Protection Officer (URPO): Gretta Roberts (telephone: ext: 42663; 0747 114 3213; e-mail: gretta.roberts@durham.ac.uk)

University Radiation Protection Adviser (URPA): as URPO

Dr Gary Sharples
Last updated 13 September 2019
Reviewed 13 September 2019

Appendix 1 - Work to be performed

Below follows a series of SOPs and details of the nuclides being used.

Carbon 14

Principle emissions:	Beta energy
Energies of principal emissions:	156 keV (maximum)
Physical half life:	5500 years
Biological half life:	12 days

Tritium 3

Principal emissions:	Beta energy
Energies of principal emissions:	18.6 keV (maximum)
Physical half life:	12.4 years
Biological half life:	9.4 days

Phosphorus 32

Principal emissions:	Beta energy
Energies of principal emissions:	1710 keV (maximum)
Physical half life:	14.29 days
Biological half life:	257 days (effective half-life of 13.5 days)

General procedure for preparation of RNA bioconjugates by *in vitro* transcription followed by bioconjugation procedures (David Hodgson)

1. *In vitro* transcription with T7 RNA polymerase.

Mix together in a 650 µL disposable Eppendorf tube:

DEPC treated water to 50 µL final volume

5 µL	10x T7 buffer
5 µL	100 mM DTT
4.2 µL	250 mM $MgCl_2$
5-10 µL	approx 2.5 mM dsDNA template
16 µL	20 mM NTPs
3.33 µL	Guanosine-based initiator for 5'-end labelling
0.2 µL	RNase inhibitor

Mix by pipetting and centrifuge briefly to collect sample.

Add:
5 µCuries (0.185 MBq) of alpha-labelled-^{32}P NTP (likely UTP)
Mix by pipetting.

Add:

T7 RNA polymerase to appropriate level (depending on enzyme prep)

Mix by pipetting.

Incubate at 37 °C for 0.5 h-3 h.

Centrifuge briefly to collect sample and add 1U RQ1 DNase. Mix by pipetting, spin down and incubate at 37°C for 30 mins.

Spin down and add 50 µL 8 M urea/EDTA “loading buffer”, vortex, place at 95° C heat block for 2 min, allow to cool, spin down.

2. Purification of RNA transcript

During or before the transcription reaction was started, an approx 6” x 4” W x H 3 mm thick slab gel was prepared. Example for a 110 mer RNA transcript, use 6-10% Urea Polyacrylamide gel. Make with 10 x 100 µL volume wells. During DNase step above, prewarm gel (run in 1x TBE).

In certain cases, spin column (size exclusion e.g. Zeba spin) prep-purification is required to remove guanosine-based initiator that co-migrates under electrophoresis with the RNA transcript.

Rinse out gel wells, carefully pipette transcription/loading buffer mixture into one well. Load tracer dyes in another well. Run gel as appropriate.

Run gel for a short period to separate transcript form aborted transcripts (5-15mers), which account for about 90% of the RNA products of the reaction and therefore 90% of the radioactivity. Check lower buffer in electrophoresis apparatus for hotness—should be cold because short running time ensure ALL isotope is still trapped in the gel.

Once the gel has been run, check buffer reservoirs for hotness, dispose as appropriate. Remove gel plates from apparatus and check for residual hot buffer contamination.

Prize plates apart. Put plastic wrap on top of gel; flip over; peel off the other glass plate; cover that side of the gel with wrap too.

At this point relative RNA yields can be gauged by exposing the gel to an IP plate for ~30 seconds.

3. Isolation of transcript

Visualise transcript band by UV shadowing (use a plastic-wrapped fluorescent silica TLC plate and a standard UV lamp). Mark transcript with permanent marker pen. Remove gel to glass plate and cut out band with a razor blade.

Transfer cut band to 2 mL Eppendorf tube. Add 450 µL 0.3 M NaCl. Close tube and allow to passively elute overnight at room temperature.

Spin down. Collect supernatant avoiding gel pieces. Add 2 volumes of ethanol. Centrifuge at 10000 g for 15 min. Decant. Wash pellet with 70% ethanol solution and allow to dry. Redissolve pellet in 10-20 µL DEPC-treated water or equivalent.

4. Bionjugation experiments

Usually up to 5000 cpm of material from step 3 is taken into an appropriate buffer and exposed for 1-2 h to a bioconjugation reagent e.g. NHS-biotin, thiol-ractive agent, biotin hydrazide. After this time, the RNA is ethanol-precipitated, an appropriate loading buffer is added, and up to 1000 cpm per lane as gauged on GM is loaded on to the gel. It is important to make sure that each lane has a similar activity within it in order to allow good quantitation to take place. A 0.4 m-spaced gel is used for this quantitative work. After electrophoresis, the gel plates are separated and the gel is peeled onto blotting paper. The gel is then covered with wrap and dried. Quantitation takes place using an image plate and phosphorimager.

5. Adenylyl cyclase assays

1) Purified adenylyl cyclase protein is incubated in a glass borosilicate tube with other assay components (buffer, ATP, enzyme activators) and 25 kBq [alpha^{32}P] ATP as radioactive tracer.
2) After the prescribed assay period the contents of the glass tube are poured into a matrix containing BioRad 'PolyPrep' column. The column is held in a plastic support over a radioactive waste collection container. The empty borosilicate tube is rinsed and the non-radioactive tube discarded in the normal waste. The radioactive reaction poured onto the column is a mixture of unused [alpha^{32}P] ATP and [alpha^{32}P] cAMP reaction product. Washing this column, through the support matrix, and over a subsequent column separates [alpha^{32}P] ATP from [alpha^{32}P] cAMP.
3) Eluted [alpha^{32}P] ATP is collected in the waste collection container and discarded as liquid waste.
4) Eluted [alpha^{32}P] cAMP is collected in scintillation vials, counted, and discarded as liquid waste. Empty scintillation vials are rinsed and discarded in the normal waste.
5) The chromatography columns are washed and reused. There is therefore no solid waste.

6. cAMP radioassay

1) 1 µCi/well [^{3}H]-adenine is added to tissue culture cells in a 12 well plate under sterile conditions.
2) The tissue culture plate is incubated 24 hours in a sterile environment 37^{o}C/5% CO_2 or 37^{o}C/atmospheric CO_2.
3) Tissue culture media is discarded and cell contents released into 5% tricarboxylic acid in the 12 well plate.
4) The contents of each individual well are pipetted into a matrix containing BioRad 'PolyPrep' column. The column is held in a plastic support over a radioactive waste collection container. The empty 12 well plate is rinsed and the non-radioactive plate discarded in the normal waste. The

radioactive solution poured onto the column is a mixture of [^{3}H]-adenine nucleotides and [^{3}H]-cAMP. Washing this column, through the support matrix, and over a subsequent column separates [^{3}H]-adenine nucleotides from [^{3}H]-cAMP.

5) Eluted [^{3}H]-adenine nucleotides are collected in the waste collection container and discarded as liquid waste.

6) Eluted [^{3}H]-cAMP is collected in scintillation vials, counted, and discarded as liquid waste. Empty scintillation vials are rinsed and discarded in the normal waste.

7) The chromatography columns are washed and reused. There is therefore no solid waste.

7. Labelling DNA/RNA

1) Purified DNA/RNA is incubated in an Eppendorf tube with other assay components (buffer, nucleotides, enzyme) and 25 kBq [gamma^{32}P] dCTP as radioactive tracer.

2) After the prescribed assay period the contents of the Eppendorf tube are placed in a G50 microspin column, the column centrifuged, and the flowthrough containing the probe collected. The probe will be stored appropriately at -20°C for further use or rinsed into the sink and the clean tube discarded as normal waste.

3) The G50 will be rinsed into the sink from the spin column and the column washed with a 4% Decon solution before checking with a Geiger counter and discard into the normal solid waste.

8. Probing nitrocellulose membranes.

1) [gamma^{32}P] dCTP labelled DNA probe will be incubated in hybridization buffer with nitrocellulose membrane in a sealed Perspex tube in a dedicated “hot” over in the Radiochemistry facility.

2) After incubation the used probe will be either stored appropriately at -20°C for reuse or disposed by rinsing into the sink.

3) The [gamma^{32}P] dCTP containing nitrocellulose membrane will be washed with successive buffers and each buffer will be disposed into the sink.

4) On completion of washing, the [gamma^{32}P] dCTP containing membrane will be wrapped in Saran wrap and the Perspex tube rinsed before checking with a Geiger counter. All washing areas and the oven will be checked with a Geiger counter.

5) The wrapped [gamma^{32}P] dCTP containing membrane will be blotted with tissue to check for leakage and exposed to film in an imaging cassette. Prior to film development, the wrapped membrane will be removed and the cassette checked for contamination before analysis.

6) The [gamma^{32}P] dCTP containing membrane will be discarded with liquid waste by macerating. There is therefore no solid waste.

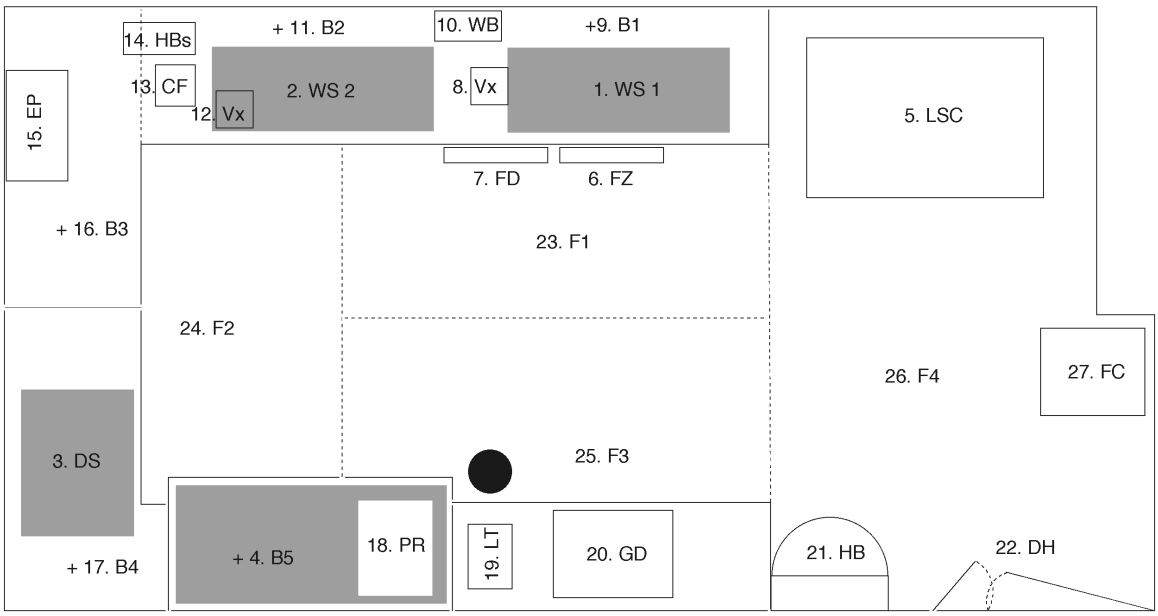
Appendix 2 RISK ASSESSMENT FOR USE OF RADIOISOTOPES

The following examples can be viewed in a separate document (Martin Cann).

RRA Regulation of Adenylyl Cyclase by Carbon Dioxide
RRA ADPase Assay
RRA *In Vitro* Adenylyl Cyclase Assay
RRA *In Vitro* Guanylyl Cyclase Assay
RRA *In Vivo* cAMP-cGMP Accumulation
RRA Probing Membranes with Radiolabelled Probes
RRA Radiolabelling DNA-RNA
RRA Prep of RNA bioconjugates by *in vitro* transcriptions
RRA Regulation of Adenylyl Cyclase by Carbon Dioxide - P_i uptake

Appendix 3 - CONTAMINATION MONITORING PLANS FOR LABORATORY 217A

Lab 217A Monitoring map (revised January 30/1/12)



Number	Description
1. WS 1 (Hot area)	Workstation 1
2. WS 2 (Hot area)	Workstation 2
3. DS (Hot area)	Disposal sink
4. B5 (Hot area)	Bench 5 (fume hood floor)
5. LSC	Liquid scintillation counter (including keyboard)
6. FZ	Freezer
7. FD	Fridge
8. Vx	Vortexer
9. B1	Bench 1 (surrounding WS1)
10. WB	Waterbath
11. B2	Bench 2 (surrounding WS2)
12. Vx	Vortexer
13. CF (Hot area)	Centrifuge
14. HBs	Heater blocks
15. EP	Electrophoresis kit (inc powerpack buttons)
16. B3	Bench 3 (surrounding EP)
17. B4	Bench 3 (surrounding DS)
18. PR	Platereader
19. LT	Laptop (including keyboard)
20. GD	Gel dryer
21. HB	Hand basin
22. DH	Door handle
23. F1	Floor area 1
24. F2	Floor area 2
25. F3	Floor area 3
26. F4	Floor area 4
27. FC	Filing cabinet

Appendix 4 - Hot area monitoring

Hot area monitoring

CONTAMINATION MONITORING RECORD HOT AREAS													LABORATORY 217A
Date	Name	Isotope	Monitor	Contamination (Bq. cm^{-2})									Notes
				WS1	MCF1	WS2	DS1						

Monitoring before session

Monitoring after session

Appendix 5 - Monthly Laboratory Contamination Monitoring

LABORATORY CONTAMINATION MONITORING										**LABORATORY 217A**			
Date													
Isotope													
Monitor													
Hot areas													
WS1													
MCF1													
WS2													
DS1													
FD													
CF													
EP													
Warm areas													
LSC													
FZ													
WB1													
Vx1/2													
B1													
B2													
B3													
B4													
B5													
F1													
F2													
F3													
HEB 1/2/3/4													
Performed by:													

Appendix 6 - Decontamination Log

Decontamination Log

Laboratory 217A

Date	Action taken	Name