Document Number: ANP039	Title: **EXPERIMENTAL ANIMAL PROCEDURES INVOLVING RADIOACTIVE PRECURSORS**	Effective Date: January 2005
Section: Animal Research		Supersedes Date:
Subsection: Facility	***CONFIDENTIAL INFORMATION*** **MOLECULAR MEDICINE RESEARCH INSTITUTE**	Page: 1 of 3

1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedures for the use of radioactive precursors in experimental animals at the MMRI Animal Research Facility.

2.0 SCOPE

2.1 This procedure applies to animals used for experimentation.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

5.1 SOP# ANP005, Euthanasia of Rats and Mice

5.2 MMRI Radiation Safety Manual

5.3 SOP # ANP021, Radioactive Carcass Disposal.

5.4 SOP # ANP012, Collection of Blood, and Urine and Fecal Samples.

6.0 SAFETY PRECAUTIONS

6.1 It is the responsibility of all personnel to use good judgment and safe practices in the laboratories. Protective clothing (e.g., laboratory coats, coveralls, boots, face masks, aprons, rubber gloves and safety glasses) is provided by the company.

6.2 Two layers of disposable rubber gloves, closed toe shoes, shoe covers, and safety glasses are worn when handling radioisotopes. Following each experimental manipulation using an isotope, hands will be monitored using a calibrated Geiger-Muller counter. If monitoring indicates that contamination has occurred the outer layer of gloves will be removed and placed in the disposal container appropriate for the isotope being used. Shoe covers will be removed when leaving the designated radiation area and hands and shoes will be monitored for contamination.

6.3 Disposable plastics used will be disposed of in the appropriate disposal container.

6.5 All injury accidents are promptly reported to the appropriate Supervisor.

7.0 EQUIPMENT AND MATERIALS

7.1 Preparation of Animal Housing for Containment of Radioisotope.

7.1.1 On the day of study disposable cages (Ancare, Bellmore, NY) will be lined with disposable absorbent diapers in preparation to receive animals. Diapers will act to contain urine excretion and fecal pellets.

7.1.2 If water provision is called for, a hydration gel pack ("Napa Nectars" from Systems Engineering, Napa, CA) is placed in each cage immediately prior to placing animals in cages.

7.2 Equipment and other supplies

7.3.1 Disposable: syringes, gauge 15 x3" gavage needles (Popper and Sons, New Hyde Park, NY), 20-200 uL sterile pipette tips, 200-1000 uL pipette tips, 15 mL graduated, conical plastic centrifuge tubes, 50 mL graduated, conical plastic centrifuge tubes, sterile 1 mL tuberculin syringes, 5 ml pipettes.

7.3.2 Plexiglas shielding, minimum of 1 cm thickness. Sufficient shielding is required to protect the experimenter while isotopes are being manipulated and animals are being gavaged, and to house all cages and animals for the duration of the experiment. Shielding is also required for use as refrigerator inserts for storage of animal carcasses.

7.3.3 Radioisotopes. These will vary depending on the study, and end-points to be measured. The isotope being used must comply with the approved isotope list found in the MMRI radiation license.

8.0 PROCEDURE

8.1 Radioactive precursors introduced in vivo by oral gavage. Radioisotopes will be administered in trace amounts, at concentrations defined by individual studies and isotopes being used. Studies will proceed in the designated MMRI radiation room. Rodents will be given the appropriate dose of isotope by oral gavage, and will remain in the cage for a designated period (no longer than 4 hr, and as outlined in the protocol).

Following each study, diapers will be disposed of in the appropriate dry waste container for decay. Cages will be monitored for residual isotope contamination, and if required will be rinsed with water from the designated sink disposal area, and trace amounts of this liquid waste stream will be disposed of into the sink. All incidences of cage levels higher than background that require washing will be recorded. Contaminated cages where contamination is not removed by washing, will be stored to allow for decay of

the isotope to background levels before re-use in the instances of short half-life isotopes, or stored for removal if the isotope is either C^{14} or H^3. All animal carcasses will be stored at –20^{o}C, behind Plexiglas shielding, and will be stored to allow for decay for short half-life isotopes, or stored for removal if the isotope is either C^{14} or H^3.

Animal tissue or fluid samples collected from these studies will be handled in the MMRI designated radioisotope room only.

Work areas will be monitored as outlined in the MMRI radiation safety manual.

8.2 Radioactive precursors introduced ex vivo using the intestinal sac model. 7-8 week old SD rats (Charles River) are starved overnight. Animals are made unconscious by CO2 inhalation and euthanized using a guillotine. The intestine is dissected out and washed the inside with saline. The first 12.5 cm from pylorus (duodenum) is removed. The second 12.5 - 17 cm segment containing the jejunum is tied to a glass rod using surgical thread, and is everted so that the mucosal surface facing out side. The inside out intestine is then filled with the 1.7 ml of buffer and three equal size sacs are made by tying the intestine with surgical thread and by cutting in between.

Sacs are placed in glass vials containing 2 ml of the buffer with 32P inorganic phosphate 1 μCi/10ml and incubated at 370C for 60 min with aeration (95% CO2 and 5% O2). At the end of the experiment sacs are washed with saline and contents are taken by cutting with a pair of scissors. 100 ul aliquots of inside and out side buffer are read on a scintillation counter.

Animal tissue or fluid samples collected from these studies will be handled in the MMRI designated radioisotope room only. Animal tissue or fluid samples contaminated with radioisotope will be stored to allow for decay for short half-life isotopes, or stored for removal if the isotope is either C^{14} or H^3.

Work areas will be monitored as outlined in the MMRI radiation safety manual.