Document Number: ANP017	Title: **NECROPSY PROCEDURES**	Effective Date: January 2005
Section: Animal Research		Supersedes Date:
Subsection: Facility	***CONFIDENTIAL INFORMATION*** **MOLECULAR MEDICINE RESEARCH INSTITUTE**	Page: 1 of 16

1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedures to perform necropsy, and tissue collection of animals from 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) are provided by the company.

6.2 Disposable rubber gloves, closed toe shoes and safety glasses are worn when dissecting animals or otherwise handling animal tissues. Safety glasses will be worn while observing or performing necropsies, and handling preservatives.

6.3 Surgical face masks, or equivalent, are worn upon entering the laboratory during the necropsy of a non-human primate or animals in which infective agents are known or suspected to exist.

6.4 Used disposable knife blades, hypodermic needles and syringes are placed in a disposable sharps container located in each necropsy laboratory. When full, the container is disposed of safely.

6.5 Personnel present at the necropsy of animals suspected or known to have infectious diseases or contain radioactive material diseases or contain radioactive material shall be informed of the potential hazard.

6.6 Special precautions are required for handling radioactive carcasses and instructions for handling can be found in MMRI Radiation Safety Manual and in SOP # ANP021, Radioactive Carcass Disposal.

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

7.0 EQUIPMENT AND MATERIALS

7.1 Preparation of Fixatives

7.1.1 Formalin is purchased as 37-40% formaldehyde, which is known as 100% formalin. This solution should be diluted at the ratio of nine parts water to one part formalin for a 10% solution and should be isotonic and buffered to prevent the distortion of cells and the formation of formalin pigment in the fixed tissue. Formalin is buffered by 4.0 grams of sodium monophosphate; 6.5 grams sodium disphosphate.
10% buffered formalin can also be purchased from scientific supply vendors.

7.1.2 Neutral Buffered Formalin Solution

37-40% Formalin	100 mL
Deionized Water	900 mL
Sodium phosphate monobasic (98+ %)	4.0 g
Sodium phosphate dibasic (98+ %)	6.5 g

7.1.3 Formalin-Sodium Acetate Solution

37-40% Formalin	100 mL
Sodium Acetate (98+ %)	20 g
Deionized Water	900 mL

7.1.4 Formalin-Alcohol-Acetic Acid Solution

37-40% Formalin	100 mL
Ethanol, (reagent)	90 mL
Glacial Acetic Acid (reagent)	5 g

7.2 Equipment and other supplies

7.2.1 Instruments: scalpels, scissors, forceps, necropsy board

7.2.2 Disposable: syringes, absorbent pads,

7.2.3 Other Solutions: euthanasia solution, 0.9% normal saline,

7.2.4 Storage supplies: 40 ml fomalin jars, 1 gal ziplock bags, labels, necropsy data sheets, shipping box, hazard sticker

7.2.5 Balance, perfusion apparatus,

8.0 PROCEDURE

8.1 Definitions

8.1.1 The Necropsy area is an area established for the purpose of conducting procedures associated with postmortem examination of animals. It is designed for safe necropsy activity, data acquisition and tissue handling.

8.1.2 A Necropsy technician is an individual qualified by experience, education, or training to dissect an animal and evaluate the gross appearance of the various tissues and organs. Individuals shall also have knowledge of animal physiology as well as the technical aspects of the necropsy laboratory operation, i.e., balance, computer data entry , preservative preparation, record keeping, specimen collection and tissue sampling during scheduled necropsies. Only personnel with appropriate training will be responsible for a necropsy procedure.

8.1.3 A Pathologist is a physician, veterinarian or specialist who interprets and diagnoses the changes caused by diseases or toxicity in tissues and organs.

8.1.4 Necropsy is a systematic approach to the dissection of animals to examine organs and tissues.

8.1.4.1 A complete necropsy is the systematic dissection of an animal to allow examination of body cavities, tissues and organs. The organ systems to be examined in a complete necropsy are:

Cardiovascular	Musculo-Skeleton
Digestive	Nervous
Endocrine	Reproductive
Hematopoietic	Urinary
Integumentary	Respiratory

8.1.4.2 A partial necropsy is the dissection of an animal to make visible for examination a limited set of specified body cavities, tissues and organs.

8.2 Tissue Preservation: The tissues/organs to be preserved from each animal are to be specified by the study protocol. For definition purposes the following will apply.

8.2.1 Complete: Tissue specimens are preserved so that all organ systems are represented from a complete necropsy.

8.2.2 Partial: Preservation of specimens from selected organ systems only.

8.2.3 Altered: Preservation of tissues found to be altered at necropsy.

8.2.4 None: No tissues preserved during necropsy even though gross pathological changes may be observed and described.

8.3 Special Terms

8.3.1 Altered Tissue: Tissue that is found to be altered during collection or manipulation.

8.3.2 Tissue Mass: Any enlargement of tissue that is not associated with or originating from a recognized organ is a tissue mass. The words "Tissue Mass" or letters "TM" are used for identification. If the mass is attached to a recognizable organ, it can be described as a mass of that organ and is subject to pathologist microscopic verification.

8.4 Abbreviations are sometimes used to record data or to identify tissues and organs in tissue cassettes. Explanations of abbreviations used will be provided and maintained in the raw data of the specific study.

8.5 General Considerations

8.5.1 Perform a necropsy on all animals found dead (or submitted for euthanasia due to morbidity) prior to study termination. Collect and preserve all tissues specified in the specific study protocol. Determine a gross diagnosis as to the probable cause of death, if possible.

8.5.2 Animals that reach scheduled euthanasia and animals that die during or after blood collection must have all tissues collected, weighed and preserved as required by the protocol.

8.5.3 Ensure all animals in a study have a unique identity. The identity of each animal is an individual cage card and/or ear tag or tattoo. Label each preservative container and specimen slide with the unique animal number, study number, type of tissue, initials and date.

8.6 Euthanasia

8.6.1 Verify each animal's identification at the time of euthanasia. Compare the cage card information with the ear tag, ear punch or tattoo, etc.

8.6.2 Euthanize the animal following SOP# ANP005. The study specific protocol specifies the type of euthanasia. Record the method of euthanasia in the original raw data for the study.

8.6.3 At the time of euthanasia and necropsy for study animals, the order of euthanasia must progress across dose groups. All animals in one group must not be euthanized before those in another group, unless otherwise specified.

8.6.4 Timing the euthanasia of an individual animal in a series of animals is critical to expedite the necropsy and to preclude postmortem changes. Discretion for the timing is left to the Study Director in charge of the necropsy. No more than three animals are euthanized in advance of the time of necropsy.

8.7 Preservation of Tissues

8.7.1 Standard Tissue Preservative: Formalin, 10% neutral buffered. The terms "formalin", "formalin solution" or the abbreviation "NBF" or "10% NBF" refer to this standard fixative solution. The ratio of organ volume: fixative volume must be at least 1:10.

8.7.2 Other preservatives may be required. Study specific protocols may call for other preservatives. Documentation of these preservatives will be maintained in the raw data for that study.

8.7.3 Refer to section 7.1.1 for preparation of fixatives.

8.8 Animal Dissection

8.8.1 While no single approach to dissection is established, it is stressed that each technician establish routine procedures to systematically expose, examine, remove and prepare tissues for examination, weighing and preservation. Rapid handling and processing is critical to properly preserve tissues and organs.

8.8.2 During any of the dissecting procedures, gentle handling of the tissues and organs is of paramount importance. Water or other non-fixative liquid must not come into contact with small tissues and organs because of potential induction of artifacts.

8.8.3 Generally, one technician will necropsy a mouse or rat. A team approach (two or more persons) may be used for larger species).

8.8.4 At the end of each animal's necropsy, the tissues preserved are reviewed by the technician to assure that all tissues specified in the protocol were collected. When the team approach is used for larger species, the list of tissues to be preserved is read for affirmative responses by the person placing the tissue into the preservative. The reader documents under comments whether or not all the specified tissues were preserved and initials and dates the entry.

8.9 Organ/Tissue Examination and Preservation

8.9.1 Organs and tissues to be harvested and preserved are specified in the study protocol; however, tissue sampling must be standardized relative to region. In general, organs less than 0.5-0.7 cm thick can be preserved intact by immersion in preservative. Larger tissues may require additional handling, i.e., trimming or sectioning for fixation.

8.9.2 Cardiovascular System

8.9.2.1 Heart: Trim the heart of associated great vessels and pericardial sac. Weigh the heart and inspect it externally. Hearts from mice and rats are not examined internally. For larger species, open the heart to visualize all ventricular endocardial surfaces. After weighing, preserved the entire heart.

8.9.2.2 Aorta: Take a tube segment of 1-3 cm from the mid-thoracic region. Take care not to stretch the aorta during removal. Remove the mouse's aorta with the spinal column attached.

8.9.3 Digestive System

8.9.3.1 Salivary gland: After inspection, preserve the entire gland or section. In small animals, excise the mandibular salivary gland and mandibular lymph node together unless specified otherwise.

8.9.3.2 Small intestine: Collect all three regions of the small intestine (duodenum, jejunum, ileum). If the intestines are too large, they may be cut up into sections, but each section should be weighed. Insert a blunt needle into the duodenum and insert sufficient fixative. Alternatively, slice the intestine lengthwise and fix in an "open" manner.

8.9.3.3 Large intestine: Collect, examine and prepare all three regions (cecum, colon, and rectum) as outlined for the small intestine.

8.9.3.4 Pancreas: For large species, preserve a short strip from the caudal lobe. For small species preserve most or all of the gland.

8.9.3.5 Liver: Excised and weigh the whole liver. Harvest the whole liver of the mouse. For rats and larger species, take one 0.5 - 0.7 cm section (including the free borders of the lobes) from at least two different lobes.

8.9.3.6 Gall bladder: Remove, drain, examine and preserve the gall bladder as specified by the study protocol. Rats have no gall bladder.

8.9.4 Endocrine System

8.9.4.1 For these purposes, this system consists of the pituitary gland, thyroid glands, parathyroid glands, adrenal glands and pancreatic islets.

8.9.4.2 Sample the endocrine pancreas with the exocrine pancreas.

8.9.4.3 Harvest the parathyroid glands with the thyroid gland. Take the thyroid gland from rodents with the tracheal/esophageal block.

8.9.5 Hematopoietic System

8.9.5.1 The organs and tissues that produce blood elements include bone marrow, spleen, lymph nodes and thymus gland.

8.9.5.2 Bone Marrow: Air dried, methanol fixed smears and/or formalin fixed sections of sternum or femur are preserved if required. When specified, bone marrow smears will be taken from animals killed for cause. For rodents, the marrow section will be included with femur; other marrow may be presented but will not be reported unless altered. The spleen, mesenteric and mandibular lymph node(s) (or sections thereof) and thymus gland are preserved.

8.9.6 Integumentary System

8.9.6.1 Organs and tissues of this system consist of skin, subcutaneous tissues, ex orbital and auditory sebaceous glands. Take routine skin specimens from the mid-abdominal region.

8.9.6.2 For dermal studies, take the skin sample from the middle of the application site. Labeled tissue containers are provided to identify the skin-application site.

8.9.7 Musculo-Skeletal System

8.9.7.1 Tissue sampling includes muscles from the rear leg usually with the sciatic nerve attached. Examine the surface of the coxo-femoral joint and preserve a portion if abnormalities are seen.

8.9.7.2 For rodents when required by protocol, preserve the total knee joint (femorotibial joint) together with femur and femoral bone marrow. A short segment of tibia and/or fibula may also be part of the total joint sample.

8.9.7.3 For larger species, preserve one joint surface and/or joint capsule from the knee.

8.9.7.4 For rodents, the bone sampled is the femur. The cross-sectional sample from larger species is taken at the junction of the middle and distal thirds of the femur.

8.9.8 Nervous System

8.9.8.1 The components of this system include the eyes, brain, spinal cord and sciatic nerve.

8.9.8.2 Eyes: Remove the eyes with minimal tension applied to the optic nerve and minimal pressure applied to the entire globe. A small portion of orbital tissue, including the Harderian /lacrimal gland, may remain attached to the eyes.

8.9.8.3 Brain: Weigh the entire brain. Preserve the brain when required. When tissue preservation is not required, the cerebral hemispheres and cerebellum are cross-sectioned and examined.

8.9.8.4 Spinal Cord: Excise and preserve a section of the spinal cord with the vertebral column when stated in the study protocol.

8.9.8.5 Sciatic Nerve: The sciatic nerve is routinely preserved and left attached to the skeletal muscle specimen. When specified by protocol, the nerve can be separated.

8.9.9 Reproductive System

8.9.9.1 This system consists of the following organs or structures:

Male	Female
epididymides	cervix
mammary glands	clitoral gland
penis	clitoris
preputial gland	mammary glands
prostate gland	ovaries
seminal vesicles	oviducts
scrotum	uterus
testes	vagina
urethra	vulva

8.9.9.2 Males: Preserve only the testes unless otherwise stated in the protocol. Preserve the testes of rodents and immature animals intact; incise the capsule to allow fixation. For larger animals, section transversely before preservation.

8.9.9.3 Females: Preserve only the ovaries and uterus unless otherwise stated in the protocol. Preserve the entire uterus except for large domestic animals. For large domestic animals, preserve tubular segments from the midsection of each uterine horn approximately 3.0 cm long. Weigh and preserve the cervix with the uterus in all species. Preserve the vagina and vulva in tubular sections.

8.9.10 Urinary System

8.9.10.1 These organs and tissues include kidneys, ureters, urinary bladder and urethra. Unless specified, only the kidneys and urinary bladder are harvested.

8.9.10.2 Kidneys: Cross-section (parallel incisions) the kidney starting from the lesser curvature and on either side of the hilus. Generally, the capsule is left intact, however, it may be removed to facilitate examination. Routine kidney samplings are the central hilar section (0.5-0.7 cm thick) and one pole.

8.9.10.3 Bladder: Preserve the entire bladder for the common laboratory animal species.

8.9.11 Respiratory

8.9.11.1 Excise the lungs and preserve in the appropriate fixative. Slice them into smaller sections, if necessary. Lungs may also be filled with formalin, via the trachea, and tied with a suture, to preserve the 'normal' physiologic cavity dimensions of the lung.

8.9.12 Special Tissues/Procedures/Cassette Labeling

8.9.12.1 During the course of a study, tissues or organs may have changes that are of potential neoplastic origin and/or other disease states that warrant pathologic examination beyond gross evaluation. Samples of those alterations may be taken for further pathologic examination even though no mention of such procedure is made in the study protocol.

8.9.12.2 Tissue masses may be seen for the first time prior to or at the time of necropsy. Those tissue masses identified by the technician and those found at necropsy are described, sampled and preserved in a manner to facilitate further examination. Sampling should include representative areas of each tissue mass and may require multiple labeled cassettes.

8.9.12.3 If a tissue mass is identified in-life, but then subsequently disappears, examine the anatomic region in question at necropsy.

8.9.12.4 When a mass is found during necropsy, the prosector must decide whether or not it represents one described during the "in-life" phase, or if it is a new and distinctly different mass. Gross description of the mass will be entered under the appropriate organ or location.

8.9.12.5 All "in-life" identified masses present at the time of the animal's demise must be addressed in the gross data by giving a gross description of the mass.

8.9.12.6 Regional lymph nodes associated with the drainage fields for the tissue masses are located, if possible, and saved in labeled cassettes to maintain their identity.

8.10 Documentation

8.10.1 All observations of the necropsy will be documented on the Gross Necropsy Sheets (Appendix I). The sheets will contain the Study Number, Study Type, Date of Necropsy, Animal ID, Species, Strain, Group Number, Dosage and End Weight. The weight, color, shape and texture of each tissue/organ examined will be documented.

8.10.2 The following will be marked as normal or described, if abnormal: external surface, nasal and paranasal sinuses, injection site, cranial cavity, abdominal cavity, thoracic cavity and pelvic cavity and viscera. When the necropsy and documentation is complete, the person performing the necropsy will sign and date the forms.

8.11 Cassette labeling

8.11.1 The organ, animal identification and, if sufficient space is available, the study number is written on the cassette in pencil.

8.12 Tissues/Specimens Not Preserved

8.12.1 Tissues with certain conditions or alterations may not require preservation. Examples of such conditions are haircoat staining with feces, urine, or staining associated with dacryorrhea or otorrhea.

8.12.2 Staining from a mammary gland may be observed in a pregnant female on a teratology study. Alopecia can be seen as a normal event due to nesting instincts. Unless these changes are seen with distinct dosage-related patterns, they need not be described or preserved. Torn or scabby ears from ear tagging or notching, tail encrustations associated with scratching, biting or self-mutilation will be described but may not be preserved unless specified otherwise. Small lobules of liver may be seen in the rat and mouse to protrude into the thoracic cavity. This is also considered to be normal variation and need not be described or harvested. Tail nodules are described, if multiple nodules are present, a representative sample will be taken.

8.13 Organ Weighing

8.13.1 Organs to be weighed are specified in the study specific protocol. During the course of any study, animals may die or be euthanized for cause due to debilitating or moribund condition. Unless specified otherwise, organ weights are not taken for those animals, since concurrent controls are usually not necropsied at that time.

8.14 Balance Failure

8.14.1 If the balance fails during necropsy and another balance is not immediately available, organs that have been harvested but not weighed, are immediately placed in preservative. Those organs not weighed are recorded on the Necropsy Form. Organs are usually not weighed after preservation. Subsequent necropsies will not be conducted during that session until repair or replacement of the balance is completed or until the supervisor and/or the study director has been notified.

If data is being input directly into a computer or other type of processor and it fails, organs may continue to be weighed on the balance and the data recorded manually. The manually recorded data forms are signed and dated as original data by the weigher and counter-signed by the prosector. Organ weights recorded manually may be entered into the computer at a later time.

8.15 Transfer of Specimens to Histology Laboratory

8.15.1 Specimen containers used to hold preservative and tissues after necropsy are identified by the study number, animal number, and specimen identity.

8.15.2 Prepare the specimens for shipment by placing 6-8 labeled containers in a 1 gallon zip lock bag. Seal the bag and place all bags in a sturdy shipping box. In the box, include a specimen list and processing instructions.

8.15.3 Seal the box with packaging tape. Place a shipping label and a hazardous substance sticker on the box. Complete the FedEx air bill and place the box at the FedEx Pick-up area.

8.15.4 When the specimens have been processed and slides are returned from the histology lab, place the slides and their cover letter in the room with microscopes.

APPENDIX I

GROSS NECROPSY					
Study No:		Study Type:			
Animal ID:		Species:		Strain:	
Group No:		Dosage:		End Wt:	
TISSUE/ORGAN	WEIGHT	COLOR	SHAPE	TEXTURE	COMMENTS
Skin					
Spleen					
Pancreas					
Lt. Kidney					
Rt. Kidney					
Adrenal Glands (2)					
Liver					
Gall Bladder					
Ovaries					
Uterus					
Lt. Testes					
Rt. Testes					
Urinary Bladder					
Sm. Intestines					
Lg. Intestines					
Stomach					
Aorta					
Heart					
Thymus Gland					
Lung					
Thyroid/Trachea					
Skel. Muscle					
Fat					
Femur w/Marrow					
Lymph Nodes					
Salivary Glands (2)					
Eyes					
Harderian/Lacrimal					
Brain					
Pituitary Gland					
Spinal Cord					
COMMENTS					
SIGNATURE:				DATE:	

Form # 10a (Jan. 1, 2005) SOP# ANP017

Appendix II

STUDY NO. _____ STUDY TYPE: _____

ANIMAL ID _____ SPECIES _____ STRAIN _____

GROUP NO. _____ DOSAGE _____ END WEIGHT _____

	NORMAL	DESCRIPTION IF ABNORMAL
EXTERNAL SURFACE		
NASAL AND PARANASAL SINUSES		
INJECTION SITE		
CRANIAL CAVITY		
ABDOMINAL CAVITY		
THORACIC CAVITY		
PELVIC CAVITY AND VISCERA		
COMMENTS		
SIGNATURE:		DATE:

Appendix III

Specimen List

CTRX STUDY NO.
PRINCIPAL INVESTIGATOR / CONTACT:
PURPOSE OF STUDY:
ANIMAL SPECIES:
DATE TISSUE FIXED
Number of containers
Sequence of numbered containers
Total number of tissues
TISSUES OR SAMPLES OF INTEREST:
Method of Fixation
SECTIONING INSTRUCTIONS
STAINING INSTRUCTIONS
REQUIRED NO. OF SECTIONS PER SLIDE:
STAINING REQUIRED PER LUNG
SPECIAL INSTRUCTIONS:
NAME OF HISTOPATHOLOGY LAB:
ADDRESS:
PHONE NO.:
CONTACT PERSON:
DATE TISSUES SENT TO LAB:
DATE TISSUES RETURNED:
CTRX: Animal Numbers: