Document:	**IACUC –Special Procedures**		
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Purposes

1. This Standard Operating Procedure (SOP) describes the special procedures for IACUC protocols. Proposals with deviations from these policies will require PIs to compel with written scientific justification (including references) provided to IACUC for approval.

2. Compelling written scientific justification must be provided to the IACUC and approved if deviations from these policies are proposed.
   Exceptions will be considered on a case-by-case basis.

Procedure

1. **Tumor Size:** No greater than 1.5 cm tumor size (at largest dimension) or 2000 mm3 in total volume. Animals must be euthanized if tumors become ulcerated, infected or interfere with mobility.

2. **Physical Restraint:** Should be the minimum period required to accomplish the research objective. No more than 6 hours of physical restraint, without opportunity to exercise for at least 1 hour.

3. **Tail Clip (mice):** A single tail clip of up to 5mm is allowed. No anesthesia required up to 2 weeks of age.

4. **Toe Clip (mice):**

(i) In accordance with NACLAR Guidelines and IACUC guidebook, Singhealth IACUC discourages the use of toe clipping as a method of identification. Principal Investigators must provide strong scientific justification in their protocol application and prior IACUC approval must be obtained before performing the procedure

(ii) “As a method of identification of small rodents, toe clipping should be used only when no other individual identification is feasible”- The Guide for the Care and Use of Laboratory Animals

(iii) When individual pup requires “both” early identification and determination of genotype, toe clipping is allowed in neonatal mice up to 7 days of age without anesthesia. Anesthesia is required when performed on mice between 8-14 days of age.

Note: In recent studies, (iii) is considered a refinement by making it unnecessary to perform 2 different procedures. **(Bonapart et al,** FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents

(iv) Toe clipping is restricted to the most distal bone of the toe (3rd phalanx). Instruments for toe clipping must be aseptic and appropriately disinfected in between use and the procedure must be performed by a trained personnel.

(v) If toe clipping is performed, a maximum of one toe per foot maybe clipped do not clip 1st digit or thumb on each forepaw.

(vi) If toe clipping is used for genotyping but not for permanent identification, only one digit per animal is allowed to be clipped (except for the 1st digit or thumb of each forepaw). This may be followed at a later date by a form of permanent identification

Note: recent study also advocates early, pre-weaning genotyping. This approach has many advantages: the animals are easier to handle; some tissue samples yield more DNA as they are less ossified; and genotyping results are available before the weaning date, allowing for the better planning of experiments and management of the colonies.

5. **Blood Collection:** The amount of blood collected should be no more that 10% of blood volume in a single bleed and no more that 20% in a two week period. Exceptions may be granted when fluid replacement is included to prevent hypovolemia and hematocrit is monitored to detect anemia.

6. **Food and/or Water Restriction:** Overnight fasting, up to 16 hours, is generally allowed. Behavioral projects must consider alternative methods of motivation.

7. **Multiple Survival** Surgeries: Generally discouraged, but considered if required to achieve the desired physical or physiological effect and there is no alternative available. Sufficient time between surgeries must be allowed for proper recovery.

8. **Weight Loss:** Weight loss should not exceed 20% of body weight over an extended period or no more than 10% over a short period. Weight of growing animals should be compared to age matched cohorts.

9. **Death as an endpoint: Death as an endpoint is not approved. I**nvestigators must humanely euthanize all moribund animals rather than allowing them to die spontaneously.

10. **Human Endpoints:** Investigators must propose humane endpoints based on potential complications of the specific study. If unexpected adverse outcomes occur during the conduct of a study resulting in death, euthanasia or therapeutic intervention this should be reported to the IACUC in an

amendment. The humane endpoints of the study should be revised in the amendment to reflect the potential for these adverse outcomes recurring. (describing therapeutic intervention)

11. **Physical Methods of Euthanasia:** Decapitation of mice and rats using a guillotine and cervical dislocation should be done under anesthesia. In rare cases, with acceptable scientific justification, the IACUC may approve these methods without anesthesia, but must assure that they are performed by a skilled individual. Neonates up to 10 days of age may be euthanized by decapitation (complete separation of the head and body) or cervical dislocation without anesthesia when performed by skilled personnel.

12. **Trio Mating:** Mating systems using one male and two female mice in a standard sized cage is allowed as a housing space exception to the ILAR Guide, 8th ed. An in-house study demonstrating that there was no adverse effect on suckling and weanling mice housed in a trio-mating cage have been reviewed by the IACUC prior to adopting this policy.

13. **Social Housing and Environmental Enrichment:** All social species should be socially housed in pairs or groups whenever possible. If social housing is not possible environmental enrichment should be provided to solitary animals. Nonhuman primates that must be singly housed should have visual, auditory and olfactory contact with other NHPs, in addition to environmental enrichment. Justifications for single housing should be reviewed periodically by the veterinary staff.

14. **Non-pharmaceutical grade drugs:** A pharmaceutical-grade compound is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognized national or regional pharmacopeia. (The Guide, 8th ed). Whenever possible, pharmaceutical-grade compounds should be used for clinical or research purposes. For the clinical treatment of animals and to prevent or reduce/eliminate animal pain or distress pharmaceutical-grade compounds, which are generally available, must be used.. When the use of non-pharmaceutical-grade preparations is necessary and scientifically justified, (i.e., to accomplish the aims of a study) the investigator must assure that the chemical properties of the compound are appropriate for the study. For example this may include the route of administration, the purity, grade, stability in and out of solution, solution vehicle properties, pH, osmolality, and compatibility of the solvent and other components of final preparation The method of preparation, labeling, administration and storage of formulations should be appropriately considered with the aim of maintaining their stability and quality (i.e., to prevent inadvertent co-administration of infectious agents or contaminants).

15. **Harm Benefit:** During protocol review the IACUC will weigh the objectives of the study against potential animal welfare concerns in accordance with the principles of the “three R’s” (replacement, reduction, refinement). The potential benefits that are likely to accrue as a result of the research must outweigh the potential adverse effects of the study on the animals.

16. **Use of Expired Drugs:** Expired anesthetics and analgesics must not be used for either survival or non-survival procedures. Other expired drugs shall not be used for survival procedures, but may be used for acute procedures. Expired drugs should be stored separately from other drugs in a location designated for expired drugs. A manufacturers' statement that a drug is usable beyond the expiration date may be acceptable.

17. **Rodent Surgeries:** Sterile technique should be applied to survival surgeries in rodents. This includes removal of fur from and scrubbing of the incision, use of sterile instruments and some form of drape. A dedicated operating room is not required, but the area where surgery is performed (e.g., investigator's lab) should be clean and dedicated for that purpose while surgery is performed. Other activities in the surrounding areas should be minimized to prevent contamination of the surgical site.

18. **Animal Welfare Concerns:** Individuals who have specific concerns about animal care and treatment are encouraged to report their observations of suspected deficiencies verbally or in writing to the IACUC or Group Director, Research, or veterinary staff. Reports may be submitted anonymously, if desired. Mechanisms for reporting concerns will be posted in prominent locations in the facilities. Individuals reporting animal welfare concerns shall not be discriminated against or be subject to any reprisal for generating the report.

19. **Non-compliance:** Reports of non-compliance and/or animal welfare concerns will be investigated by the IACUC. The immediate welfare of the animals will be the first priority during the investigation. Corrective actions may range from amending procedures and re-training individuals to suspension of the protocol activities. In cases of chronic, willful non-compliance the individual's privileges to conduct animal research may be withdrawn.

20. **Deviations from Standard Environmental Temperatures:** Protocols approved for exposure of animals to extreme temperatures (e.g., cold exposure) require special monitoring. Core body temperature must be monitored and recorded. Humane endpoints must be established for intervention in the event animals do not adapt adequately.