NTU-IACUC Policies for Special Procedures

Before submitting an Animal Use Protocol (AUP) application, applicants should refer and adhere to the NTU-IACUC Policies for Special Procedures. For any exemptions from the following, there must be convincing scientific justification provided to the NTU-IACUC.

1. Tumour Size (mice)

For tumours that were induced by injection, the sizes must not be greater than 1.5cm (at largest dimension) or 2000mm^3 in total volume. Formula for the calculation of tumour volume: π/6 x L(mm) x W(mm) x H(mm).

2. Physical Restraint

a) Restraints must not be used as a convenience for managing/handling animals or as a substitute for competency training in handling animals.

b) The use of short-term restraint devices is acceptable as it limits the movement of the animal and allows the researcher to carry out examinations, collect samples, and administer drugs, chemicals or biological agents. It also addresses safety or injury concerns to both the researcher and the animal.

c) Prolonged restraint for a continuous period exceeding 15 minutes should be avoided, unless required for accomplishing the research objectives. In such cases, the researcher must provide to the IACUC the type and duration of restraint, how often the animals will be monitored, the animal's need for rest and exercise, and where possible the acclimatization program.

d) Veterinary care must be provided if severe behavioral change, lesions or illness is observed in the animals under restraint. Temporary or permanent removal of restraint may be necessary. The IACUC must be informed about any welfare concerns should they arise.

3. Tail Clip (mice)

A single tail clip of up to 1cm is allowed. No anesthesia required for mice up to 2 weeks of age.

4. Toe Clip (mice)

Generally discouraged, but allowed with justification. No anesthesia required for mice up to 12 days of age.

5. Blood Collection

A blood volume no more than 10% of the body weight in a single bleed and no more than 20% in a two week period can be collected (~100µl – 200µl). Drawing of blood from the retro-orbital vein.

6. Food and/or Water Restriction

Overnight fasting is generally allowed for a period up to 16 hours. Behavioral projects must consider alternative methods of motivation.

7. Multiple Survival Surgeries

Generally discouraged, but can be 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, as advised by Attending Veterinarian.

8. Weight Loss

No more than 20% weight loss over an extended period or no more than 10% weight loss over a short period (e.g. 5 days) Weight of growing animals should be compared to age matched cohorts.

9. Death as an endpoint

Death as an endpoint is not approved unless adequately justified and approved by the NTU-IACUC. Variable endpoints with estimated numbers should be included in the protocol and this will be reviewed on a case-by-case basis. Investigators must humanely euthanize all moribund animals rather than allowing them to die spontaneously.

10. Humane 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.

12. 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.

13. 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.

14. Footpad injections

Footpad injections in rabbits are prohibited but allowed in mice. Where scientific justification is provided, footpad injections may be permitted in rodents, but only in one hind foot, and with the animals housed on soft bedding. Suggested maxi-mum injection volumes can range from 0.01 to 0.05 for mice and 0.10 ml for rats. The need for footpad injections must be critically evaluated by the IACUC.

15. Induction of Diabetes Mellitus

Once hyperglycemia develops, mice are to be kept euglycemic with daily insulin injections of 5ul/kg administered via IP injection. When STZ is first injected, drinking water provided is to contain sugar; typically 30% concentration of sucrose in drinking water is recommended. For [1]low dose STZ, this sucrose solution is to be given for 5 consecutive days; [1]high dose STZ administration is generally discouraged. Upon onset of diabetes, regular bedding change and monitoring have to be conducted, along with regular checks for presence of ants in cages with sucrose solution; this must be filtered before use.

[1]Refer to article "**Streptozotocin-Induced Diabetic Models in Mice and Rats**" for more information.

16. Breeding

For highly fertile mice (e.g. ICR strain), one cage is limited to have 1 male and 1 breeding female. A maximum of 1 male and 2 breeding females are permitted to be held in a cage but should both females become pregnant, one of the pregnant females is removed.

17. Irradiation

In case of lethal or sub-lethal animal total-body irradiation, the dosage of radiation should be carefully considered and adjusted according to biological variables like type of animal strain, the age, and health status of used animals. Since irradiated animals remain immune compromised for an extended period of time, strict veterinary and husbandry care requirements are needed to ensure their well-being. Animals should be handled only under a HEPA-filtered flow hood or in a HEPA filtered laminar flow room and caretakers or research staff should always use aseptic techniques handling them. Administration of antibiotics in the drinking water should be considered to minimize the chance of potential bacterial infections and decrease the burden of gastrointestinal bacteria.

18. Micro Implantations

Animal implants generally should not exceed 5%-7% of the animal's overall body weight. For larger implants, proper justification must be presented to the IACUC. A general guide for size of implants in mice and rats is as follows:

Weight of Implant	0.4g	1.1g	5.1g
Minimum Animal Weight: Mice			
Subcutaneous	10g	20g	n/a
Intraperitoneal	20g	n/a	n/a
Minimum Animal Weight: Rats			
Subcutaneous	10g	20g	150g
Intraperitoneal	20g	150g	300g

19. Use of non-pharmaceutical grade drugs or other substances

The use of pharmaceutical grade drugs or substances in animals ensures that unwanted side effects are not introduced into the study, hence they should be used whenever such grade drugs or substances are available.

Should non-pharmaceutical grade drugs or substances be used, their use must be described in detail as well as justified in the Animal Use Protocol submission and approved by the IACUC. Instances where such use may be necessary would be to achieve the scientific goals of the project or when pharmaceutical grade drugs or substances are not available.

In addition, the following details should also be provided for IACUC consideration:

- Grade
- Sterility
- Osmolarity
- Site of administration
- Formulation and storage conditions
- Potential animal welfare and scientific issues relating to their use.