University Animal Care Committee Standard Operating Procedure		
Document No: **7.31**	**Subject: Tamoxifen Administration to Mice**	
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Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the procedures that are to be followed when administering tamoxifen to research mice.

1. Introduction and Definitions:

- Tamoxifen is a selective estrogen receptor modulator used in the treatment of breast cancer. In mice, tamoxifen is used to trigger tissue specific gene expression in genetically modified animals. It is commonly administered PO via gavage or in a special diet formulation.
- When working with Tamoxifen the facility SOP on the handling of Tamoxifen must be read and followed.
- Tamoxifen is a well-known human carcinogen that can lead to the formation of DNA adducts and possible teratogenicity, genotoxicity and reproductive toxicity. It causes sterility and late abortions.
- An Animal Welfare Assessment is required for all mice receiving tamoxifen. Anorexia, body condition loss and weight loss are expected and monitoring with supportive care will be required.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI,** subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

2. Monitoring Requirements and Supportive Care:

- An Animal Welfare Assessment will be required for all mice that are receiving tamoxifen, ***see Appendix 1***. Anorexia, body condition loss and weight loss are expected.
- Prior to administration of tamoxifen, a baseline body weight must be taken and recorded. The mice will then need to be weighed daily during administration and continue until the body weight returns to the baseline.

- For chronic administration in food or water, body weight should be recorded twice weekly for the duration of administration to monitor for inappetence and weight loss.
- An initial reduction in food intake and subsequent weight loss is expected when mice are initially fed a tamoxifen diet. Weight loss of 10% is common. If mice lose more than 15% of their body weight, tamoxifen administration must be stopped and nutritional supplements (diet gels) offered until body weight returns to baseline.
- If weight loss is more than 10% of the original body weight for your mouse model, consider alternative administration routes and/or decreasing the dose of tamoxifen.

3. Dosing Tamoxifen:

- Tamoxifen needs to travel to the target tissue at the appropriate concentration at the correct time to induce mutation.
- Dose rates quoted in the literature for gene activation (e.g., up to 100mg/kg) are far higher than those used therapeutically in humans (0.4mg/kg). This high dose may not be necessary and will increase the toxicity and adverse effects on the mouse. ***There may be a cumulative effect in tissues on repeat dosing.***
- Consider:
  - What organ is being targeted – higher doses may be required to penetrate the brain and activate the gene.
  - Age, strain and genotype of the mouse. Young mice may experience more adverse effects.
  - The dose rate carefully – recommend no more than 2mg per adult mouse.
  - Running pilot studies with lower doses to investigate effectiveness at doses closer to therapeutic levels, literature doses may be extremely high.

4. Preparation and Storage:

- Dissolve tamoxifen in corn oil to the desired concentration by shaking overnight at 37 degrees Celsius.
- Tamoxifen is light sensitive and must be stored in an amber-coloured bottle or wrapped in foil at 4 degrees Celsius for a maximum of 7 days or at -20 degrees Celsius for a maximum of 30 days. Expired tamoxifen must be discarded and cannot be used in live mice. Once thawed to room

temperature, the solution may require warming and mixing to ensure accurate dosing.

- Reconstructed solution must be labelled with the following information:
  - Drug Name
  - Concentration
  - Manufacturer/Lot#
  - Expiry Date
  - Date Reconstituted
  - Discard Date

5. Method of Administration:

- **Tamoxifen Diets:** *This is the recommended route and should be tried before other routes are attempted. Advantages are reduced handling and no restraint or injections as well as fewer adverse effects.*
  - Expected weight loss <10% with this method.
  - Diet dose is typically 40-80mg/kg of Body weight per day.
  - On average the treatment ranges from 1-2 weeks to 1-2 months
  - Giving tamoxifen in the feed reduces peak/variation in plasma levels if the mouse is eating well.
  - Older mice and certain strains may be more reluctant to eat the diet.
  - Staff and users must not overfill the food hoppers and the cages must be appropriately labelled. Tamoxifen diets require storage at 4 degrees Celsius or lower, frequent replacement of the diet in the hopper is recommended (e.g. replace diet once weekly).
  - Tamoxifen diet should be introduced gradually to minimize weight loss:
    - **Option 1:** Gradually acclimate mice by providing a mixture of tamoxifen containing pellets with regular feed pellets for 3-4 days. Gradually increase the ratio of Tamoxifen pellets to regular diet over 5-7 days.
    - **Option 2:** Feed tamoxifen daily Monday-Friday. Feed regular diet on weekends and holidays.
    - **Option 3:** For longer treatments: Feed tamoxifen on alternative weeks – two weeks on tamoxifen diet, one week on regular diet.

- **Oral Pipette or Gavage:**
  - Peak plasma concentrations are achieved after 3-6 hours following oral administration but will depend on the solvent used. Plasma half-life is approximately 12 hours in mice.
  - More adverse effects (anorexia, gut stasis) may be experienced.
  - Requires handling and restraint; risks associated with oral gavage.
  - Neonatal pups (5-10 days of age): use <10uL and place in mouth by oral pipette to allow mouse to swallow rather than oral gavage.
  - If repeat oral gavage is required, consider tamoxifen diet.

- **Subcutaneous Injection:**
  - Often administered in oil to achieve a depot effect; effect is detectable for weeks after injection.
  - The mixture is viscous and may require a slight warming to inject and the needle size may need to be 23 gauge.
  - Deliver injections at body temperature.

- **Intraperitoneal Injection:**
  - The potential impact on animal welfare is high. May result in peritonitis, inflammatory effects, precipitation in the peritoneal region (sterile peritonitis), and general risks associated with IP injection (penetration of organs, misdirected injection, adhesions).
  - Recommend single injection only (if other routes are not possible).
  - Usually tamoxifen is dissolved in alcohol/oil mixture before administering IP.
  - Must use newly prepared sterile solution every time.
  - Literature suggests tamoxifen is detectable in plasma around 12 hours.

Appendix 1:

ANIMAL WELFARE ASSESSMENT SCORE SHEET

Score Sheet: Assigning Scores A-E:

WELFARE PARAMETERS	SCORE
Body Weight Score ***(Weigh mice and compare to initial body weight)***	
Weight loss of 0-5%	0
Weight loss of 6-10%	1
Weight loss of 11-15%	2
Weight loss of 15% or more*	3
Mobility and Ambulation ***(Compare movement to that of a healthy animal)***	
Normal ability to ambulate	0
Reduced and slow mobility, but not visibly lame - can access food and water	1
Hindered ability, lame on one limb - can access food and water	2
Cannot ambulate – unable to use a limb, cannot access food or water	3
Responsiveness Score ***(Compare behaviour of mice to that of normal mice of that breed)***	
Bright, alert and responsive	0
Quiet but alert, ruffled fur and/or slight dehydration	1
Lethargic, hunched, sunken eyes, moderate dehydration and/or less responsive	2
Moribund	3
Respiration ***(Listen closely and observe if breathing seems laboured)***	
Normal	0
Mild increase – increased effort visible on the chest	1
Moderate increase – increased effort obvious on the chest	2
Sustained tachypnea or dyspnea (distress). Severe; open mouth breathing or holding elbows out from chest	3
Tumour Score ***(Measure with calipers and observe skin surrounding the tumour)***	
Noticeable size <0.5cm, skin overlying growth is normal	0
Small size 0.5-1.0cm or skin red/inflamed over tumour	1
Mid-size 1.0-1.4cm or skin red/inflamed over and around tumour	2
Large size 1.5cm or larger or skin is ulcerated/opened over tumour	3

** can be a score of 2 ONLY if prior approval from the Animal Care Committee*

WELFARE STATUS	TOTAL SCORE
Acceptable welfare. No mitigation required. Monitor	**0**
Mild welfare concern. Humane intervention may be required as per the AUP and Queen's SOPs and policies.	**1-2**
Moderate welfare concern. Humane intervention required as per the AUP and Queen's SOPs and policies.	**3-4**
Serious welfare concern. Severe stress or pain. Humane intervention by the Veterinarian required as per the AUP and Queen's SOPs and policies. If treatment or supportive care does not improve or resolve the welfare concern, humane euthanasia is required.	**5-8**
Unacceptable welfare. Immediate euthanasia is required.	**9**

Use the score sheet guide to determine intervention points and endpoints.

Date							
Initial Body Weight							
Current Body Weight							
% Body Weight Change							
A. Body Weight Score							
B. Mobility and Ambulation							
C. Responsiveness Score							
D. Respiration							
E. Tumour Score							
TOTAL SCORE (add A to E)							

References:

- **The Centre of Phenogenomics (TCP) SOP on Tamoxifen Administration**

SOP Revision History:

Date	New Version
September 9th, 2024	Added in Monitoring Requirements
October 30th, 2024	Updated the Score Sheets