**Humane Intervention Points**

This Standard Operating Procedure (SOP) is intended to provide instructions on how to set objective end points for humane intervention with animal research models. It is designed for use by research and veterinary personnel, and the NUS Institutional Animal Care and Use Committee (IACUC). This SOP is approved by the IACUC. Any exemption must be submitted for approval by the IACUC prior to its application.

**TABLE OF CONTENTS**

1. Introduction
2. Procedures
3. References
4. Appendix

**1. INTRODUCTION**

a. General

In using animals for research and teaching, prompt intervention to alleviate pain and distress is essential to protect animal welfare while promoting quality research outcomes. This SOP defines the planning and implementation components necessary to provide prompt assessment, treatment, and documentation for all animals used in research and teaching.

b. Requirements

Ensuring appropriate intervention points involves the combined efforts of the principal investigator, the IACUC, and Comparative Medicine (CM) to:

i. Determine the humane interventions that are appropriate for the study.
ii. Ensure that humane intervention points are clearly defined in the protocol.
iii. Ensure all personnel responsible for making animal observations have been adequately trained to observe, recognize, and document the intervention point parameters as approved in the protocol.
2. PROCEDURES

a. Instructions for research personnel

   i. Review literature of established models and alternative methods and determine the likely adverse effects and criteria for the assessment of pain and distress.
   ii. Consult CM veterinarians for possible study refinements designed to minimize pain and distress.
   iii. Apply relevant refinements as an alternative to choosing experimental endpoints that result in unrelieved or severe animal pain and distress.
   iv. Ensure the experimental endpoint is the earliest time point at which statistically valid results can be obtained.
   v. Choose intervention points that are balanced with experimental endpoints which are most likely to ensure that animals can be humanely maintained on study until they reach the pre-determined scientific endpoint.
   vi. Choose assessment criteria that are relevant to the nature of experiments.
   vii. Establish all responsible individuals involved in the care and observations of the animals and assign a clear chain of consultation for dealing with unanticipated events.
   viii. Schedule animal observations at an appropriate frequency to detect early signs of discomfort and facilitate implementation of early interventions.
   ix. Document all health parameters and procedural activities, including all observations and specific measurements or data (e.g. body weight, body condition scores).
   x. Ensure individuals assessing animals have appropriate training to recognize assessment criteria.
   xi. Increase the frequency of observation and measurements in response to a decline in the animal’s condition and during pre-determined critical periods during the study.
   xii. Review intervention points throughout the study to ensure they are appropriate and amend if required.
   xiii. Include intervention information when publishing study results.

b. Instructions for CM veterinary personnel

   i. Perform daily observation of animals on study.
   ii. Provide guidance and consultation to researchers in choosing humane interventions that are appropriate to their research and have the least impact on scientific outcomes.
   iii. Document relevant clinical findings on the health and procedural records.
   iv. Apply humane interventions where applicable and in consultation with relevant research personnel.
c. Instructions for IACUC

   i. Review intervention end points in protocols.
   ii. Approve appropriate intervention points listed in the protocol.
   iii. Refer the principal investigator to CM veterinarians for consultation if intervention points in the protocol need revision.

d. General guidelines for choosing humane intervention points.

   i. Use objective, measurable parameters whenever possible, with specific intervention. For example:
      1. Body weight loss expressed as a percentage of target or baseline body weight.
      2. Targeted level of liver enzymes or other blood markers.
      3. Targeted tumor growth rate or size. Use quantitative terms or numerical scores/measurements to assign objective measures of animal comfort.

   ii. Avoid use of general or ambiguous terminology and phrases, such as “analgesia will be administered as needed.” Specifically indicate what actions will occur at given time points e.g. “analgesia will be administered every 8 hours for 48 hours post operatively”.

   iii. Create score sheets or assessment checklists. Refer to instructions below on designing scoring systems:
      1. Refer to literature searches, previous experiments or pilot studies to determine optimal indicators of animal discomfort relevant to the study.
      2. Assign a numerical score for each observation e.g. provide a range of scores from 0 (normal to mildly affected) to 5 (significant change from normal).
      3. Monitor and record all scores at regular time points as predetermined through literature searches and pilot studies.
      4. Add the scores for all designated observations and use the cumulative score to derive the level of pain and distress each animal is experiencing.
      5. Use the cumulative scores as predictors of further deterioration and indicators for intervention.

e. Pilot Studies

   i. If experimental effects are unknown, a pilot study is recommended to establish clinical effects and time course for progression or resolution.
      1. Use the minimum number of animals/groups necessary to gather information from the pilot study.
      2. Ensure the evaluation methods represent an adequate frequency of observations for early detection of adverse effects.
3. Use pilot study results to establish scoring systems and humane intervention points for future research in similar areas.
   ii. Refer to the appendix of this document for examples of assessment criteria used to set intervention points.

3. References

APPENDIX A – DEFINITIONS

Humane intervention points: clear criteria set to define the point at which humane interventions must be implemented to prevent or relieve unnecessary distress and/or pain to a research animal.

Humane interventions: actions might include any of the following:

- Provide adequate veterinary treatment such as analgesia and/or supportive therapy to the animal(s);
- Cease performing procedures that contribute to distress &/or pain;
- Remove the animal(s) from the study;
- Modify the experimental procedures to minimize discomfort to the animal(s);
- Modify the housing and husbandry practices to improve the comfort of the animal(s);
- Increase the frequency of observation to ensure early identification of pain or discomfort;
- Humanely euthanize the animal.

Experimental endpoint: the point at which the scientific aims and objectives have been reached.

Pilot study: a preliminary study used to determine the intervention points in cases where the course of disease, the experimental effects, or the indicators of discomfort are otherwise unknown.

NOTE: An IACUC protocol is required to perform a pilot study.

Scoring system: a method of evaluation of an animal in which objective values are assigned to specific clinical signs &/or behavioral observations. These values are used to calculate a numerical score for each animal. Typically, humane interventions are predetermined and based on the total numerical score. Numerical scores are used to determine when intervention is necessary.

Death: The stages leading to death can be characterized as:

- Predictable Death: presence of clinical signs indicative of death before the planned end of the experiment; for example: inability to reach water or food.
- Impending Death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, recumbency, and tremor.
- Moribund: state or process of dying or inability to survive, even if treated
Pain: Pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.

Pain can be:
- Acute nociceptive pain: pain response evoked by a brief noxious stimulus which produces no tissue damage. This form of pain is not regarded as severe.
  Example: pedal reflex
- Persistent (chronic) inflammatory pain: the pain resulting from tissue damage and the ensuing inflammatory process, and may persist after local tissue damage has healed. This type of pain may be severe or distressing, particularly if long lasting or permanent.
  Example: self mutilation, localized infection
- Neuropathic pain: pain as a result of compromised function or abnormal activation of the peripheral or central nervous system. Neuropathic pain is always considered to be severe and distressing pain.
  Example: the presence of a large internal tumor that compresses nerves.

Objective signs of pain can include vocalization, evidence of infection, aversion or avoidance by active withdrawal from stimuli, guarding affected body parts, or self mutilation. Reduced food intake may be a sign of chronic pain.

Distress: An aversive state resulting from maladaptation or inability to adapt to behavioral alterations

- Acute stress is not regarded as a cause of distress; it may be necessary to optimize vigilance. Distress is usually associated with a change in mobility, locomotion, and environment, and can result in stereotypic behavior.

- The major stressors associated with distress are situations that may give rise to marked pain, fear, or anxiety. Retreating to the corner of the cage or excessive struggling or vocalizing are examples of distress in anticipation of an experimental procedure.

Suffering: A negative emotional state that in human beings is produced by persistent pain and/or distress. It should be assumed that persistent pain or distress in animals leads to suffering of animals in the absence of evidence to the contrary. If something is known to cause suffering in humans, it should be assumed to cause suffering in animals.
### Common Clinical Observations and Assessment Criteria used to define Humane Intervention Points

Note: Appropriate assessment criteria may differ between species. Be aware of the characteristic behavior of the species under observation.

#### Observe for Changes in Behavior

- Non-specific behaviors (i.e., change in frequency of behaviors or activities such as exploratory behavior, food hoarding or other feeding behavior changes, grooming, resting, sleeping, eating, drinking)
- Changes in unprovoked behavior
- Response to external stimuli (e.g., exaggerated response or lack of response when the animal is gently touched; unusually aggressive or docile when handled)
- Change in the normal group behavior
- Changes in the frequency of behaviors that might occur as a result of pain or discomfort (e.g., vocalizations, excessive licking or scratching, guarding, biting, teeth grinding)
- Adverse response relevant to the problem being studied (e.g., for an arthritis study in rodents: lameness and swelling)
- Self-trauma

#### Observe for Changes in Appearance

- Loss of body condition
- Postural changes (e.g. hunched posture)
- Sunken eyes (indicative of advanced dehydration)
- Changes in mucous membrane color (e.g. jaundice, anemia, cyanosis)
- Head tilt
- Missing anatomy (e.g., tail sloughing, digit missing)
- Obvious tissue swelling or masses
- Excessive porphyrin excretion (red staining around the eyes and nose of rodents)
- Rectal or vaginal prolapse (note if tissue necrosis has occurred)
- Bleeding from any orifice
- Piloerection
- Unkempt or ruffled hair coat

#### Monitor for Changes in Weight

- Always consider weight measurement in relation to the following:
  - Activity/ demeanor
  - Baseline weight (at the start of the study)
  - Age (e.g. still growing versus adults)
  - Concurrent muscle wasting
  - Comparison to animals of similar age, sex, and physiological status
Ascites or tumor growth which can mask weight loss
- 20% weight loss (emaciated appearance; rapid weight loss over two to four days; or progressive weight loss over a few weeks)
- Begin interventions such as nutritional supplements as weight loss is detected rather than allowing weight loss to progress.

**NOTE:** An increased frequency of measurements may be required to detect rapid weight loss.

### Monitor Body Condition Score (BCS)

- Monitoring the BCS is useful for animals that have conditions that negate body weight assessment e.g. pregnant animals, animals with enlarged organs or tumor growth (particularly internal tumors).

Body condition scoring, BC 1 through 5, as described by Ullman-Culler and Foltz

| BC 1 | Mouse is emaciated.  
|      | - Skeletal structure extremely prominent; little or no flesh cover.  
|      | - Vertebrae distinctly segmented. |

| BC 2 | Mouse is underconditioned.  
|      | - Segmentation of vertebral column evident.  
|      | - Dorsal pelvic bones are readily palpable. |

| BC 3 | Mouse is well-conditioned.  
|      | - Vertebrae and dorsal pelvis not prominent; palpable with slight pressure. |

| BC 4 | Mouse is overconditioned.  
|      | - Spine is a continuous column.  
|      | - Vertebrae palpable only with firm pressure. |

| BC 5 | Mouse is obese.  
|      | - Mouse is smooth and bulky.  
|      | - Bone structure disappears under flesh and subcutaneous fat. |

A "+" or a "+" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 3, 2+-).
### Changes in Other Measurable Clinical Signs
- Heart rate and character
- Respiratory rate and character
- Body temperature
  - Hypothermia
  - Hyperthermia

### Changes in appetite or hydration status
- Anorexia or partial anorexia
- Changes in the observed and/or measured amount of food and water intake
- Observe for weakness and inability to obtain food and water (e.g., inability or reluctance to stand or chew) assuming that the animal has recovered from anesthesia
- Inability to ambulate that prevents the animal’s easy access to food and/or water

### Observe for Signs of Infection
- Overt signs such as purulent or mucoid discharge
- Less obvious signs such as:
  - Increased body temperature
  - Elevated WBC parameters
  - Failure to respond to antibiotic therapy within an appropriate time
  - Systemic signs of illness

### Neurological Signs
- Frequent seizure activity
- Paresis
- Paralysis
- Head tilt

### Respond to Signs of Pain or Distress
- Sudden pain or distress unalleviated by the use of analgesics, sedatives, or tranquilizers
- Signs of severe organ system dysfunction non-responsive to appropriate therapy, or with poor prognosis as determined by a CM veterinarian
  - Severe medical conditions that cannot be controlled with appropriate therapy (e.g. severe systemic infections, kidney, liver or heart failure, respiratory distress)
  - Hematological or biochemical parameters that indicate organ failure incompatible with life (e.g. changes in heart rate, respiratory rate and quality)
## Moribund State

- Near death
- Depression coupled with:
  - Markedly reduced body temperature e.g. cold to the touch.
  - Non-responsive to stimulation (i.e. toe pinch withdrawal test), assuming the animal has recovered from anesthesia
  - Evident unconsciousness
## Special Conditions: Common assessment criteria and relevant information for defining humane intervention points for animals with tumors

<table>
<thead>
<tr>
<th>Study Requirements</th>
<th>Tumor Size and Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Principal investigator must determine the degree of tumor development that is</td>
<td>• Maximum Allowable Size:</td>
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<tr>
<td>required to meet scientific objectives of the research protocol.</td>
<td>• 1.5 cm in mice</td>
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<tr>
<td>• Determine whether the extent of tumor growth can be minimized while still</td>
<td>• 2.5 cm in rats</td>
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<td>maintaining the scientific objectives</td>
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<tr>
<td>• Prevent death as a result of excessive tumor growth as this may result in</td>
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<td>unnecessary pain or distress as well as loss of animals from the study (i.e.</td>
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<td>negating tissue or blood collection).</td>
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<td></td>
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<tr>
<td><strong>General assessment of animals with tumors</strong></td>
<td></td>
</tr>
<tr>
<td>• Assess tumor size and appearance in conjunction with any adverse impact on the</td>
<td></td>
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<tr>
<td>animal’s overall condition and behavior</td>
<td></td>
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<tr>
<td>• Assess the animal’s body condition scores (BCS) and intervene to prevent or treat</td>
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<tr>
<td>further decline in body condition e.g. provide moist rodent chow in an accessible</td>
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<tr>
<td>location in the cage</td>
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<tr>
<td>• Account for the weight added by the tumor when measuring body weight</td>
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<tr>
<td>• Take note of the tumor location and impact on normal bodily functions (e.g.</td>
<td></td>
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<tr>
<td>ambulation, ability to eat or drink or impairing vital functions)</td>
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<tr>
<td>• Assess the animal for signs of pain or distress</td>
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<tr>
<td>• Increase frequency of observation when tumors are fast growing, nearing endpoint</td>
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<tr>
<td>size or causing any adverse impact on the animal</td>
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<tr>
<td><strong>Ulcerations of tumors</strong></td>
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<tr>
<td>• Significant ulceration or necrosis of superficial tumors</td>
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<tr>
<td>• Ulceration unresponsive to treatment</td>
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<tr>
<td>• When touched, ulcerated tissue produces a response consistent with pain (e.g.,</td>
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<tr>
<td>vocalization, flinching, withdrawal)</td>
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<tr>
<td><strong>Other Effects</strong></td>
<td></td>
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<tr>
<td>• Interference with normal gait or movements</td>
<td></td>
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<tr>
<td>• Interference with vital functions such as breathing, eating and drinking</td>
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<tr>
<td>• Causing persistent self-trauma</td>
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<tr>
<td>• Causing signs of systemic illness</td>
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<tr>
<td>• Palpation of tumors induces response consistent with pain (e.g., vocalization,</td>
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<td>flinching, withdrawal)</td>
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<tr>
<td>• Animal found unexpectedly to be moribund, cachectic, or unable to obtain food or</td>
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<tr>
<td>water</td>
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**Special Conditions: Common Assessment Criteria used to define humane intervention points for studies involving moribund states or death as an endpoint**

### Death as an Endpoint

- Use humane intervention points other than death whenever possible.
- Use the minimum number of animals necessary to achieve statistical significance.
- Ensure scientific justification has been made to the IACUC in special circumstances in which death as an endpoint is necessary.
- Determine if pain relieving measures can be used without impacting negatively on research results.
- Always do a search for alternatives to keep abreast of recent literature since recent developments of alternatives are possible.
- Determine whether animals will be euthanized when moribund (if not, determine what information will be gained in the interval between the animal being moribund and death).
- Keep written records of all monitoring sessions including the time of the observation, the person making the observation, and entries regarding the animal’s condition as described in the Vet Request Form (yellow form).

### NOTE

- Any approved use of death as an experimental endpoint will be noted on all protocol forms and regulatory papers as being the highest pain level category “E,” indicating that analgesic or anesthetics are not provided to alleviate pain or distress.

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**Reduce the need to use death as an endpoint by using the following clinical indices:**

- Ensure that trained personnel monitor the animals at least once daily when signs of morbidity become evident.
- Increase the frequency of observation whenever there is a lack of clear criteria known to indicate when death may occur. Increased observation frequency may improve the ability to observe events that occur outside of regular working hours.
- House individually if injury from cage mates is likely.
- Provide easy access to food and water for any animals with clinically abnormal behavior.
- Monitor body temperature whenever possible as hypothermia can predict imminent death.
## Special Conditions: Assessment criteria and relevant information for the determination of humane intervention points for animals with ascites

### Ascites in general
- The maximum number of taps is 3 with the last collection procedure performed on euthanized mice.
- For animals injected with hybridoma cells, trained personnel should observe these animals daily for signs of ascites fluid accumulation.

### Body weight
- Weight loss can be masked by an accumulation of body fluid in the abdomen.
- The weight of the animal plus the ascites should not exceed 20% baseline body weight.

### Animal appearance:
- Observe daily for abdominal swelling.
- Increase assessment frequency to 2 times every 24 hours (at regularly spaced intervals) once the abdominal swelling appears (usually at 7 to 10 days after hybridoma injection).
- Alleviate pressure by harvesting the ascetic fluid.
- Euthanasia is recommended when ascites accumulation exceeds the size of normal pregnancy mass.