1. PURPOSE

This Standard Operating Procedure (SOP) provides instruction on how to set humane intervention points for animal research models.

2. INTRODUCTION

2.1. Humane intervention points are clear criteria used to indicate the need for either treatment of an animal or removal of an animal from a study based on objectively defined clinical signs, to prevent or relieve unnecessary pain or distress to a research animal.

2.2. A guiding principle of the Canadian Council on Animal Care (CCAC) is that, “animals must not be subjected to unnecessary pain or distress”. The experimental design must offer them every practicable safeguard, whether in research, in teaching, or in testing procedures” (CCAC 1989) An endpoint is defined by the CCAC as the point at which an animal’s pain and/or distress is terminated, minimized or reduced, by taking actions such as euthanizing the animal humanely, terminating a painful procedure, or giving treatment to relieve pain and/or distress.”

2.3. Humane interventions are defined as actions or instructions, including, but not limited to, the following:

- Adequate veterinary treatment, analgesia and/or supportive therapy to the animal(s)
- Termination of painful procedures
- Removal of the animal(s) from the study
- Modification of the experimental procedures to minimize the discomfort to the animal(s)
- Increasing the frequency of animal observations
- Modification to the housing and husbandry practices to improve the comfort of the animal(s)
- Euthanasia

2.4. Whether using animals for teaching or research, any actual or potential pain, distress, or discomfort should be minimized or alleviated by choosing the earliest point of intervention that is compatible with achieving the scientific objectives of the research, or educational objective for teaching.

2.5. The majority of early intervention points rely on regular observations of animal behaviour to find the earliest clues of distress. Waiting until stressors are manifested in illness or death is not satisfactory unless it can be rigorously defended under the scientific objectives of the study design (CCAC 1998) e.g. by a proportionate benefit.

2.6. Pilot studies should be used to determine intervention points in cases where the course of disease, the experimental effects or the indicators of discomfort are otherwise unknown.

2.7. Ensuring appropriate intervention points involves the combined efforts of the PI, the veterinarian and the ACC to carry out the following instructions:

2.7.1. Determine the humane intervention points that are appropriate for the study.

2.7.2. Ensure that humane intervention points are clearly defined in the Animal Use Protocol (AUP).

2.7.3. Ensure all personnel responsible for making animal observations have been adequately trained to observe and recognize the intervention points in the approved AUP.

3. PROCEDURE

3.1. Establishing intervention points:
3.1.1. Review literature and perform web-based searches of established models and alternative methods; understand side-effects of the experimental manipulations. Implement the alternatives whenever possible.

3.1.2. Consult with veterinarian on study refinements designed to minimize pain and distress.

3.1.3. Schedule regular animal observations at an appropriate frequency to ensure early detection of signs of pain and discomfort.

3.1.4. Increase the frequency of observations and measurements in response to a decline in the animal’s condition and during pre-determined critical periods during the study.

3.1.5. Keep records of all observations including specific measurements or data (e.g., body weight, clinical signs, etc.). A monitoring sheet is highly recommended (see e.g. in Appendix 1).

3.1.5.1. This is required for particular studies and should be outlined in the approved Animal Use Protocol (e.g. protocols involving surgery) or if a study proves to have unexpected deleterious effect on the animals additional monitoring and observations will be required.

3.2. Below is a list of signs and symptoms that may require a humane intervention (as defined in 2.3). The PI should specify if any of these, or other known, signs or symptoms is expected as direct result from the experimental manipulation. If so, the PI, in consultation with the veterinary staff or ACC should indicate a level of severity that requires a humane intervention. This list is not exhaustive but is meant to assist in establishing criteria for humane interventions:

- Weight loss exceeding 15% of age-matched control animals.
- Body condition score (BCS) at or less than 2 (see Appendix 2 and 3)
- Uncontrolled seizures.
- Impaired mobility that interferes with normal eating, drinking, ambulating or grooming.
- No or weak response to external stimuli.
- Hypothermia.
- Respiratory distress: laboured breathing, increased or decreased respiratory rate, cyanosis
- Hunched posture, lethargy and lack of grooming.
- Incoordination, paralysis
- Abnormal vocalizations
- Pale eyes and/or extremities (rodents) or mucous membranes
- Uncontrolled hemorrhaging
- Self-mutilation
- Dystocia
- Prolapsed uterus or anus
- Specific organ failure assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc.).
- Mass that is ulcerated, necrotic or impairing normal function (e.g., eating, drinking) or exceeding acceptable size endpoints:
  - Mice: \(2\text{cm}^3\) or 10% of the baseline bodyweight
  - Rats: \(5\text{cm}^3\) or 5% of the baseline bodyweight
3.3. Monitoring

3.3.1. Once animals start to display clinical signs of illness (see SOP: VET-06 - Recognizing Pain and Distress in Rodents), research personnel must monitor their subjects frequently (at least once daily, depending on severity of condition), this includes weekends and holidays.

3.3.2. During periods in which morbidity and mortality are expected to increase, animals must be evaluated a minimum of two times daily (every 8-12 hours). Those animals that are not expected to survive until the next scheduled evaluation should be humanely euthanized.

3.3.3. Investigators need to make every effort to identify and humanely euthanize moribund animals that have not responded to treatment.

3.3.4. In general, experiments should be designed such that all procedures are completed prior to animals reaching a moribund state.

3.3.5. Should an animal appear ill or in distress a veterinarian must be contacted for further assessment.

3.3.6. Written records of monitoring sessions may be required, depending on the protocol and the endpoints and need be made available to the veterinarian, ACVS or ACC on request.

3.3.7. If ACVS has examined an animal and expects that it will not survive until the next scheduled examination, a reasonable attempt will be made to contact the Principle Investigator (P.I.) to obtain permission to euthanize the animal. If the ACVS is unable to contact the P.I., the veterinarian will be contacted to obtain permission to euthanize the animal.

3.4. Veterinary Authority

3.4.1. The ACC delegates to the Veterinarian the authority to treat, remove from a study, or euthanize, if necessary, an animal according to the veterinarian's professional judgment.

3.4.2. The veterinarian must attempt to contact the animal user whose animal is in poor condition before beginning any treatment that has not previously been agreed upon, and must also attempt to contact the ACC Chair, but the veterinarian has the authority to proceed with any necessary emergency measures, whether or not the animal user and ACC Chair are available.

3.4.3. A written report should be sent by the veterinarian to the animal user and to the ACC following any such event.

4. REFERENCES

SOP: VET-06 – Recognizing Pain and Distress in Rodents


5. APPENDIX 1

5.1. Sample scoring sheet

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight Changes</td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 &lt; 10 percent weight loss</td>
<td></td>
</tr>
<tr>
<td>2 10-15 percent weight loss</td>
<td></td>
</tr>
<tr>
<td>3 &gt; 20 percent weight loss</td>
<td></td>
</tr>
<tr>
<td>Physical Appearance</td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Lack of grooming</td>
<td></td>
</tr>
<tr>
<td>2 Rough coat, nasal/ocular discharge</td>
<td></td>
</tr>
<tr>
<td>3 Very rough coat, abnormal posture, enlarged pupils</td>
<td></td>
</tr>
<tr>
<td>Measurable Clinical Signs</td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Small changes of potential significance</td>
<td></td>
</tr>
<tr>
<td>2 Temperature change of 1-2C, cardiac and respiratory rates increased up to 30 percent</td>
<td></td>
</tr>
<tr>
<td>3 Temperature change of &gt; 2C, cardiac and respiratory rates increased up to 50 percent, or markedly reduced</td>
<td></td>
</tr>
<tr>
<td>Unprovoked Behavior</td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Minor changes</td>
<td></td>
</tr>
<tr>
<td>2 Abnormal, reduced mobility, decreased alertness, inactive</td>
<td></td>
</tr>
<tr>
<td>3 Unsolicited vocalizations, self mutilation, either very restless or immobile</td>
<td></td>
</tr>
<tr>
<td>Behavioral Responses to External Stimuli</td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Minor depression/exaggeration of response</td>
<td></td>
</tr>
<tr>
<td>2 Moderately abnormal responses</td>
<td></td>
</tr>
<tr>
<td>3 Violent reactions, or comatose</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
</tr>
</tbody>
</table>

0 = normal: no action
1-4 = moderate changes: should be monitored daily
5-9 = significant changes: monitor twice daily
>10 = euthanize

*** A score of 3 in any one category: euthanize

6. APPENDIX 2

6.1. Mouse – body condition score

**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+, 2, 2-...)
6.2. Rat – body condition score

**BC 1**
Rat is emaciated
- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

**BC 2**
Rat is under conditioned
- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.

**BC 3**
Rat is well-conditioned
- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.

**BC 4**
Rat is overconditioned
- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

**BC 5**
Rat is obese
- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.