

**1. PURPOSE**

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

**2. RESPONSIBILITY**

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Principal investigator (PI) and their research staff, veterinarian, veterinary care staff, Facility Animal Care Committee (FACC).

**3. INTRODUCTION**

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- 3.1. Humane intervention points are clear criteria set to prevent or relieve unnecessary pain or distress to a research animal.
- 3.2. Humane intervention points do not necessarily lead to euthanasia of the animal, rather they result in interventions to alleviate the stressful/painful experimental procedure.
- 3.3. Humane interventions are defined as actions or instructions including, but not limited to, the following:
  - 3.3.1. Adequate veterinary treatment, analgesia and/or supportive therapy to the animal(s)
  - 3.3.2. Termination of painful procedures
  - 3.3.3. Removal of the animal(s) from the study
  - 3.3.4. Modification of the experimental procedures to minimize the discomfort to the animal(s)
  - 3.3.5. Increasing the frequency of animal observations
  - 3.3.6. Modification to the housing and husbandry practices to improve the comfort of the animal(s)
  - 3.3.7. Euthanasia
- 3.4. Pilot studies can be used to determine intervention points in cases where the course of disease, the experimental effects or the indicators of discomfort are otherwise unknown.
- 3.5. Ensuring appropriate intervention points involves the combined efforts of the PI, the veterinarian and veterinary care staff, and the FACC to carry out the following instructions:
  - 3.5.1. Determine the humane intervention points that are appropriate for the study.
  - 3.5.2. Ensure that humane intervention points are clearly defined in the Animal Use Protocol (AUP).
  - 3.5.3. Ensure all personnel responsible for making animal observations have been adequately trained to observe and recognize the intervention points in the approved AUP.

**4. PROCEDURES**

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- 4.1. Establishing intervention points:
  - 4.1.1. Review literature and perform web-based searches of established models and alternative methods. Implement the alternatives whenever possible.
  - 4.1.2. When available, use scoring grids based on published methods for monitoring specific animal models; these can be useful tools in setting and monitoring humane intervention points.
  - 4.1.3. Consult with the veterinarian on study refinements designed to minimize pain and distress.
- 4.2. Monitoring:
  - 4.2.1. Schedule regular animal observations at an appropriate frequency to ensure early detection of signs of pain and discomfort.

- 4.2.2. 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. Animals that can be expected to deteriorate, should be examined more frequently and more carefully.
- 4.2.3. Monitoring can consist of undisturbed observation, handling, body condition scoring, response to external stimuli, blood and urine assays, weighing, and visual inspection of the environment.
- 4.2.4. Score sheets can be used to record the findings. Keep records of all observations including specific measurements or data (e.g., body weight).
- 4.3. The determination of humane intervention points is not necessarily based on clinical signs but could also start from pre-clinical signs or from physiological or molecular biomarkers predictive of pain/distress later on in the disease process.
- 4.4. Humane intervention points should be balanced against the scientific endpoints to be met. Pain and distress might be intrinsic to a certain experimental model, e.g., arthritis model. However, the humane intervention points should never be beyond the scientific endpoints.
- 4.5. Recommended general intervention points, i.e., the point at which an experimental animal's pain and/or distress is terminated, minimized, or reduced:
  - 4.5.1. Weight loss exceeding 15% of baseline (pre-study) bodyweight
    - 4.5.1.1. For young animals, failure to maintain normal weight gain within 15% of age-matched control animals
    - 4.5.1.2. For overweight/obese primates, weight loss exceeding 15% of targeted weight
  - 4.5.2. Body condition score (BCS) less than 3
  - 4.5.3. Anorexia
  - 4.5.4. Diarrhea or vomiting
  - 4.5.5. Medical conditions, e.g., organ failure, respiratory distress, sepsis
  - 4.5.6. Uncontrolled convulsions or tremors
  - 4.5.7. Impaired mobility which interferes with normal eating, drinking, ambulating, or grooming.
  - 4.5.8. No or weak response to external stimuli.
  - 4.5.9. Hypothermia or hyperthermia
  - 4.5.10. Mass that is ulcerated, necrotic or impairing normal function (e.g., eating, drinking) or exceeding acceptable size endpoints:
    - 4.5.10.1. Mice: 2cm<sup>3</sup> or 10% of the baseline bodyweight
    - 4.5.10.2. Rats: 5cm<sup>3</sup> or 5% of the baseline bodyweight
  - 4.5.11. Respiratory distress: labored breathing, increased or decreased respiratory rate, cyanosis
  - 4.5.12. Hunched posture, lethargy, and lack of grooming.
  - 4.5.13. Incoordination, paralysis
  - 4.5.14. Abnormal vocalizations
  - 4.5.15. Pale eyes and/or extremities (rodents) or pale mucous membranes
  - 4.5.16. Uncontrolled hemorrhaging
  - 4.5.17. Self-mutilation
  - 4.5.18. Self-injurious behavior
  - 4.5.19. Complications secondary to medical/surgical interventions or other experimental manipulations
  - 4.5.20. Specific organ failure assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc.)
- 4.6. Recommended intervention points for aging rodents (over 18 months of age):
  - 4.6.1. Refer to SOP 412.

- 4.7. Recommended intervention point for rodent cancer models:  
 4.7.1. Refer to SOP 415.
- 4.8. Recommended intervention point for rodent models involving a progressive ascending paralysis:  
 4.8.1. Refer to SOP 419.

## 5. REFERENCES

- 5.1. Canadian Council on Animal Care (CCAC). Identification Of Scientific Endpoints, Humane Intervention Points, And Cumulative Endpoints. March 2022; Available online.
- 5.2. Association of Primate Veterinarians' Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research. J Am Assoc Lab Anim Sci. 2020;59(1):6-8.
- 5.3. 3Rs-Centre Utrecht Life Sciences, Department of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, [Humane Endpoints](#) website, accessed April 2023.

## SOP REVISION HISTORY

DATE	NEW VERSION
2023.03.17	2. RESPONSIBILITY Principal investigator (PI) and their research staff, <b>veterinarian</b> , veterinary care staff, Facility Animal Care Committee (FACC).
2023.03.17	3.4. Ensuring appropriate intervention points involves the combined efforts of the PI, the <b>veterinarian and</b> veterinary care staff, and the FACC to carry out the following instructions:
2023.03.17	<b>4.1.2. When available, use scoring grids based on published methods for monitoring specific animal models; these can be useful tools in setting and monitoring humane intervention points.</b>
2023.03.17	4.1.3. Consult with the <b>veterinarian veterinary care staff</b> on study refinements designed to minimize pain and distress.
2023.03.17	<b>4.6. Recommended intervention point for rodent models involving a progressive ascending paralysis: 4.6.1. Refer to SOP 419.</b>
2023.03.17	<b>4.3. Recommended intervention points for non-human primates: 4.3.1. Weight loss exceeding 15% of pre-study bodyweight or targeted weight for obese animals 4.3.2. Body condition score (BCS) less than 2 4.3.3. Anorexia 4.3.4. Diarrhea or vomiting 4.3.5. Medical conditions, e.g., organ failure, respiratory distress, sepsis 4.3.6. Hyperthermia 4.3.7. Complications secondary to medical/surgical interventions or other experimental manipulations 4.3.8. Self-injurious behavior</b>
2023.03.17	<b>5. REFERENCES 5.1. Canadian Council on Animal Care (CCAC). Identification Of Scientific Endpoints, Humane Intervention Points, And Cumulative Endpoints. March 2022; Available online. 5.2. Association of Primate Veterinarians' Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research. J Am Assoc Lab Anim Sci. 2020;59(1):6-8.</b>
2023.04.16	<b>3.2. Humane intervention points do not necessarily lead to euthanasia of the animal, rather they result in interventions to alleviate the stressful/painful experimental procedure.</b>
2023.04.16	<b>4.2. Monitoring: 4.2.1. 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. Animals that can be expected to deteriorate, should be examined more frequently and more carefully. 4.2.2. Monitoring can consist of undisturbed observation, handling, body condition scoring, response to external stimuli, blood and urine assays, weighing, and by visual inspection of the environment. 4.2.3. Score sheets can be used to record the findings. Keep records of all observations including specific measurements or data (e.g., body weight).</b>
2023.04.16	<b>4.3. The determination of humane intervention points is not necessarily based on clinical signs but could also start from pre-clinical signs or from physiological or molecular biomarkers predictive of pain/distress later on in the disease process. 4.4. Humane intervention points should be balanced against the scientific endpoints to be met. Thus, pain and distress might be intrinsic to a certain experimental model, e.g. arthritis model. However, in this case the humane endpoint should never be beyond the scientific endpoint.</b>
2023.04.16	<b>4.5. Recommended general intervention points, i.e., the point at which an experimental animal's pain and/or distress is terminated, minimized, or reduced: 4.5.1. Weight loss exceeding <del>20%</del> 15% of baseline bodyweight. For young animals, failure to maintain normal weight gain within 15% of age-matched control animals. 4.5.2. Body condition score (BCS) less than <del>2</del> 3. 4.5.3. Anorexia 4.5.4. Diarrhea or vomiting 4.5.5. Medical conditions, e.g., organ failure, respiratory distress, sepsis 4.5.6. Uncontrolled <del>seizures</del> convulsions or tremors 4.5.9. Hypothermia or hyperthermia 4.5.18. Self-injurious behavior 4.5.19. Complications secondary to medical/surgical interventions or other experimental manipulations</b>
2023.04.16	<b>5.3. 3Rs-Centre Utrecht Life Sciences, Department of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Humane Endpoints website, accessed April 2023.</b>