

Procedures involving Pain or Distress

Background

Sociological studies have shown that the majority of US citizens support the use of animals in medical research except when there are matters of unrelieved pain or distress to the animals. US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, IV, V, and VI published in the Federal Register May 20, 1985 serve to unify US policy on the ethical significance of pain and distress in laboratory animals:

IV Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain, when consistent with sound scientific principles, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V Procedures with animals that cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

The NIH ARAC has developed useful guidelines regarding recognition and minimization of pain and distress and the IACUC's responsibility for the intramural research program (http://oacu.od.nih.gov/ARAC/documents/Pain_and_Distress.pdf). The CCR ACUC supports this guidance and has developed the following additional guidelines relevant to NCI's animal program.

Cancer pain in humans is recognized as the result of multifaceted mechanisms, particularly metastasis to bones. Tumor growth models and metastasis assays in rodents typically do not produce signs of pain until the tumors become advanced and invasive, produce debilitation, and/or cause release of mediators of inflammation. Left to progress and grow without intervention, experimental tumors and blood cancers would typically cause sickness and death. The majority of animal cancer studies in lab animals, however, can be carried out to completion causing minimal to no pain or distress by selection of experimental endpoints that precede the onset of clinical illness, by early recognition of humane endpoints, and by judicious use of anesthetics and analgesics.

In the past, radiation, toxicological, cancer, and infectious disease studies utilized death of animals as a study endpoint, often to demonstrate the benefit of therapeutic intervention. Such an endpoint is not normally approved if a moribund condition can be recognized, at which time the animal may be euthanized for data collection. For those scientific studies in which animals are expected to experience pain or distress, analgesics and other therapeutic drugs should be considered and utilized as recommended by the veterinary staff. In cases where therapeutic interventions are scientifically contraindicated or are unlikely to relieve pain or distress, the study is classified as a Category 3 (USDA Category E), which requires justification and

documentation of consideration of less painful alternative approaches and strict minimization of animal numbers. Efforts are also taken to limit pain or distress to that which is necessary to answer the scientific questions.

The CCR ACUC recognizes its role to reduce pain and distress in animal studies of cancer and AIDS and requires the following items in order to comply with NIH ARAC Guidance and the US Government Principles:

- Require use of humane endpoints and encourage selection of pre-moribund experimental endpoints, which precede development of advanced disease, when possible
- Describe monitoring plan in the ASP if illness is anticipated or likely and include increased monitoring during critical phases
- Require use of post-operative analgesics unless scientifically contraindicated – including multi-modal analgesia, with doses appropriate to the species and to the procedure
- Encourage use of pilot studies to optimize animal welfare for later experiments, if applicable
- Utilization of the Veterinary staff to ensure adequate care by supporting approved veterinary care protocols and relying on the judgment of the attending veterinarian about magnitude and/or acceptability of unanticipated pain or distress in research animals.

The following procedures and techniques are not considered to cause significant pain or distress, given the stipulations provided:

Procedure/Technique	Considerations
Brief physical restraint or restraint in an animal acclimated to restraint.	<i>The period of restraint should be minimized, and animals should be monitored through the period of restraint.</i>
Fasting or water restriction for 24 hours or less (most species, including mice)	<i>Caloric restriction to 75% of ad lib or feeding every other day is acceptable with monitoring and if scientifically justified. See ARAC Guideline (http://oacu.od.nih.gov/ARAC/documents/Diet_Control.pdf)</i>
Use of small amounts of Complete Freund’s adjuvant (single dose only) when scientifically justified and minimized.	<i>See ARAC Guideline (http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf)</i>
Subcutaneous tumors that do not exceed 2 cm in any dimension, unless they interfere with physiological functions such as food consumption, breathing, or urination. The sum of tumors or	<i>Tumor ulceration is also frequently considered a humane endpoint since it often reflects a large tumor that has outgrown its blood supply, though ulceration may be a characteristic of the tumor’s biology or response to therapy. Necrosis of tumor tissue is not typically associated with pain in humans or animals. When tumor necrosis is</i>

calculated mass should not exceed 10% of the animal's body weight.	<i>anticipated and unavoidable, documentation of daily monitoring and evaluation for other signs of pain is required.</i>
Lung metastasis assays that precede the onset of labored breathing or acute death	<i>Experimental endpoints must be chosen based on dose of cells delivered and growth characteristics unique to the particular tumor cell line</i>
Properly performed parenteral injections, oral gavage, blood collection, and euthanasia methods consistent with the AVMA Guidelines on Euthanasia.	<i>Housing of healthy research animals in compatible groups, and daily care in accordance with Guide for the Care and Use of Laboratory Animals should also help to avoid discomfort or distress.</i>

Recognition of Unanticipated Pain or Distress in Rodents and the NCI/LASP Response (*Action codes apply to NCI facilities, other facilities use similar action codes*)

	Behavior and Clinical Signs	Examples	Comments
Mild Pain (Action Code 2): <i>Investigator is alerted and treatment or special monitoring commences within 24 hours.</i>	No obvious signs of pain or distress. Quiet, but mobile and responsive after stimulation; Weight loss up to 15%; Tumor may have reached size limitation described in ASP (investigator must euthanize within 24 hours); Tumor may have ulceration or necrosis requiring daily monitoring (investigator must euthanize within 24 hours) Hunched posture; Rough haircoat; Piloerection	Ascites without respiratory compromise; dermatitis without self-mutilation; superficial bite wounds; rectal prolapse; abscess; signs of intracranial tumor growth; Stereotypical behavior that is not severe	Condition may be progressive, but is unlikely to progress quickly. Adaptive mechanisms, including behavior changes can keep pain or distress to minimal levels.
Moderate Pain (Action Code 3): <i>Communication to investigator and some form of treatment commence the same day as recognition; Veterinary intervention (treatment,</i>	Hunched posture and decreased activity levels; Clinical dehydration; Less mobile and alert; responsive only following moderate stimulation; Eyes partially closed or squinted; Tachypnea; Shallow respirations with abdominal component; Guarding, scratching, or	Moist dermatitis with pruritis or self-mutilation; Dystocia; severe bite wounds ; dehydration evident by skin turgor test	Respiratory rate changes may be the direct result of changes in ventilation capacity due to space-occupying lung lesions, rather than the result of pain. If pain is likely and no treatment is likely to be effective,

<i>analgesia, or euthanasia) must occur before the close of business</i>	<ul style="list-style-type: none"> mutilating potentially painful area; lameness; Tumor infected or necrotic and being mutilated by one or more animals 		euthanasia should occur the same day as detection.
<p>Severe Pain or Moribund (Action Code 4):</p> <p><i>Communication to investigator followed by euthanasia immediately, within the hour, or as determined by the facility veterinarian</i></p>	<ul style="list-style-type: none"> Not eating or drinking Cachexia; Hunched posture with head down; non-responsive to manual stimulation. Inactive, lying prone or on side; Eyes closed or squinted with ocular discharge Profound, easily detected hypothermia; Inability to access food and water 	<ul style="list-style-type: none"> Paralysis, Chronic, severe inflammation producing joint swelling; stupor; Severe blood loss; Labored, open-mouth breathing; sepsis; severe metabolic derangements 	If death is clearly imminent within a few seconds or minutes (uncommon), immediate euthanasia is the most humane response, followed by contacting the investigator.

Definitions

Pain = cognitive and emotional recognition of tissue damage or potential tissue damage, mediated by nociceptive pathways

Distress = an aversive, negative state in which physiological and behavior responses cannot return the organism to homeostasis. The inability to cope or adapt may lead to maladaptation, making the animal's condition worse. This is a defining distinction between stress and distress.

Moribund= a severely debilitated state that precedes imminent death.

Pre-moribund = physical state that may be characterized by signs of illness which are expected to worsen and progress to a moribund state.

References

Defining the Moribund Condition as an Experimental Endpoint for Animal Research
http://dels-old.nas.edu/ilar_n/ilarjournal/41_2/Defining.shtml

Guidelines for the welfare and use of animals in cancer research
<http://www.nature.com/bjc/journal/v102/n11/full/6605642a.html>