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MOLECULAR MEDICINE RESEARCH INSTITUTE

MMRI Guidelines for Pain Assessment and Classification for Animal Use Protocol Design

The goal of this document is: 1.) to help investigators design humane experiments utilizing designated endpoints that minimize the potential for pain and distress and 2.) to assist IACUC members when assigning USDA pain and distress category classification for proposed experimental procedures using mice purchased or bred by MMRI. This document uses relevant guidelines from the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* and from the ACLAM *Guidelines for the Assessment and Management of Pain and Distress*, as well as other international guidance documents for the standard of care & use of laboratory animals.

An overview on recognizing pain for investigators designing animal use protocols

Pain has been defined by the ACLAM panel as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, and should be expected in an animal subjected to any procedure or disease model that would be likely to cause pain in a human.

It is important to note that assessing the degree of pain or discomfort experienced by an animal can be difficult and subjective. While the physiologic mechanisms of pain perception are similar in all mammalian species, the ability to tolerate and cope with pain may be vastly different from one species to the next and in one individual or situation to the next. For example, prey species such as rodents have adapted to hide overt signs of pain to avoid signaling to a predator that they are ill and would be an easy meal. Therefore, a rodent that is experiencing mild to moderate pain may display no clinical signs associated with its discomfort. Moderate to severe pain in rodents frequently leads to subtle changes in normal physiology or behavior. Accurate recognition of these changes requires that animal facility personnel and investigative staff are familiar with the normal behavior and physiology for the species, strain, sex and age or social group they are using. Therefore only qualified and trained animal facility or research personnel will be allowed to manage studies that have the potential to cause pain and distress. The examples of behavioral changes listed below serve to create a common ‘baseline’ for investigators designing studies with the potential for pain. This baseline can be referenced in Section III of the MMRI Animal Care and Use Protocol Form, and it can be used as a guide to help explain when to carry out euthanasia of mice that are considered untreatable, or when to administer treatments, either pharmacologic or non-pharmacologic, aimed at alleviating or ameliorating pain.

Some examples of behavioral changes associated with pain in mice:

1. Changes in activity level: reduced spontaneous motor activity or nesting behavior, delayed response to handling.

2. Changes in appearance: hunched position, ruffled fur, accumulation of normal or abnormal secretions
3. Changes in feeding behavior: decreased food and water consumption, reduction in body weight or fecal mater
4. Significant attention given to a surgical, injection or inoculation site: excessive licking or chewing, erythema, swelling, redness or discharge from the site.

See table below from EU guidance document

Table 2 Signs of acute pain in animals

Guarding	Attempting to protect, move away, or bite
Crying	Movement or palpation
Mutilation	Licking, biting, scratching, shaking
Restlessness	Pacing, lying down and getting up, shifting weight
Recumbency	Unusual length of time
Ambulation	Reluctance to move, difficulty in rising
Abnormal positions	Head down, tucked abdomen
Increased respiration rate	

Modified from Soma (1987)

Experimental considerations for investigators designing protocols or procedures with the potential for causing pain

1. Avoiding and minimizing discomfort, distress, and pain, when consistent with sound scientific practices, is imperative. Investigators should consider that procedures that cause pain or distress in human beings or other domestic animals may cause pain or distress in laboratory animals

2. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate equipment, personnel, acclimatization and/or sedation, analgesia, or anesthesia (unless justified). Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents (e.g. if using paralytics to inhibit spontaneous motor activity for scientific purposes must also use analgesics and anesthetics).

3. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly sacrificed at the end of the procedure or, if appropriate, during the procedure. Early predictors of declining condition should be well thought out and clearly delineated.

4. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures using mice. All personnel involved in overseeing a given experiment shall receive adequate training on the humane care and use of laboratory animals.

5. Where exceptions are required in relation to the provisions of these principles, the decisions should not rest with the investigators directly concerned but should be made by an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration nor for purely economic reasons.

The following table is an example of non-pharmacologic pain amelioration techniques which should be considered whenever pain alleviating drugs can not be employed (table from p105 of ACLAM guidelines)

Minimal to Mild	Mild to Moderate	Moderate to Severe
Wound care	Wound care	Wound care
House singly until ambulatory	Soft, absorbent bedding, nest material	Soft, absorbent bedding, nest material
	Modified food and water access	Modified food and water access
	House singly until ambulatory	Increased food palatability
	Supplemental heat	Supplementary heat and hydration, SC or IP
		House singly until ambulatory

General approach to pain/distress categorization in animal use protocols

Investigators and reviewers should employ the following sets of questions and criteria:

1. Comparison with humans or other common domestic animals:

   a) What would be an equivalent or comparable procedure or state in the majority of humans or common domestic animals?
   b) Would it cause more than minimal or transient pain or distress?
   c) If pain might be expected, would it be necessary to treat it and how?
   d) What would the consequences be of not treating the pain?

2. Animal procedures for which the human or veterinary equivalent causes very transient, minor, or no pain or distress, or treatment is not necessary for the majority of individuals, should generally be classified as Category C procedures, unless it is established or documented that animals show objective signs of pain or distress (see examples above).

3. Procedures that cause animals to exhibit objective signs of pain or distress spontaneously and to a significant extent should generally fall under Category ‘E’

(or Category D if treated appropriately with analgesics or anesthetics or other pain ameliorating measures) unless an investigator can justify variance. Also the combination of several parameters of pain and distress is given greater significance than any one of the individual signs

The following table from the ACLAM guidelines helps predict degree of pain based on common laboratory procedures and the next table from the EU uses target tissues sensitivity as a predictor of potential pain & distress

← PAIN POTENTIAL→

Minimal to Mild Pain	Mild to Moderate Pain	Moderate to Severe Pain
Catheter implantation	Minor laparotomy incisions	Major laparotomy/ organ incision
Tail clipping	Thyroidectomy	Thoracotomy
Ear notching	Orchidectomy	Heterotopic organ transplantation
Superficial tumor implantation	C-section	Vertebral procedures
Orbital sinus venotomy	Embryo transfer	Burn procedures
Superficial lymphadenectomy	Hypophysectomy	Trauma models
Ocular procedures	Thymectomy	Orthopedic procedures
Multiple ID antigen injections		
Intracerebral electrode implantation		
Vasectomy		
Vascular access port implantation		

Table 3 Sensitivity of tissues and organs to pain

Eyes, ears, teeth	+ + +
Nerves	+ + +
Testes	+ + +
Spinal cord	+ + to + + +
Skin	+ + to + + +
Serous membranes	+ + to + + +
Periosteum	+ + to + + +
Blood vessels	+ + to + + +
Viscera	+ to + + +
Muscles	+ to + +
Joints and bones	+ to + +
Brain tissue	–

Modified from FELASA (1994)

USDA Pain Categories

MMRI uses three Pain and Distress Categories C, D, and E (corresponding to the USDA reportable pain
categories) as follows:

Category C
Research, experiments, or tests involving potential for no more than momentary pain or distress to the animals more severe than routine injection or blood collection. Use of anesthetic for restraint, to reduce handling stress and/or the potential for accidental injury to the animals, is permitted in this category.

Category D
Research, experiments, surgery or tests having the potential to produce accompanying pain or distress to the animals more severe than a routine injection or blood collection, and for which appropriate anesthetic, analgesic, or tranquilizing drugs are used.

Category E
Research, experiments, surgery, or tests having the potential to produce accompanying pain or distress to the animals more severe than a routine injection or blood collection, for which appropriate anesthetic, analgesic, or tranquilizing drugs will not be given because use of such drugs would affect the procedure, result, or interpretation of the research, experiments, surgery, or tests. Also included in this category are any procedures resulting in a permanent disease state or impairment which if left untreated would be thought to cause pain/distress in humans or common domestic species

Please note that pain categories are meant to describe the nature of procedures an individual animal will undergo. While an individual animal may experience procedures fitting under several categories, they should be listed under the most severe category they qualify for. Also noted that prospective categorization of procedures and protocols is based on the intent and once in place may need to be adjusted for individual animals, groups or the entire protocol via amendment or annual review

Examples for Category C
(procedures that are minimal, transient, or involve no pain or distress**)**

1. Select common veterinary or medical procedures performed correctly by trained personnel such as the intravenous (I.V.), subcutaneous (S.C.), intramuscular (I.M.), intraperitoneal (I.P.) injections, or retro-orbital blood collections without anesthesia.
2. Euthanasia performed in accordance with the recommendations of the most recent AVMA Panel on Euthanasia, utilizing procedures that produce rapid unconsciousness and subsequent humane death (e.g. tissue collection after death).
3. In vivo subcutaneous propagation of a tumor that is not expected to result in spontaneous death nor result in a significant disease state or physical impairment. Tumor size not to exceed 1500mm^3 (measured using the formula V=L*W*H/2).
4. Animal identification (ear notching)

5. Tail clipping of mice less than 4 weeks old; ≤ 1 cm

Examples for Category D
(procedures with the potential to produce pain or distress in animals, but which are performed using
appropriate and adequate anesthetics, analgesics, or tranquilizers to alleviate the pain or distress)

1. Injection or blood collection by relatively invasive routes such as intracardiac or central vessel, while under anesthesia
2. Perfusion under anesthesia.
3. Laparotomy or vasectomy under anesthesia
4. Embryo transfers under anesthesia

Examples for Category E
(potentially painful or distressing procedures that are performed without appropriate and adequate
anesthesia, analgesia, or tranquilizers because they would adversely affect the results or interpretation of
data)

1. Studies with significant mortality or death as an endpoint (LD50 studies)
2. Procedures leading to clinically significant weight loss (>10%)
3. Use of paralyzing agents or immobilizing drugs without anesthesia
4. Studies of burns or trauma without anesthesia
5. Studies of systemic autoimmune diseases affecting multiple organs and/or systems
6. maintaining animals in a chronic disease or other state of significant impairment without appropriate treatment (ie maintaining a breeding colony of diabetic animals without insulin treatment)

Pain and distress justification

Some procedures that are known to cause pain and/or distress can be justified for scientific reasons (e.g. if the scientific aims cannot be properly interpreted when pain is alleviated). This justification must be provided by the investigator, and reviewed by the IACUC, before a painful procedure is carried out. There are provisions for such justification in the IACUC Animal Use Protocol, amendment and renewal forms. Such justification must be significantly compelling and supported by the current body of scientific, veterinary and or medical literature and prove that suitable alternatives are not readily available. The IACUC may also request a face-to-face review or small supervised pilot studies of certain procedures, if warranted. It is important to note that all procedures, justified or not, have the potential to be terminated (via euthanasia), suspended or revoked, if the animal care staff, with appropriate consultation, determines them to be outside the scope of the approved procedure. Such action would require documentation and review by the IACUC and possible reporting to IO and other accrediting and regulatory bodies

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MMRI GUIDELINES FOR PAIN ASSESSMENT

Care and Use of Animals in Research

ACKNOWLEDGEMENT OF POLICIES AND PROCEDURES

I, the undersigned, acknowledge reading and understanding the regulations and standards applicable to the Molecular Medicine Research Institute.

Company Name (print): _____

Name (print): _____

Signature: _____

Date: _____