

Central Michigan University
Institutional Animal Care and Use Committee
Policy/Guideline/Principle of Care in Animal Pain
And Distress Management

A key aspect of the animal welfare regulations is that pain and distress be minimized whenever possible. Therefore, it is necessary to design and perform experiments in such a way as to prevent the animals from experiencing problems unless necessary to achieve the goals of the study. Experiments that require animals to experience pain and distress that cannot be relieved with analgesics due to interference with the experimental protocol or that require the use of death as an endpoint require special consideration by the IACUC and scientific justification by the Project Director.

Definitions:

- **Pain**: a feeling of distress, suffering or agony, caused by stimulation of specialized nerve endings Ref.9. Pain is a complex experience that typically results from stimuli that damage or have the potential to damage tissue; Such stimuli prompt withdrawal followed by, the ability to experience and respond to pain is widespread in the animal kingdom and extends beyond vertebrates (Sherwin 2001) Ref.3
- **Distress**: physical or mental anguish or suffering Ref. 9, an aversive state in which an animal fails to cope or adjust to various stressors with which it is presented. Stress may not induce an immediate and observable pathologic or behavioral alteration, making it difficult to monitor and evaluate the animal's state when it is present. Recognition and Alleviation of Distress in Laboratory Animals (NRC 2008) is a resource with important information about distress in experimental animals. Ref.3.
- **Analgesia**: absence of sensibility to pain Ref 10, temporary abolition or diminution of pain perception Ref 9
- **Analgesic**: relieving pain, pertaining to analgesia, a drug that relieves pain Ref. 10
- **Anesthesia**: absence of sensitivity to stimuli Ref. 10, state of controllable, reversible insensibility in which sensory perception and motor responses are both markedly depressed. Ref.9
- **Euthanasia**: the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Ref. 10.

Regulatory References:

1. USDA Animal Welfare Act
2. Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS)
3. The Guide for the Care and Use of Laboratory Animals "*Guide*", National Academy Press, Eight Edition, Updated 2011, Washington, D.C.
4. (The *Guide* endorses: 1. USA Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research and Training. 2. Federal Animal Welfare Regulations 3. P.H.S. Policy on Humane Care and Use of Laboratory Animals and 4. Other applicable federal, state, local laws, regulations, and policies.)
5. CMU's IACUC Policy
6. Recognition and Alleviation of Pain in Laboratory animals (NRC 2009)
7. AVMA Guidelines on Euthanasia (AVMA 2007)
8. NIH Guidelines for Pain and Distress in Laboratory animals

Other:

9. Laboratory Animal Anesthesia A Practical Introduction for Research Workers and Technicians P. Flecknell second edition 2005
10. Dorland's Medical Dictionary 21st Edition
11. Recognition and Alleviation of Pain in Laboratory Animals, The National Academy of Sciences 2009
12. Handbook of Experimental Animals "The Laboratory Mouse" Elsevier Academic Press

Regulatory Requirements

It is the ethical and legal obligation of all personnel involved with the use of animals to reduce or eliminate pain and distress in teaching and research animals whenever such actions do not interfere with the outcome of the scientifically justified project objectives. The Institutional Animal Care and Use Committee (IACUC) has the delegated responsibility and accountability for ensuring that all animals under its oversight are used humanely and in accordance with regulations. Laws and regulation require that investigators adequately control pain in research animals, unless the outcomes of an experiment would be negatively impacted. Alleviating pain in research animals typically refers to reducing its duration and /or intensity as those two characteristics affect averseness.

USDA Animal Welfare Act Regulations 2.31(d) (1) (iv) (A) and (ix): “Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics, or anesthetics, unless withholding such agents is justified for scientific reasons, in writing, by the principal investigator and will continue for only the necessary period of time... Activities that involve surgery include appropriate provision for peri-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices.

PHS policies IV.C.1.a-b: Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design. Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator.

PHS policy IV: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, 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.

PHS policy V: Procedures with animals that may 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.

Guide for the Care and Use of Laboratory animals, p. 120: “An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols... Pain is a stressor and, if not relieved, can lead to unacceptable levels of stress and distress in animals. Furthermore, unrelieved pain may lead to ‘wind up,’ a phenomenon in which pain sensitization results in a pain response to otherwise nonpainful stimuli... For these reasons, the proper use of anesthetics and analgesics in research animal is an ethical and scientific imperative... In general, unless the contrary is known or established, it should be assumed that procedures that cause pain in humans also cause pain in animals.”-*PHS Policy IV*

Institutional Animal Care and Use Committee Guidebook, 2nd Edition, 2002

AVMA Guidelines on Euthanasia (AVMA June 2007) to be updated with final update of AVMA Guidelines of 2012

“The Three Rs” (Russell and Burch 1959) provide the underlying principle to the ethical care and use of laboratory animals:

- **Refinement** of experimental procedures to reduce or eliminate pain and distress. When the use of animals is unavoidable; one must minimize pain, distress, or other threats to animal welfare e.g., researchers should ensure that animals’ needs are met; use pain treatment drugs; and specify humane endpoints –which would determine when a study design should be changed or a study ended early due to concerns about animal pain, distress, or welfare.

- **Reduction in the number of animals being used. Use methods that enable equivalent information to be obtained from fewer animals or more information from the same number of animals, such as through the use of advanced imaging techniques.**
- **Replacement of animals with other reliable models. Use alternative methodologies, such as computer modeling, or replace higher order animals with those of lower order (such as the use of amphibians or invertebrates).**

Recognition of Pain and Distress

All vertebrates should be considered capable of experiencing the aversive state of pain. Animals should be monitored for pain and distress as appropriate for the condition, procedure and degree of invasiveness. Critical to the assessment of the presence, or absence, of pain or distress is having the ability to distinguish between normal and abnormal animal behavior. Project Directors, research personnel, and animal care staff must be trained to distinguish these differences and make good observations, provide post op monitoring and perform adequate record keeping. This is especially true when dealing with species that often exhibit pain and distress with only subtle changes in their behavior.

Certain procedures are always assumed to have the potential for causing pain and distress. These are the basis for the numerous experimental guidelines that are intended to prevent pain or distress. These include:

- Surgery
- Repeated use of large volumes of, or intradermal injections of Freund's complete adjuvant
- Intraperitoneal implantation of ascites-producing hybridomas for monoclonal antibody production
- Prolonged physical restraint
- Malignant neoplasms
- Prolonged food or water deprivation
- Distal tail biopsy in animals over 3 weeks of age (tail snipping)
- Electrical shock or other adverse stimuli that are not immediately escapable
- Paralysis or immobility in a conscious animal
- Inflammatory disease
- Organ failure resulting in clinical signs
- Non-healing skin lesions
- Whole body irradiation at high doses
- Withdrawal of more than 10% of an animal's blood volume
- Studies that require an animal to reach a moribund state or die spontaneously as the endpoint of the study

Assessment of pain or distress may be based on many different criteria including:

- Increased or decreased activity
- Abnormal postures, hunched back, head pressing, muscle flaccidity or rigidity
- Poor grooming (erected, matted, or dull hair coat)
- Decreased food or water consumption
- Decreased urine or fecal output
- Weight loss (generally 20% of baseline), failure to grow, or loss of body condition (in mice this may not be greatly affected, and this parameter is useful as objective measures of pain Ref.12.
- Dehydration, skin tenting, sunken eyes
- Decrease or increase in body temperature
- Decrease or increase in pulse or respiratory rate
- Physical response to touch (withdrawal, lameness, abnormal aggression, vocalizing, abdominal splinting (rigidity of muscles occurring as a means of avoiding pain caused by movement of the part), increase in pulse or respiration)
- Teeth grinding
- Self-aggression, trauma, and mutilation
- Inflammation
- Photophobia

- Diarrhea
- Objective criteria of organ failure demonstrated by hematological or blood chemistry values, imaging, biopsy or gross dysfunction
- Self-imposed isolation or hiding
- Rapid breathing, opened-mouth breathing, abdominal breathing
- Tearing (including Porphyria), lack of blinking reflex
- Twitching, trembling, tremor
- Redness or swelling around surgical site

Policy Regarding Non-surgical and Surgical Procedures:

1. Assessment of pain and distress in animals, including late-term fetuses, is difficult and can be subjective. As such, procedures that cause pain or distress in humans should be assumed to cause similar effects in animals, unless the contrary is established. In general, animals whose biological niche is that of a prey species are less likely to alter their behavior in response to pain than would a predatory animal, as doing so would make them a target for predation.
2. “Pain is a stressor and, if not relieved, can lead to unacceptable levels of stress and distress in animals. Unrelieved pain may lead to “wind up” a phenomenon in which central pain sensitization results in a pain response to otherwise nonpainful stimuli Thus, the proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative”.
Ref. 3
3. Fundamental to the relief of pain in animals is the ability to recognize its clinical signs in the specific species. Species vary in their response to pain and criteria for assessing pain in various species differ. Pain should be appropriately treated based on observation and the likelihood that the procedure will cause pain. The U.S Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training states “that in general, unless the contrary is know or established, it should be considered that procedures that cause pain in humans may also cause pain in other animals”.
4. When possible, pain should be treated pre-emptively (the administration of preoperative and intraoperative analgesia, before the cause) enhancing intraoperative patient stability and optimizing postoperative care and wellbeing by reducing postoperative pain. Analgesic agents become effective before the animal emerges from anesthesia when administered before surgery and re-dosed as needed during the procedure. **Local anesthetic/analgesic drugs are not to be used as the sole analgesic for pain.** Contact the veterinarian to determine the best form of analgesia for a particular animal/project during the planning phase of the project or when an animal is observed to be in pain.
5. Analgesics must be provided to all animals following survival surgery to avoid or minimize the discomfort, distress, and pain when consistent with sound scientific practices, unless scientific justification for withholding post-operative analgesics is provided by the investigator and approved by the IACUC or if a veterinarian examines the animal and determines that analgesic administration is no longer necessary.
6. The use of local pain-relieving drugs may be indicated for topical, local, regional and spinal analgesia. Lipid solubility and protein binding in the axons determine the potency of these drugs. These agents block the action potential of axons by reducing or preventing the influx of sodium ions. Local analgesics are **not** intended for use in lieu of systemic analgesics.
7. If the withholding of routinely administered intra, post-operative or post-procedural systemic analgesia has been scientifically justified in an IACUC approved protocol, the protocol will be listed as a Category E study (studies in which the animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not

receive pain relieving agents). At this time if animals cannot receive systemic analgesia with IACUC approved justification they must be listed in Category E. Special exceptions must include the “3R’s with justification from experts.

8. **Minor** procedures require at least 24 hours of post-operative analgesia, and then as needed if the animal still appears to be in pain. When animals may experience more than momentary or slight pain or distress (i.e. pain in excess of that caused by injections or other minor procedure), the investigator must provide, in the animal care and use protocol, a detailed description of how pain or distress will be assessed and how pharmaceutical agents, supportive therapies and validated non-chemical interventions (e.g. laser therapy, application of ice/cold therapy, thick bedding, acupuncture) will be used to alleviate pain and distress.
9. **Major** survival surgeries require at least 48 hours of post-operative analgesia, and then as needed if the animal still appears to be in pain. Surgeries that involve the potential for significant pain or distress (e.g. spinal cord transection, bone fracture induction, or open abdominal surgeries) may require the use of multimodal analgesia. A major survival surgery is defined as any procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic function, or any procedure that requires the use of more than a single application of a short-term anesthetic. When animals are subjected to major survival surgery, routine provision of post-surgical analgesia is required.
10. Investigators are encouraged to consult with the veterinarian during the course of protocol planning concerning appropriate use of drugs for control of potential pain and distress pertinent to Category D and E protocols. The attending veterinarian under APHIS/AC Policy 3 and PHS Policy (IV.A.3.b.1) has the authority to ensure the provision of adequate sedation, analgesia, or anesthesia. Investigators are also encouraged to consult with the veterinarian as needed to arrive at appropriate methods of treatment that meet the clinical needs of the animals and do not compromise the scientific integrity of the experiments. The relief of pain and distress in research animals is ethically sound, humane and promotes good science.

Responsibility

The ultimate responsibility for the ethical use of animals lies with the investigator. Those persons listed on an approved Animal Protocol that are responsible for procedures, surgeries, and post-procedure care are to provide close monitoring and documentation. Also, they are to contact the CMU’s Attending Veterinarian if animals show any signs of pain, infection, illness and for advice regarding the use of analgesics.

The IACUC has the ultimate responsibility for ensuring that pain and distress in research animals are limited to that which is necessary in the course of approved teaching and experimentation. They are authorized to suspend an activity involving animals in accordance with the specifications set forth in PHS IV.C.6

Background Information

Anesthesia, analgesia, and pain management are crucial components of research involving animal subjects. The standard of care at Central Michigan University is to prevent and to treat animal pain and distress whenever diagnosed. This policy is to provide guidance to researchers/instructors using animals, including rodents, which may experience more than momentary or slight pain or distress. Such animals require appropriate sedation, analgesia, and/or anesthesia unless there are suitable scientific justifications to withhold such agents which may be determined by the process of internal and/or external scientific review. Exceptions to these principals are permitted only in the minority of protocols approved by the Institutional Animal Care and Use Committee as a USDA Category E with adequate scientific justification.

One of the guiding principles in the care and use of laboratory animals is to minimize pain and distress (PHS US Govt. Principles IV). Efforts should be made to refine a procedure to reduce pain and distress or to minimize the number of potentially painful procedures animals undergo. When judging the potential effect of any procedure, it should be assumed that what would be painful to humans is painful to animals (US Govt. Principle IV and Lawson, 2009, Pg. 15). Pain and distress in laboratory animals are major animal welfare concerns that must be addressed in order to apply the principle of Refinement which is to reduce to an absolute minimum the pain and distress experienced by those animals that are used in teaching and research procedures (Flecknell, 2000).

Animals can experience pain and distress. Management of pain in animals requires anticipating and preventing the pain (pre-emptive) or recognizing and alleviating the pain (post-inductive). Procedures or situations that produce distress (stress that will alter or has the potential to alter an animal's homeostasis and disrupt biological functions critical to the animal's well being) should include analgesics, anesthetics, tranquilizers or alternative means—such as training, acclimation, enrichment, or group housing for social species to reduce or mitigate the distress

Preemptive analgesia (the administration of pre-op and intra op analgesia) enhances intraoperative patient stability and optimizes post-op care and well being by reducing post-op pain (Coderre et al. 1993, Hedenquist et al. 2000, Pg. 121)

Moribund Animals and Death as an Endpoint

Background

The OLAW/ARENA *Institutional Animal Care and Use Committee Guidebook, 2nd Edition, 2002* states that “Endpoints other than death must always be considered and should be used whenever the research objective can be attained with non-lethal endpoints.”

Policy

The routine use of death as an endpoint rather than euthanasia is strongly discouraged. Alternative endpoints other than death must always be considered whenever the research objective makes it possible.

Guidelines

- A. Investigators who wish to use death as an endpoint as part of their study must consult with the veterinarian prior to protocol submission.
- B. The protocol **must provide a scientific justification for using death as an endpoint and a discussion of alternative endpoints and why those endpoints are not acceptable.**
- C. If scientifically possible, animals should be humanely euthanized at the time the alternative endpoint is reached, or if they demonstrate signs of being moribund. Moribund is defined as “in a dying state” (Webster's New World Medical Dictionary, 3rd ed., 2008). Animals are considered moribund if they manifest **one or more** of the following clinical signs and **recovery is not expected:**
 - Inability to maintain an upright position
 - Prolonged (>24 hours) physical inability to obtain food/water
 - Prolonged(>24 hours) anorexia or clinical dehydration
 - Uncontrollable or chronic diarrhea, vomiting, constipation
 - Agonal breathing or cyanosis
 - Unconsciousness with no response to external stimuli (e.g. toe-pinch test)
- D. If death as an endpoint must be used, the following stipulations must be met:
 - Animals reaching the moribund state must be monitored at minimum every 1-2 hours including weekends and holidays)
 - Written records must be made of all monitoring sessions indicating the time and date of observations, the person performing the observations, and any findings (such as number of animals demonstrating clinically abnormal behavior, number of animals found dead, etc.) The records must be kept on file and available to the IACUC or facility personnel upon request.

- Moribund animals must be provided easy access to food and water (e.g. moist mash on the cage floor) as well as supportive therapy (e.g., IP/SC fluids, supplemental heat source, etc.)
- Cage cards indicating moribund state or death as an endpoint must be placed on every cage at the onset of experiments. A “Study Endpoint Information” sheet must also be completed and maintained in the animal room. This sheet must include the required research endpoint (moribund or death) and current contact information.

Summary

Key to fulfilling the responsibilities for both the principal investigator (PI) and the IACUC are:

- ***Understanding the legal requirements***
- ***To distinguish a normal animal from one in pain and distress.***
- ***Relieving or minimizing the pain and distress appropriately***
- ***Education:*** “Investigators, veterinarians, and animal care staff must be aware of the basic principles, causes, and signs of pain, and should be knowledgeable about pain treatment options and their potential deleterious effects. As the field of pain medicine benefits from new insights and methods for prevention and treating pain in humans, so should laboratory animals benefit from the research for which they are currently an indispensable underpinning. The ability to minimize pain in laboratory animals can proceed in tandem with advancing scientific progress”. Ref. 10

The IACUC must assure that all aspects of the animal protocol that may cause more than momentary pain and/or distress are addressed, alternatives to painful or distressful procedures are considered, and that methods, anesthetics and analgesics to minimize or eliminate pain and distress are included when these methods do not interfere with the research objectives. A written scientific justification is required to be included in the Animal Protocol for any painful or distressful procedure that cannot be relieved or minimized. The obligation to reduce pain and distress ***does not end*** with the review of the AP. It is the responsibility of the animal care staff, the research staff, the IACUC, and veterinarians to continue to monitor animals for pain, distress, illness, or mortality during the course of the research study. The animals should be observed a minimum of once daily or more often based on veterinary input.

The veterinarian has the authority to assure that handling, restraint, anesthesia, analgesia and euthanasia are administered as required to relieve pain and suffering, provided such intervention is not specifically precluded in protocols reviewed and approved by the IACUC.

Animals should be monitored for evidence of pain or distress, and should be administered analgesics or have procedures instituted to relieve pain or distress, unless scientifically justified. Observations and actions taken to relieve pain or distress must be documented. ***These documents, surgery records, post-operative records, IACUC approved score sheets must be available to the IACUC, veterinarians, and animal care staff.***

Pain scoring systems have evolved and been customized for use in both companion and research animals (Grimm and Hardie, 2004; Hawkins, 2002; Petersen, 2004; Roughan, 2003). A panel of behaviors observed by the clinical and/or research staff is formulated to designate inferred animal pain and justify decisions on alleviation of the pain. Each behavior /criteria that is chosen by observers as indicative of pain perception is given a weighted value (often ranging from 1-5) and summed to create an overall pain score. Scales of pain scores are then created, with the higher total scores indicative of higher pain states and frequently used to justify the need for potent analgesic treatment, humane endpoints or euthanasia and study exclusion.

Effective pain scoring in research animals requires training of observers to assure the scores are sensitive (e.g., will not miss animals in pain), specific (e.g., will not confuse nonpainful states, such as paralysis, with reluctance to move due to pain) and will be reproducible between different observers. In rodents in particular, it is also important that pain scoring take into account the normal nocturnal behavior of this species and not assume that inactivity during the day is solely due to pain. It is also best to formulate pain

scoring rubrics in a proactive format, in which the criteria and pain scale is formulated during study design and often with IACUC input. A team approach is also valuable, including input from veterinarians trained in recognition and methods to detect pain in laboratory animals, as well as input from scientists and research technicians experienced in working with the specific animal model in question. Not to be forgotten is input from the animal care staff that is accustomed to observing the animals during the acclimation period and is thus aware of how the animals manifest normal species-specific behaviors.