FLORIDA ATLANTIC UNIVERSITY

Division of Research

SUBJECT:	**Effective Date:**	**Policy Number:**
Recognition and Alleviation of Pain and Distress in Laboratory Animals	11/17/2017	10.4.13
	Supersedes:	**Page** **Of**
	8/25/2017 9/1/2014 4/29/2005	**1** **8**
	Responsible Authorities:	
	Vice President, Research Attending Veterinarian Institutional Animal Care and Use Committee Director, Research Integrity	

I. Background

The *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training* state "proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative". Both the *Animal Welfare Act* (AWA) and the *Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals* mandates the Institutional Animal Care and Use Committee to review and approve any animal research protocols before an animal study can be conducted. This includes determining whether pain/distress is involved and if so that alternatives are considered, described provision for alleviation of pain/distress is appropriate or if this would confound the research strong scientific justification is provided. In addition, the IACUC is also obligated to monitor the studies after approval including the implementation of the safeguards for minimization of pain/distress. The Attending Veterinarian has to be consulted during the design of a study whenever experimental procedures have the potential or real propensity to induce discomfort, pain and/or distress (AWA §2.33, b, 4).

II. Purpose

To establish a policy that provides guidance to Research Personnel how to address anticipated potential or real pain/distress during the design and conduction of a scientific experiment using an animal model. To minimize the impact on the study animals through timely recognition of pain or distress as well awareness of species-specific measures that can be implemented to alleviate it by Research as well as Animal Care Staff.

III. General Statement

A key aspect of the animal welfare regulations is that pain and distress in laboratory animals be prevented or minimized whenever possible. The safeguarding of the well-being of the animals is the legal and moral obligation of everybody involved in animal use and care including IACUC members, veterinary and animal care staff as well as research personnel. **Prevention and lessening of pain/distress is likewise imperative for**

animal welfare and quality of research conducted. Pain and distress can represent uncontrolled experimental variables with significant effects on research results since it has been demonstrated that pain and stressors result in production of cytokines and activation of an endocrine state of catabolism often translating into immunosuppression and development of chronic pain states.
Many laboratory animal species will hide their pain or distress and it is often difficult to recognize it early enough. Therefore, it is generally accepted and expressed in animal welfare regulations to assume that any procedures that would cause pain or distress in humans will cause the same in other animal species as well unless evidence to the contrary has been established.

IV. Policy

1. Researchers have to consider the impact that any proposed experimental procedures have on the animals used in research or teaching and need to describe this in the animal care and use protocol. Alternatives to studies that might cause pain and/or distress have to be explored.
2. To minimize pain and/or distress, experiments have to be designed and performed in such a way as to prevent the animals from experiencing problems unless necessary to achieve the goals of the study.
3. Any measures that can alleviate pain and/or distress in animals need to be considered whenever experimental procedures, induced or spontaneous disease states studied are anticipated to include pain and/or distress. Those measures that improve animal welfare without compromising the scientific integrity of the project need to be implemented.
4. Experiments that require animals to experience pain and/or distress that CANNOT be relieved with anesthetics, analgesics and supportive care methods due to interference with the experimental protocol or that necessitate the use of death as an endpoint require a strong scientific justification by the PI and special consideration by the IACUC before approval can be granted.
5. Researchers have to consider humane end-points when animals have to be removed from the study and/or need to be euthanized for any study involving more than momentary or slight pain. Humane end-points are study specific and need to be described in the IACUC protocol whenever possible.
6. If painful and/or distressful conditions occur during the conduct of a study that were not anticipated, the protocol needs to be amended to reflect these conditions in conjunction with appropriate measures to alleviate pain and/or distress as well as humane end-points or a strong scientific justification why pain and/or distress cannot be relieved.
7. The IACUC might require the performance of a pilot study in certain circumstances when it is not clear whether pain and/or distress is to anticipate or what level of pain and/or distress will be involved. Such circumstances might include but are not limited to introduction of a novel animal model or experimental procedure and newly created genetically modified animals.
8. The IACUC has adopted the USDA pain categories not only for AWA covered species but any vertebrate animal used in the FAU animal care and use program.

9. The PI in conjunction with the help of the AV has to assign the different experimental groups of animals to the appropriate pain category. The assigned pain category should be reviewed at appropriate intervals.
10. All personnel working with animals must be qualified and trained to recognize signs of pain and/or distress and to administer appropriate measures of relief in the particular animal species involved in their studies.
11. The AV or his/her designee has to be engaged whenever pain and/or distress occurs in study animals that was either not anticipated at all or is not relieved by the measures described in the animal care and use protocol.

V. Definitions

1. **Pain** is defined for non-human animals as "an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species-specific behavior, including social behavior" by Zimmerman. **Nociception** is a measurable physiological event of a type usually associated with pain. A sensation of pain can exist in the absence of nociception.
2. **Stress** is the effect produced by physical or environmental events (i.e. external stimuli) or physiologic or psychological factors (i.e. internal)
3. Stress can lead to **distress** if not relieved producing mental or physiological reactions leading to illness. Distress is also defined as great pain, anxiety and sorrow. For animals distress includes conditions that are disagreeable, and which the animal would choose to avoid if possible.
4. **USDA Pain Categories**
   A. (Note: there is no USDA level A.)
   B. Level B: Breeding or Holding Colony Protocols, no experimental procedures performed on animals
   C. Level C: Experimental procedures that do not induce more than momentary or slight pain or distress.
   D. Level D: Any experimental procedures that will induce pain or distress and the pain/distress will be relieved with anesthetics, analgesics and/or supportive care methods.
   E. Level E: Pain or distress or potential pain or distress that is **not** relieved with anesthetics, analgesics, anxiolytic drugs or other methods for relieving pain or distress.
5. **The "3 R's":** The approach to minimize any pain and distress associated with animal research models has been described most fully in Russell and Burch's classic text "the Principles of Humane Experimental Techniques". Since then it has been popularized as the "3 R's" of animal experimentation: **Reduction** of the numbers of animals used, **Replacement** of animals with nonsentient alternatives or with human subjects when appropriate, **Refinement** of experimental design to minimize pain and distress.
6. The term **Humane Endpoints** describes the setting of clear, predictable and irreversible criteria that allow early termination of the experiments before the animal experiences significant harm while still meeting the experimental objectives. Humane endpoints are chosen to minimize or terminate the pain or distress of the experimental animals via euthanasia rather than waiting for their deaths as the endpoint.
7. **Death as an Endpoint** refers to projects in which the animals' non-experimentally induced death is required as a measured data point. It does not refer to projects in

which the animals will be euthanized prior to non-experimentally induced death for tissue collection or project termination.

8. **Moribund** is defined as "in a dying state". Animals are considered to be moribund if they are in a debilitating physical state where death is imminent and treatment ineffectual.
9. **Morbid** state is a condition relating to, or typical of, disease or illness.
10. **Analgesia** is the absence of the sense of pain without loss of consciousness. An analgesic, or painkiller, is any member of the group of drugs used to achieve analgesia.
11. **Supportive Care** can be defined as any intervention to reduce pain and/or distress that is not drug based.

VI. Accountability

The Principal Investigator (PI) will be responsible for:

- Describing all experimental procedures in the animal care and use protocol considering whether any of the procedures can induce pain and/or distress, measures to alleviate pain and/or distress if applicable and humane endpoints.
- Assuring that personnel are appropriately trained to recognize pain and/or distress or any abnormalities not expected with the animal model in the species they are using.
- Assure that research personnel follows the animal care and use protocol, especially in regards to use of described anesthetics, analgesics and other supportive care methods and that any anesthetic, sedative or analgesic drug administered to animals is within the manufacturer provided expiration date.
- Involving the AV or his/her designee in the design of the study pertaining to painful/distressful procedures and whenever any pain/distress is recognized that is unexpected or cannot be relieved with the measures described in the animal care and use protocol.

The IACUC will be responsible for:

- Reviewing and approving, requiring modifications in (to secure approval) or withholding approval of IACUC protocols and/or amendments, especially assess the appropriateness of the use of painful/distressful procedures, the measures to be implemented to alleviate pain/distress and proposed humane endpoints.
- Providing oversight for all animal procedures conducted including painful/distressful procedures and conditions as well as provision of pain/distress relief.
- Develop and direct an appropriate training program.

The Research Integrity office will be responsible for:

- Administrative support of the IACUC members to facilitate their regulatory function
- Maintaining policy and assure regular review and update as necessary by the IACUC
- Keeping relevant training records and provide to the IACUC for review

The Office of Comparative Medicine (CM) will be responsible for:

- Veterinary review of IACUC protocol(s) and advice PI on appropriate study design concerning painful/distressful procedures as well as appropriate methods for anesthesia, analgesia, other supportive care measures and humane end points.
- Providing support and training for all personnel including animal care staff regarding recognition of pain/distress in particular animal species and

administration of anesthetics, analgesics and supportive care including hands on training, verification of proficiency of personnel in particular methods via observation and support regarding proper usage of equipment and supplies.

- Daily observation of all animals housed in VS managed vivaria and notification of the AV or his/her designee and the research personnel whenever unusual signs of pain/distress are observed in any of the animals under their care.

VII. Procedures

A. Study Design and IACUC Protocol

1. The PI has to consult with the AV or his/her designee whenever an animal model will be utilized that involves or has the potential of painful/distressful procedures or experimental disease states. The consult should start already during the design of the study and includes the veterinary review of the animal care and use protocol.
2. All experimental procedures have to be assigned a USDA pain category and the highest pain category needs to be entered into the protocol by species. Control groups might be exposed to procedures with a lesser pain category, which should be described in the procedure(s) section of the IACUC protocol (see examples for USDA pain/distress categories at http://www.fau.edu/research/comparative-medicine/support-material-guidelines.php).
3. Death as an endpoint has to have a strong scientific justification and should only be proposed if euthanizing a moribund animal would invalidate the study. In the IACUC protocol it needs to clearly state why alternatives are not appropriate. The minimum number of animals necessary to achieve statistical significance should be used. Death as an endpoint needs to be avoided whenever an earlier endpoint can provide the necessary scientific outcome. Death as an endpoint is always considered pain category E.
4. Measures to alleviate pain and/or distress must be described in detail in the IACUC protocol including timely interventions such as administration of anesthetic/analgesic/tranquilizing drugs and supportive care methods. Note that analgesic, sedative, and analgesic drugs cannot be administered to animals beyond the manufacturer provided expiration date. Frequency of monitoring must be stated for the time range pain/distress is anticipated, which needs to correlate with the severity of such expected. The frequency might have to be increased whenever pain/distress cannot be alleviated or death as endpoint for scientific reasons. Responsible research personnel need to be identified.
5. The earliest clinical endpoint (i.e. humane endpoints leading to early euthanasia) that will contribute to the resolution of the hypothesis must be identified for procedures or induced and spontaneous experimental disease states including pain and/or distress.
6. Investigators have to consider increased monitoring for animals involved in protocols that have the potential for pain/distress that may either not be reliably controlled or anticipated. Examples for such situations are new and/or unique phenotypes of transgenic, knock-in, knock-out, or otherwise genetically-engineered rodents, performance of novel procedures and/or newly introduced animal models.
7. Investigators must continuously refine methods and search for alternatives to painful/distressful procedures and models. Protocols have to be amended

whenever changes in pain category and frequency of monitoring appear essential as well as improved measures of alleviation of pain/distress and/or refined experimental procedures become available amongst other necessary changes in the study.

B. Recognition of Pain and/or Distress

1. Investigators should feel confident in judging the condition of their animal subjects, differentiating between animals well-managed for pain control, morbid and moribund animals (see http://www.fau.edu/research/comparative-medicine/support-material-guidelines.php for a link to the FAU formulary for Guidelines for Recognition of Pain/Distress, Use of Anesthetics and Analgesics in Laboratory Animals identifying morbid and moribund animals), and be prepared to perform approved euthanasia when necessary.
2. The presence of pain in animals can be recognized by alterations in animal behavior (e.g. reduced activity and grooming, hunched-up posture, changes in temperament, removal from social interactions, reduced food and water intake) and in physiological variables (e.g. reduced depth of respiration, increased heart rate, dehydration at varying degrees).
3. The evaluation of potential pain and distress is complex due to the variation of thresholds and manifestations of pain and distress among species and even individuals within a species. See guidelines for assessing pain and distress in various animal species (http://www.fau.edu/research/comparative-medicine/support-material-guidelines.php for a link to the FAU formulary for Guidelines for Recognition of Pain/Distress, Use of Anesthetics and Analgesics in Laboratory Animals). Personnel have to be trained appropriately to recognize early species specific signs of pain/distress.
4. The diverse task of alleviation of pain and distress requires various measures and most often a combination of such including drugs, adjustments to the environmental conditions such as light, temperature and noise, provision of specific environmental enrichment, social housing, modification in research protocols and other humane strategy as feasible (http://www.fau.edu/research/comparative-medicine/support-material-guidelines.php for a link to the FAU formulary for Guidelines for Recognition of Pain/Distress, Use of Anesthetics and Analgesics in Laboratory Animals). Some of these measures might interfere with research results.
5. Animal pain/distress can produce a range of undesirable physiological changes, which may radically alter measured responses to experimental stimuli as well as the rate of recovery from surgical/experimental procedures. Therefore, its avoidance and alleviation are imperative for animal welfare and sound science.

C. Clinical Oversight of Animals

1. The PI and his/her research staff are responsible for the clinical oversight of animals involved in their studies. This includes regular monitoring of their animals to assess discomfort, pain, distress and/or health, appropriate administration of anesthetic/analgesic/tranquilizing drugs and supportive care methods as described in the protocol. The clinical oversight is aided through the daily observations, husbandry and care by animal care personnel.
2. It is the responsibility of the research personnel to involve the veterinarian whenever necessary, especially if health concerns occur including increased

morbidity/mortality and/or pain/distress cannot be appropriately alleviated with the measures described and approved in the IACUC protocol.

3. Reducing post-procedural/post-operative pain/distress is accomplished by good nursing (i.e. supportive) care (e.g. keeping animal warm, clean, well padded, in a quiet environment with con-specifics if possible) and administration of analgesic drugs. It is best to provide preemptive (i.e. before the painful insult) analgesics to avoid "wind-up" pain that is often difficult to control and monitoring for pain should continue during and beyond the approved analgesic regimen. Supplementary analgesics need to be provided to individual animals on an as needed basis. In addition, it is important to prevent and/or treat post-anesthetic complications such as aspiration pneumonia, dehydration, infection. Note that any drug administered to animals, especially anesthetics, analgesics, and euthanasia agents cannot be expired at the time of administration.
4. If death as an endpoint has been approved by the IACUC, the research personnel is responsible for monitoring the animal(s) at minimum twice daily when the moribund state has been reached (in the early morning and late afternoon) including weekend and holidays. Moribund animals must be provided easy access to food and water. Written records must be made of all monitoring events indicating date/time of observation, any findings (such as number of moribund animals and animals found dead) and initial of the person performing the monitoring. These records have to be easily available to the IACUC and CM personnel upon request.
5. The assignment of pain categories has to be reviewed at appropriate intervals and changes may be recommended after additional observation. Amendments to protocol should be made either if the pain category is suggested to be reduced or increased.
6. The PI and research staff should maintain written records of activities anticipating painful/distressful outcomes or the possibility of it. Records should be kept within the animal facility. The entries should describe when the painful or distressful outcome is first recognized, wheat measures are instituted, and when the adverse outcome is resolved or the animal euthanized.
7. The PI and research staff must conduct clinical and post mortem investigations whenever animals experience morbidity or mortality not anticipated in the protocol.

VIII. Policy Renewal Date

11/17/2020

IX. References

1. PHS Policy on Humane Care and Use of Laboratory Animals2.
2. Animal Welfare Regulations, 9 CFR Ch. 1
3. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
4. Guide for the Care and Use of Laboratory Animals, 8th edition, 2011
5. Russell, VMS and Burch, RL. The Principles of Humane Experimental Technique. Methuen, London, 1959.
6. Beilin, B. et al. The Effects of Postoperative Pain Management on the Immune Response to Surgery. Anesth. Analg. 97, 822, 2003.
7. Desborough, JP. The Stress Response to Trauma and Surgery. Br. J. Anaesth. 85, 109, 2000.
8. Zimmerman, M. Physiological Mechanisms of Pain and its Treatment. Klinische Anaesthesiol Intensivether, 32:1-19, 1986.

POLICY APPROVAL

Initiating Authority

Signature:_____ Date:_____

Name: Daniel C. Flynn, Ph.D., Vice President for Research

Executed signature pages are available in the Initiating Authority Office(s)