

## Guidelines for Pain Category Classification of Animals

All animals used on an approved Animal Study Proposal [ASP] must be assigned a pain and distress category. The NCI at Frederick Animal Care and Use Committee is tasked with reviewing the proposed experimental procedures and ensuring that the animals are properly categorized based upon the procedures they will undergo during the course of the protocol. Any procedures that could cause pain or distress in humans should be assumed to also cause pain or distress in animals.

The pain categories in the table below are based upon the USDA Classification of Pain Categories and are reported in Section G of the ASP form. The NCI at Frederick uses the category designations 1, 2, and 3, with their corresponding USDA category listed in parentheses. All animals should be placed in the highest level of pain or distress that they are expected to experience on the approved ASP.

Category 1 (USDA C)	Category 2 (USDA D)	Category 3* (USDA E)
Animals that are subject to procedures that cause no pain or distress, or only momentary or slight pain or distress and do not require the use of pain-relieving drugs.	Animals subjected to potentially painful or stressful procedures for which they receive appropriate anesthetics, analgesics and/or tranquilizer drugs.	Animals subjected to potentially painful or stressful procedures that are <b>not</b> relieved with anesthetics, analgesics and/or tranquilizer drugs. Withholding anesthesia/analgesia must be scientifically justified in writing and approved by the IACUC.
Examples	Examples	Examples
<p><b>Tumor Production:</b> Animals do not show any clinical signs of pain/distress and are euthanized before the humane endpoints are reached.</p> <p><b>Animal Models with Abnormal Phenotypes:</b> Models that do not elicit pain or distress thus do not require any treatment or anticipate the need for humane euthanasia.</p> <p><b>Animal Identification:</b> Ear-tagging; ear-punching; microchipping; tattooing; toe-clipping rodents (only in mice aged 5-17 days).</p> <p><b>Blood/Fluid Collection:</b> Blood collection from peripheral vessels, collection of other fluids <u>with mild/brief restraint</u>.</p> <p><b>Euthanasia:</b> AVMA approved euthanasia procedures</p> <p><b>Food restriction:</b> that reduces the animals weight by no more than 20% of normal age matched</p>	<p><b>Tumor Production:</b> Appropriate treatment when specific clinical signs are observed or euthanasia when humane endpoint is reached; ascites production &lt;=120% baseline weight and not requiring abdominal taps</p> <p><b>Animal models with abnormal phenotypes:</b> With appropriate treatment or euthanasia when humane endpoint is reached.</p> <p>Ageing studies: Animal are euthanized when they start showing clinical signs of pain/distress related to ageing for example: weight loss of more than 20%, hunched posture etc.</p> <p><b>Blood/Fluid Collection:</b> Any blood/fluid collection procedure in rodents <u>requiring anesthesia</u> (e.g. cardiac, CSF tap, cut-down, and retro-orbital blood access).</p> <p><b>Food Scheduling:</b> Caloric restriction, fasting for &gt;16</p>	<p><b>Tumor Production:</b> Ascites production models exceeding 120% baseline weight or requiring abdomen taps to relieve ascites accumulation.</p> <p><b>Animal Models with Abnormal Phenotypes:</b> Endpoints of the model require a painful, distressful, or moribund condition for a scientifically justified period of time.</p> <p><b>Euthanasia</b> by procedures not approved by the AVMA</p> <p><b>Irradiation:</b> lethal Endpoints of the model require a painful, distressful, or moribund condition for a scientifically justified period of time.</p> <p><b>Non-Surgical Procedures with Potential for Pain or Distress:</b> Endpoints of the model require a painful, distressful, or moribund condition for a scientifically justified period of time. For example, injecting substances at doses that are known to</p>

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<p>controls</p> <p><b>Genetically Altered Animals (Genotyping):</b> Collection of limited tissue sample from weanling rodents (up to 21 days of age), with the exception of toe clipping (see “Animal Identification” above).</p> <p><b>Imaging:</b> Short-term imaging procedures with conscious animals or prolonged imaging procedures with anesthesia/sedation.</p> <p><b>Injection/Dosing:</b> Injection or oral gavage of benign agents (saline, vehicle, antibiotics) or other dosing when there is no expectation of deleterious effects.</p> <p><b>Lasers:</b> Short-term laser exposure for conscious animals.</p> <p><b>Prolonged Restraint:</b> If animals are acclimated to the device and the duration of restraint is less than 30 minutes.</p> <p><b>Radioactive Materials:</b> Administration of radioactive materials should not induce pain or distress as a stand-alone procedure.</p> <p><b>Special Diets/Liquids:</b> In most cases, provision of special food or medicated water should not induce pain or distress as a stand-alone procedure.</p> <p><b>Special Housing and Husbandry:</b> Variations on standard housing modalities that should not lead to stress/distress.</p>	<p>in rodents. Alleviation of pain/distress by removal of animal from study, providing feed or sucrose water, and/or euthanasia.</p> <p><b>Genetically Altered Animals (Genotyping):</b> Tail biopsy in rodents &gt; 21 days old under anesthesia.</p> <p><b>Irradiation:</b> Appropriate monitoring and supportive care expected humane endpoints should be described.</p> <p><b>Injection/Dosing:</b></p> <ul style="list-style-type: none"> <li>• Intranasal inoculations in rodents under anesthesia.</li> <li>• Footpad injections in rodents under anesthesia depending on the compound being injected.</li> <li>• Retro-orbital injections in rodents under anesthesia.</li> <li>• Injection of compounds, like vaccinations, toxins or adjuvants [excluding Complete Freund’s Adjuvant (CFA)] that may be irritating or may cause local or generalized inflammation or Repeat injections - Necessitating increased monitoring of injection sites and overall health with potential removal from study.</li> </ul> <p><b>Lasers</b> - Anesthesia, if required to reduce distress from awake prolonged restraint.</p> <p><b>Prolonged Restraint</b> - If not acclimated, if longer than 30 minutes, or if animals are removed from the device, then treated (if applicable) if agitation and/or distress are noted.</p> <p><b>Radioactive Materials:</b> Dose or duration-associated adverse effects that are relieved with supportive care or removal from the study (or euthanasia).</p> <p><b>Special Diets/Liquids:</b> If associated with a potentially painful or distressful procedure (e.g.</p>	<p>cause deleterious effects [LPS, injecting a toxin, adjuvant, etc or foot pad injection with Complete Freund’s Adjuvant (CFA)]</p> <p><b>Special Housing and Husbandry:</b> Husbandry conditions that may precipitate pain/distress for a scientifically justified period of time with no alleviation of signs.</p> <ul style="list-style-type: none"> <li>• Constant darkness for diurnal species</li> <li>• Hypoxia, hyperoxia, hypercapnia</li> <li>• Prolonged heat or cold exposure</li> </ul> <p><b>Survival Surgery:</b> Post-op analgesia withheld if it will interfere with the model and is scientifically justified. (Note: surgical plane of anesthesia always required).</p>

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	<p>tumor production, DSS-induced colitis, etc.) and additional treatments (including analgesia) to alleviate distress.</p> <p><b>Special Housing and Husbandry:</b> Appropriate and frequent monitoring and humane endpoints are in place to provide alleviation that is specific to the protocol (i.e. nestlets provided to mice undergoing cold exposure if unable to thermoregulate).</p> <ul style="list-style-type: none"> <li>• Constant darkness for diurnal species</li> <li>• Hypoxia, hyperoxia, hypercapnia</li> <li>• Prolonged heat or cold exposure</li> <li>• No contact bedding or nesting material (mice)</li> </ul> <p><b>Non-Surgical Procedures with Potential for Pain or Distress:</b> Anesthesia, analgesia, other appropriate treatment or euthanasia when humane endpoint is reached.</p> <p><b>Survival Surgery:</b> Anesthesia and post-op analgesia</p> <p><b>Terminal Surgery:</b> Anesthesia +/- pre-op analgesia</p> <p><b>Water Scheduling:</b> Provision of additional water or parenteral fluids with signs of dehydration or otherwise indicated.</p>	

\*Please note that this is not a comprehensive list of procedures that could warrant a Category 3 designation. The ACUC reviews each study on a case-by-case basis to determine if animals are undergoing procedures that require Category 3 classification. If a procedure is classified as Category 3, the investigator is required to provide the following information to the ACUC for consideration:

- *A scientific justification as to why the appropriate use of anesthetics, analgesics, sedatives, tranquilizers, or timely euthanasia are contraindicated in the study;*
- *A description of his/her consideration of alternatives and the determination as to why alternatives are not available; and*
- *A literature search statement to include the database(s) searched, the date of the search, the period covered, and the keywords that were used.*

Certain situations [reviewed by the ACUC on a case by case basis] may require pilot studies under post approval monitoring by the Laboratory Animal Medicine Veterinary Staff. The following journal articles provide guidance on the effects of buprenorphine:

<http://www.ncbi.nlm.nih.gov/pubmed/25650386> - Primary tumor growth (orthotopic model) not influence by analgesic treatment with buprenorphine or meloxicam (indeed improved the quality of the study by prolonging the health life-time of the mice!)

<http://www.ncbi.nlm.nih.gov/pubmed/25153315> - Buprenorphine does not affect estrous cyclicity

<http://www.ncbi.nlm.nih.gov/pubmed/24536038> - Buprenorphine does not impact inflammatory response in mice with haemarthrosis.

<http://www.ncbi.nlm.nih.gov/pubmed/16764216> reports: “chronic administration of buprenorphine led to immune parameters important for antimicrobial responses or for anti-tumour surveillance (lymphoproliferation, natural killer (NK)-lymphocyte activity, cytokine production, lymphocyte number) being unaffected.”

<http://www.ncbi.nlm.nih.gov/pubmed/15275790> “evaluated lymphoproliferation, natural killer cell activity and interleukin-2 and interferon-gamma production. Drugs were administered acutely at the equianalgesic doses of 0.25 mg/kg for fentanyl and 5 mg/kg for buprenorphine, or delivered continuously with osmotic pumps for 24 h, 3 and 7 days at the rate of 7.5 microg/h per mouse (fentanyl) and 12.5 microg/h per mouse (buprenorphine). After acute administration, a significant decrease of lymphoproliferation is observed in fentanyl-treated animals only. no immune alterations were ever present in buprenorphine-treated animals”

<http://www.ncbi.nlm.nih.gov/pubmed/14597216> “assess the effects of chronic exposure to buprenorphine on hypothalamic- pituitary-adrenal (HPA) axis activation, corticosteroid-binding globulin (CBG), the main glucocorticoid (GC) carrier, and the immune system.”

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3358986/> “investigated the effects of buprenorphine in inbred mice of both sexes undergoing cecal ligation and puncture (CLP)... The findings suggest that the effects of buprenorphine on sepsis models in C57BL/6 mice may be sex-specific.”

<http://www.altex.ch/All-issues/Issue.50.html?iid=154&aid=2> “subcutaneously implanted ALZET® osmotic pumps continuously releasing buprenorphine to reduce pain in mice with LCMV-induced meningitis. Mice treated with buprenorphine demonstrated strongly reduced symptoms of pain. The LCMV-specific cytotoxic T cell response and the immune cell infiltration into the central nervous system (CNS) were unchanged in analgesia treated mice, indicating that the LCMV-induced immune response was not altered in these mice. “

<http://lan.sagepub.com/content/33/4/328.long> suggests buprenorphine impacts TNF-alpha levels

Additionally, the following is an article on the effect of tramadol on immunity in mice:

<http://www.ncbi.nlm.nih.gov/pubmed/9313273> “we evaluated the effects of the acute and chronic administration of tramadol on nociceptive thresholds (by the hot-plate test) and on immune responses (by measuring Concanavalin A-induced splenocyte proliferation, IL-2 production and natural killer activity) in the mouse. After acute subcutaneous administration, tramadol induced antinociception starting from a dose of 20 mg/kg, whereas it significantly enhanced natural

killer activity and IL-2 production at doses as low as 1 mg/kg and splenocyte proliferation starting from a dose of 10 mg/kg. After the chronic administration, the antinociceptive effect of the drug was still present, whereas the immune modifications disappeared.”

The following is an article on the effect of tramadol on immunity in humans:

<http://cancer.cytoluminator.com/cancer-photodynamic-therapy/tramadolo-nk.pdf>

Additional information regarding alternatives to painful procedures and database reference searches can be found at

<http://ncifrederick.cancer.gov/Lasp/Acuc/Frederick/Alternatives.aspx>



## Assistance with Accurate Annual Reporting for Research Facilities

September 2015

In order to assist research facilities in accurately reporting animal use on the Annual Report, APHIS Form 7023, Animal Care is providing the following information and examples as guidance only. Research activities are often unique and specific questions not covered by these examples should be directed to the appropriate Regional Office.

### Animal Care and Use Review

When an Animal Care and Use Proposal is reviewed, the IACUC must make a determination as to whether the procedure could potentially cause more than slight or momentary pain or distress. If the IACUC determines that the procedures could potentially cause more than slight or momentary pain or distress, the investigator must consider alternatives to all the procedures in that study that may cause pain or distress.

At that time, the IACUC must also review the scientific explanation for justifying the withholding of analgesics, anesthetics or tranquilizing drugs that could be used to relieve the pain or distress animals on the study might experience. If the animals do experience pain which cannot be relieved with appropriate anesthetics, analgesics or tranquilizing drugs, because they would adversely affect the study, those animals are reported in column E with the explanation and reason why pain and distress could not be relieved.”

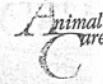
### Annual Report of the Research Facility

Occasionally, during the course of a research project unforeseen events involving animals occur, and questions arise as to how best to report these animals on the APHIS Form 7023. Unexpected pain or distress and animal incidents unrelated to ongoing research should be brought to the attention of the IACUC for purposes of adequate protocol and program review.

The following examples are not intended to address protocol review, veterinary care, or training and qualification requirements. Animal Care is providing the following examples as guidance for annual reporting purposes only.

Example 1) An animal experiences unexpected pain due to the research procedures, during the course of a study. The pain is recognized and treated with appropriate analgesics in a timely manner.

Answer: Reported in Column D.



Example 2) An animal experiences unexpected pain due to a research procedure but when the pain is recognized, the investigator determines that analgesics, anesthetics or tranquilizers would adversely affect the study.

Answer: Reported in Column E.

Example 3) An animal is unexpectedly found dead in the cage during the course of a study. The animal had been monitored appropriately and there were no pre or post mortem sign of pain or distress. The animal had not experienced pain as part of the study prior to its death.

Answer: Reported in Column C.

Example 4) An animal experiences unexpected pain or distress due to the research procedures during the course of a study. The pain is recognized and the animal is euthanized in a timely manner.

Answer: Reported in Column D.

Example 5) An animal accidentally becomes caught in a cage and experiences pain and distress which is completely unrelated to the study. The injuries are treated and appropriate analgesia is provided.

Answer: This animal should be reported in the pain category appropriate to its experiences in the study. The accident does not affect the reporting category. If the animal did not experience any pain or distress as part of the approved study it would be reported in Column C.

Example 6) An animal develops an ear infection and experiences pain or distress entirely unrelated to the study. Analgesics, anesthetics or tranquilizers would adversely affect the study so the animal is treated with palliative husbandry methods.

Answer: This is a tough one and does not fit easily into any of the classifications. Because the pain relief must be withheld due to the study, even though the pain is not caused by a research procedure, report this animal in Column E and provide a justification for not providing pain relieving analgesics.

