

University at Buffalo Institutional Animal Care and Use Program

Policy on Assignment of Pain and Distress Categories in IACUC Protocols and IACUC Review of Category D and E Procedures

Introduction

The University at Buffalo's Institutional Animal Care and Use Committee (IACUC) requires that vertebrate animals used for research, teaching or testing are assigned to a USDA pain and distress category on the IACUC protocol under which they are used. (Note that this is applied to all vertebrate species, even those not covered by USDA's Animal Welfare Act.) Numbers of animals used in each category are designated within each experiment in the Click protocol. This policy clarifies the classification of various animal procedures and provides definitions and examples of the USDA pain and distress categories. This policy is intended to ensure that animals are consistently assigned to the correct pain and distress categories. This policy also emphasizes the IACUC's review process when procedures are designated as Category D or E to align the review process with the statutory requirements of The Guide for Care and Use of Laboratory Animals, The Animal Welfare Act/Animal Welfare Regulations and PHS Policy.

Definitions

"Pain" and "Distress" have definitions that may vary based on the scientific definition of study. For the sake of this policy, the descriptions in The Guide will be considered.

Pain: Complex experience that typically results from stimuli that damage or have the potential to damage tissue; such stimuli prompt withdrawal and evasive action.

Distress: An aversive state in which an animal fails to cope or adjust to various stressors with which it is presented.

Painful Procedures: Any procedure that would reasonably be expected to cause **more than momentary or slight pain or distress** in a human being to which that procedure is applied, that is, pain in excess of that caused by typical injections or other minor procedures.

USDA Category B: Animals being held, bred, or conditioned for use in teaching, experiments, research or surgery, but not yet used for such purposes. This category does NOT apply if animals have undergone identification (i.e. ear tagging, ear punching, etc.) or genotyping (tail snip, etc.) procedures.

USDA Category C: Animals are subjected 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.

USDA Category D: Animals are subjected to potentially painful or stressful procedures for which they receive appropriate anesthetics, analgesics and/or tranquilizer drugs

USDA Category E: Animals subjected to potentially painful or stressful procedures that are not relieved with anesthetics, analgesics and/or tranquilizer drugs. Withholding anesthesia/analgesia must be scientifically justified in writing and approved by the IACUC.

Effective Date: 8/18/2025

Next Review Date: 8/2028

Approved: 8/1/2022

Revised: 8/18/2025

Policy

1. Efforts must be made to properly categorize the number of animals in each pain and distress category in each protocol. The tables that follow should serve as a resource both for Principal Investigators and IACUC members. If there are questions about how to categorize a procedure that may not be included, please contact the IACUC Office.
2. During protocol review, IACUC is responsible for ensuring that discomfort, distress and pain to animals are avoided or minimized. To ensure this, the following must be done:
 - a. The protocol must document appropriate **consideration of alternatives** for all Category **D and E** procedures.
 - i. The **Animal Justification-Alternatives Section in CLICK** should list all D and E procedures.
 - ii. The **Animal Justification-Alternatives Section** must document a recent and appropriate keyword search for each procedure from a **minimum of two databases**.
 - A highly recommended resource (may request free literature searches for alternatives) is the Animal Welfare Information Center: [Animal Welfare Information Center \(AWIC\) | National Agricultural Library \(usda.gov\)](https://awic.nal.usda.gov/)
 - iii. If alternative procedures cannot be used or are not appropriate, this must be documented in written response to **Animal Justification-Alternatives, Question 3** (“If alternative procedures were identified that would result in less pain and/or distress, clarify why they cannot be used or are not appropriate for this study”).
 - An example of this would be an explanation for why positive reinforcement (highly preferred food or fluid reward) could not be used in lieu of water restriction for conditioned-response research.

Effective Date: 8/18/2025

Next Review Date: 8/2028

Approved: 8/1/2022

Revised: 8/18/2025

Validate

Compare

Alternatives ?

Basic Information & Funding

Basic Information

Protocol Team Members

Funding Sources

Experimental Design

Scientific Aims

Experiments

Procedure Personnel Assignment

Strains

Animal Justification

Animal Justification

Alternatives

Duplication

Animal Use and Housing

Housing

Dissection

1. Record all searches for alternatives for each procedure that causes pain or distress: (category D or E)

+ Add

Procedures	Search Date	Database
There are no items to display		

2. Identify any other references used to find alternatives: (such as periodicals, publications, and consultation)

3. If alternative procedures were identified that would result in less pain and/or distress, clarify why they study:

- b. All Category D and E Procedures will be reviewed by IACUC to ensure:
- Procedures that may cause more than momentary or slight pain or distress are 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.
 - Procedures are planned with consultation from the Attending Veterinarian or their designee.
 - Procedures do not include the use of paralytics without anesthesia.
 - Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of or during the procedure.
 - This is accomplished by defining **clear humane endpoints** for any Category D or E procedure by fully completing Question 4 (i, ii, iii and iv) that appears on the first page when creating or modifying any procedure (see below).
 - In some cases, monitoring charts will be required for IACUC approval requiring uploading them as supporting documents. When required, monitoring charts must regularly be submitted to the IACUC for review.

Effective Date: 8/18/2025
 Next Review Date: 8/2028
 Approved: 8/1/2022
 Revised: 8/18/2025

4. * Will administering this procedure cause any more than momentary pain and/or distress? ☐ Yes ☐ No [Clear](#)

If yes,

i. Will it be relieved by anesthesia, analgesia, or other treatments or interventions?

☐ Yes

☐ No

[Clear](#)

ii. Identify expected symptoms from administering this procedure: ?

iii. What objective criteria will be monitored to identify pain and/or distress, frequency (i.e., twice daily, daily, weekly), and duration monitoring of the experiment):

[+ Add](#)

Objective Criteria

Frequency

There are no items to display

iv. Based upon these criteria, when will animals be removed from research: ?

- c. Any experiment which includes any Category E procedure(s) should include a statement that names the procedure(s) as well as the PI's reasoning for how potential research benefit outweighs potential harm. This statement and explanation should be included in response to Question #4 in each experiment ("Justify the purpose of this experiment"). IACUC will also conduct a **Harm-Benefit Analysis** of any **Category E** procedure to determine whether research benefit is outweighed by potential animal harm. If harm is greater than perceived benefit, approval will be withheld.

4. * **Justify the purpose of this experiment:**

The following procedures are classified as Pain Category E...

Harm is expected to be outweighed by potential research benefit because...

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Approved: 8/1/2022

Revised: 8/18/2025

Procedures and Pain Categories

Category B Procedures:

<input type="checkbox"/> Breeding WITHOUT genotyping or identification
<input type="checkbox"/> Holding animals prior to use

Category C Procedures:

<input type="checkbox"/> Animal Identification	<ul style="list-style-type: none">➤ Ear-tagging➤ Ear-punching➤ Tattooing
<input type="checkbox"/> Behavioral Studies (Non-invasive)	<ul style="list-style-type: none">➤ Open Field Tests➤ Mazes➤ Conditioned Place Preference➤ Positive Reward Conditioning➤ Allodynia and Hyperalgesia Tests<ul style="list-style-type: none">○ Von Frey Test○ Hot Plate Test○ Tail Flick Test○ Hargreaves Test➤ Strength and Climbing Tests➤ Rotarod and Balance Beams➤ Object Recognition Test➤ Marble Burying Tests➤ Running Wheel➤ Food/Fluid Preference Tests➤ Novelty-Induced Hypophagia
<input type="checkbox"/> Blood Collection (peripheral vessels)	<ul style="list-style-type: none">➤ Sub-mandibular Blood Collection (mice)➤ Tail Vein/Saphenous Blood Collection (rodents)➤ Large Animal Peripheral Blood Collections (jugular/cephalic vein, etc.)
<input type="checkbox"/> Euthanasia	By definition, euthanasia is “ending the life of an animal in a way that minimizes or eliminates pain or distress”. (AVMA Guidelines)
<input type="checkbox"/> Fasting	<ul style="list-style-type: none">➤ Fasting ≤24 hours
<input type="checkbox"/> Genotyping	<ul style="list-style-type: none">➤ Ear punch➤ Tail biopsy (rodents ≤21 days)
<input type="checkbox"/> Hearing Research Tests: NON-invasive	<ul style="list-style-type: none">➤ Auditory Brainstem Response➤ Otoacoustic Emissions Test
<input type="checkbox"/> Imaging	<ul style="list-style-type: none">➤ Short term procedures on conscious animals➤ Prolonged procedures with sedation or anesthesia

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<input type="checkbox"/> Injections (with no expectations of adverse effects)	<ul style="list-style-type: none"> ➤ Intramuscular (IM) ➤ Subcutaneous (SC) ➤ Intravenous (IV)
<input type="checkbox"/> Oral Gavage	Must not expect adverse effect.
<input type="checkbox"/> Restraint < 30 minutes	Animals must be acclimated to device, purpose must not be to induce stress and time < 30 mins.
<input type="checkbox"/> Special Diets/Special Water	Must not be associated with a painful or distressful procedure.

Category D Procedures:

<input type="checkbox"/> Behavioral Studies (pain/distress possible)	<ul style="list-style-type: none"> ➤ Treadmill Tests (escapable) ➤ Morris Water Maze ➤ Fear Conditioning with ESCAPABLE foot shock ➤ Chronic Variable Stress (constant bedding changes, cage manipulations, predator scents, absence or abundance of enrichment) ➤ Self-Administration and Extinction of Drugs in Addiction Models
<input type="checkbox"/> Blood/Fluid Collection (More painful procedures requiring anesthesia)	<ul style="list-style-type: none"> ➤ Retro-Orbital Blood Collection ➤ CSF Tap
<input type="checkbox"/> Food Restrictions > 24 Hours Duration	<ul style="list-style-type: none"> ➤ Food Scheduling >24 Hours ➤ Caloric Restriction (long term)
<input type="checkbox"/> Genotyping Requiring Analgesic and/or General Anesthesia	<ul style="list-style-type: none"> ➤ Tail Biopsy in Rodents ≥ 22 days of age
<input type="checkbox"/> Injections (<u>IF</u> general anesthesia and/or increased injection site monitoring is needed.)	<ul style="list-style-type: none"> ➤ Intranasal Inoculations (rodents) ➤ Footpad Injections (rodents) ➤ Retro-Orbital Injections (rodents) ➤ Injections of compounds that may be irritating (<u>excludes</u> Complete Freund's Adjuvant, CFA)
<input type="checkbox"/> Irradiation/Dosing (partial myeloablation; complete but transient myeloablation due to planned recovery)	Requires monitoring and supportive care. Requires euthanasia if humane endpoints reached.
<input type="checkbox"/> Laser Use	Requires post-procedure monitoring.
<input type="checkbox"/> Special Diets/Special Water (When associated with pain/distress)	<ul style="list-style-type: none"> ➤ DSS in water to induce colitis ➤ FNQ in water to cause oral tumors
<input type="checkbox"/> Special Housing and Husbandry	<ul style="list-style-type: none"> ➤ Constant light for nocturnal species ➤ Constant darkness for diurnal species ➤ Hypoxia, Hyperoxia, Hypercapnia ➤ Prolonged Heat Exposure ➤ Prolonged Cold Exposure (must offer nest materials)

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<input type="checkbox"/> Survival Surgeries	Must provide anesthesia, pre-operative and post-operative analgesia.
<input type="checkbox"/> Terminal Surgery	Must provide general anesthesia.
<input type="checkbox"/> Tumor Studies	Must define humane endpoints and allow alleviation of pain and distress.
<input type="checkbox"/> Water Scheduling	Must monitor daily and provide additional water or subcutaneous fluids if dehydration develops.

Category E Procedures:

<input type="checkbox"/> Ascites production models (excessive abdominal fluid)	IF rodent gains 120% baseline weight or requires abdominal taps to relieve ascites accumulation.
<input type="checkbox"/> Behavioral Studies that REQUIRE causing pain or distress	<ul style="list-style-type: none"> ➤ Forced Aggression <ul style="list-style-type: none"> ○ Social Defeat ○ Intruder Test ➤ Sleep Deprivation > 24 hours ➤ Depression Tests <ul style="list-style-type: none"> ○ Prolonged Forced Swim Test ○ Tail Suspension Test ○ Learned Helplessness ➤ <u>INESCAPABLE</u> Foot Shock ➤ Untreated withdrawal from induced addiction
<input type="checkbox"/> Forced Exercise	
<input type="checkbox"/> Infectious Disease Models (requiring development of clinical disease)	Monitoring is required. Euthanasia is required when humane endpoints are reached.
<input type="checkbox"/> Injections (Known to cause pain/distress at doses given)	<ul style="list-style-type: none"> ➤ CFA Injections ➤ LPS Injections (dose/route dependent) ➤ LD50 Studies
<input type="checkbox"/> Irradiation-complete (lethal) myeloablation without planned recovery	Category E when there is no rescue effort via bone marrow transplant, etc. Euthanasia is required when humane endpoints are reached.
<input type="checkbox"/> Pain Models	<ul style="list-style-type: none"> ➤ CFA/Formalin Injections to Foot Pad ➤ Monoiodoacetate Joint Injections ➤ Nerve Cuff/Constriction Injuries
<input type="checkbox"/> Restraint Stress	Purpose of procedure is to cause stress or length of restraint is greater than 30 minutes.

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<input type="checkbox"/> Survival Surgery (Analgesia Withheld)	<p>General anesthesia is always required, but surgery is Category E if post-operative analgesia is withheld with scientific justification. <u>This will apply for any major survival surgery (causing potential pain) even if animals are monitored to determine the need for analgesics.</u></p>
<input type="checkbox"/> Water Restriction	<p>Total daily volume of water is restricted. This differs from water scheduling when periods of water withholding are followed by at least 1 hour of free access to water per day.</p>

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