

USDA Pain and Distress Levels¹

USDA Category B	USDA Category C	USDA Category D	USDA Category E
No pain or distress	No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions; positive reward projects; routine procedures; injections; and blood sampling.	Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.	Pain or distress or potential pain or distress that is not relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.
<i>Examples</i>	<i>Examples</i>	<i>Examples</i>	<i>Examples</i>
<ul style="list-style-type: none"> • Animals being maintained without any research manipulation, prior to euthanasia or transfer to another protocol. • Observation of animal behavior in the wild without manipulating the animal or its environment. • Physical restraint and preventative medical procedures such as routine vaccination. • Routine husbandry procedures. • Breeding or Holding Colony Protocols 	<ul style="list-style-type: none"> • Holding, weighing or transporting animals (relatively short distances under non-stressful conditions). • Injections (nonirritating), blood collection or catheterization of superficial vessels. • Collection of body fluids or tissues post mortem. • Tattooing animals. • Ear punching of rodents. • Routine physical examinations. • Observation of animal behavior. • Studies, which do not result in clinical signs of pain and/or distress. • AVMA-approved humane euthanasia procedures. • Routine agricultural husbandry procedures. • Observational studies and live trapping (traps must provide adequate shelter/food and be checked frequently to ensure survival). 	<ul style="list-style-type: none"> • Potentially stressful transportation of animals that requires tranquilization. • Survival/terminal surgical procedures. • Retro-orbital blood collection under anesthesia. • Tail biopsy in mice > 21 days old. • Exposure of blood vessels for catheter implantation. • Exsanguination and/or perfusion under anesthesia. • Genetically engineered phenotype that causes pain or distress that will be alleviated. • Use of Freund's Complete Adjuvant. • Ocular and Skin Irritancy testing where pain and distress are relieved. • Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with: decreased appetite/activity level, adverse reactions to touch, open skin lesions, abscesses, lameness, conjunctivitis, corneal edema or photophobia, but are relieved with analgesics. • Food or water deprivation beyond that necessary for normal presurgical preparation. 	<ul style="list-style-type: none"> • Ocular or skin irritancy testing. • Burns or trauma. • Radiation sickness. • Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs. Experimental induction of disease including metabolic and nutritional diseases or disease resulting from exposure to toxicants. • Mutants with chronic pain or debilitation which is not relieved with analgesics or by appropriate intervention. • Food or water deprivation that exceeds ordinary pre-surgical preparation or is stressful to the animal. • Application of noxious stimuli (i.e. electrical shock) that cannot be avoided or escaped. • Restraint using paralyzing or immobilizing drugs without anesthesia or prolonged restraint for long periods of time. • Exposure to abnormal or extreme environmental conditions. • Psychotic-like behavior suggesting

¹ Please refer to these guidelines when completing the "Humane Use Animal Categories" of protocol in eSIRIUS. As per the IACUC, the veterinary staff may update the information in the animal use protocol accordingly to be sure that the correct USDA category is selected.

		<ul style="list-style-type: none"> • Noxious electrical shock that is not immediately escapable. • Paralysis or immobility in a conscious animal. 	<p>a painful or distressful status whether or not resulting from a procedure.</p> <ul style="list-style-type: none"> • Behavior or testing resulting in injury to cage mates or self. • Studies in which animals are allowed to die without intervention. • Studies that allow endpoints that are painful or stressful (i.e., addictive drug withdrawals without treatment, pain research). • Any procedures for which needed analgesics, tranquilizers, sedatives or anesthetics must be withheld for justifiable study purposes. • Euthanasia by procedures not approved by the AVMA.
--	--	---	--

Guidelines for determining USDA classification in protocols involving tissue collection before/after euthanasia and/or animal perfusion:

If an animal will be euthanatized by an approved physical or chemical method of euthanasia solely for the collection of tissues (after the animal's death), the procedure should be classified as USDA C.

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy), and the animal will then be allowed to recover, the procedure should be classified as USDA D (survival surgery).

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy, etc.); and the animal will then be euthanatized, the procedure should be classified as USDA D (non-survival surgery). In this scenario, it is necessary to justify why the animal couldn't be euthanatized (USDA category C) rather than anesthetized.

If an animal will be anesthetized so that vital tissues can be collected (heart, both kidneys or lungs, whole liver, etc.), the animal will obviously succumb to the procedure. To determine whether this will be euthanasia or non-survival surgery, we must consider the definition of euthanasia. A critical component of this definition is "rapid unconsciousness followed by loss of cardiac, respiratory and brain function". Based on this definition, procedures which require tissue manipulation or other prolonged techniques prior to the animal's death (more than a few minutes) should be classified as non-survival surgery (USDA D). Similarly, if an animal will be anesthetized so that the tissue can be collected in the "freshest" possible state (i.e. heart) and the tissues will be rapidly excised, the procedure should be classified as euthanasia (USDA C). (Note: In this scenario, it is difficult to justify why the animal couldn't be euthanatized rather than anesthetized.)

If an animal will be anesthetized so that it can be chemically perfused, the same "test of time" applies (i.e.: long, technical manipulations should be classified as USDA D; while rapid intravascular injection of the perfusate without other manipulations should be classified as USDA C).

NOTE: Because the USDA classification system is based on the "potential for pain, distress or discomfort," the anesthetic/euthanasia drug dose becomes a critical concern. For example, if a known "euthanasia dose" of pentobarbital will be administered, drug irreversibility is assumed. Thus, once the animal is confirmed to be in an anesthetic plane (toe pinch response, etc.), tissues can be collected/ procedures can be performed without the concern about what the animal will be perceiving. This procedure would then be classified as USDA C. The Committee recommends using a euthanizing dose whenever possible. Other methods may be appropriate with proper scientific justification.