Pain and distress categorization is subject to IACUC approval. As such, the examples below are provided as general guidance to potential categorization. Do not include non-study related veterinary or clinical care when determining the USDA pain and distress category.

**Category B**
Animals being bred, conditioned or held for future use in research, testing, or teaching but not yet used for such purposes.

**Examples:**
- Animals being held, bred or housed without research or teaching activities performed prior to transfer (pain category, protocol), or euthanasia
- Activities are limited to those needed for routine herd or colony maintenance

**Category C**
Animals upon which teaching, research, experiments, or tests are conducted involving no pain, distress, or use of pain-relieving drugs (procedures do not involve more than momentary pain or distress).

**Examples:**
- Routine procedures (e.g. weighing, tattooing, subcutaneous, intramuscular or intraperitoneal injection, peripheral intravenous injections, peripheral blood collection, oral gavage, nonsurgical catheter implantation, or ear punch in mice and rats)
- Holding or brief restraint of awake animals
- Prolonged restraint with acclimation
- Manipulative procedures (e.g. palpations, skin scraping, radiography, or ultrasonography)
- Behavioral studies (if the animal is manipulated or handled before, during, or after the observation without pain or distress)
- Change in environmental parameters (e.g., special diet or water, light cycle, room temperature if within thermoneutral zone) that do not result in morbidity
- Food restriction that results in weight loss <20% of normal age-matched controls
- Tail biopsy (tail snip) for genotyping in mice and rats < 21 days old and should not exceed 0.5 cm of tail
- New phenotype with no expected morbidity or mortality
- Live trapping/electrofishing
- Progressive or infectious disease studies where clinical symptoms will not be experienced post inoculation or infection because the endpoint of the study will occur prior to the animal experiencing any clinical symptoms
- Tumor induction studies with appropriate endpoints
- Positive reward training
- Irradiation (sublethal or with reconstitution) or radioisotope use
- Superovulation
- Nonsurgical embryo collection
- AVMA approved Euthanasia (methods listed as acceptable or acceptable with conditions when conditions are met)
- Procedures performed under anesthesia, sedation or tranquilizing drugs where the drug is provided for animal immobilization and not to relieve a painful procedure

**Category D**
Animals upon which experiments, teaching, research, surgery, or tests are conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used. Other qualifying interventions will be administered. [Note that other methods may be used to relieve more than slight or momentary pain or distress. Examples include other types of pharmacologic agents, nursing care, or other palliative methods. However, the methods used must be substantively effective (able to reduce pain and distress to that which is slight or momentary) in order to change categorization from a Column E to a Column D.]
Examples:

**Procedures performed under anesthesia, etc. where the drug is provided to relieve a painful procedure; including:**
- Surgery (survival OR non-survival)
- Laparoscopy or needle biopsies
- Retro-orbital blood collection
- Surgical placement of catheters for blood collection or agent administration
- Perfusion with a non-fixative agent or surgical perfusion
- Tail biopsy (tail snip) for genotyping in mice and rats >21 days
- Paralysis or chemical immobilization with appropriate anesthesia
- Exsanguination under anesthesia
- Induction of infections or antibody production with appropriate anesthesia and post-op/post-procedural analgesia

**Procedures with analgesia or other forms of pain/distress relief, including euthanasia when humane removal criteria are met:**
- Burns
- Induction of illness
- Lavage
- Physical trauma
- Polyclonal antibody production
- Toxic substance use
- UV light exposure to induce sunburn
- Unexpected pain/distress followed by euthanasia in timely manner

**Category E**

Animals upon which teaching, experiments, research, surgery, or tests are conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. [Note: An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be provided to the USDA on the Annual Report for USDA regulated species.]

**Examples:**
- Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes
- Death as an Endpoint or lethal dose studies
- Antibody production with ascites
- Exposure to extreme environmental conditions, including hypoxia or routine housing outside of thermoneutral zone without acclimation
- Food restriction that results in weight loss of >20% of normal age-matched controls
- Footpad injections (antibody production or microorganism)
- Genetically engineered animals with phenotype that causes pain or distress that will not or cannot be alleviated
- Induction of arthritis
- Forced or involuntary exercise, e.g.: treadmill w/ shock plate or unpleasant stimulus
- Progressive or infectious disease studies where clinical symptoms will be experienced post inoculation or infection, but no clinical treatment is given for pain relief, or to prevent or alleviate symptoms
- Injury models without pain and distress relief, such as traumatic brain injury or stroke
- Noxious stimuli from which there is no escape, including electric shock
- Ocular or skin irritancy tests
- Pain, burn, sepsis study where the animal experiences unrelieved pain
- Paralysis or chemical immobilization in awake animals
- Behavior studies where pain/distress is unrelieved before, during, or after the study
- Total body irradiation (lethal or without reconstitution)
- Tumor production or implantation resulting in metastasis
• Prolonged restraint of an awake animal for >30 minutes in a natural body position or 15 minutes or longer in an unnatural body position without acclimation
• Euthanasia by procedures not approved under AVMA Guidelines (unacceptable methods, other methods not described)