When completing the protocol form, assign each animal species listed to one of the following categories: B, C, D or E. List each animal under the highest pain/distress category that will apply to the animal at any time while on the protocol. You are not required to include non-research related veterinary care in determining the category of use.

Please note that the severity or duration of the pain or distress, or the use of anesthesia or analgesia to alleviate pain or distress may result in animals being placed in different categories.

The examples provided below are representative of the types of procedures or conditions that correlate with a particular category. The example list is not intended to be all inclusive, rather a guidance tool to assist principal investigators and the IACUC.

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

- Animals being bred or housed without any research manipulations (should not include animals undergoing genotyping procedures)

**Category C:** Animal use activities that involve no more than momentary or slight pain or distress (no greater than an injection) where there is no need for use of pain-relieving drugs.

- Holding, weighing, or physical examinations of animals in teaching or research activities
- Injections, blood collection, or catheter implantation via superficial vessels
- Observation or positive reinforcement training of animals in a laboratory setting
- Pre-weaning (<21 days of age) methods of identification or genotyping (ear notching, wing banding, tail clipping, tattooing -unless general anesthesia needed)
- Feeding studies that do not result in clinical health problems
- Routine agricultural husbandry procedures approved by the IACUC
- Humane euthanasia that meets current AVMA standards
- Live trapping with minimal potential for injury
- Short-term physical restraint or chemical immobilization, such as for transport or IVIS imaging
• Studies involving clinical signs not judged to involve more than slight pain or distress

**Category D:** Animal use activities that involve accompanying pain or distress to the animals and for which appropriate anesthetics, analgesics, tranquilizing drugs, and/or humane endpoints are used to avoid pain, distress, or discomfort.

1. Survival surgical procedures where perioperative pain or distress is alleviated, such as: catheter cut-down, laparoscopy, and biopsies.
2. Application of noxious chemicals or stimuli (e.g. electrical shock) when the animal can avoid/escape the stimuli, and/or it is severe enough to cause pain or distress
3. Non-survival surgical procedures
4. Retro-orbital blood collection in mice and rats
5. Exsanguinations under anesthesia
6. Tail clipping in rodents > 21 days old, or tattooing that requires general anesthesia
7. Induction of disease, infection, or a genotype that causes pain or distress which is alleviated as soon as signs develop with the use of pain-relieving drugs or humane euthanasia

**Category E:** Animal use activities that involve accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, tranquilizing drugs; or other methods for relieving pain or distress are NOT used. Category E research, testing, or procedures will require strong scientific justification as to why pain-relieving drugs or other methods for relieving pain cannot be used on animals. This includes citation(s) to published studies if applicable, describing what alternatives were considered and how alternatives will be used whenever possible, clarifying whether animals will be euthanized when moribund, and if not, what information is to be gained in the interval between moribundity and death.

1. Research, testing, or procedures that require death as an endpoint or continuation without pain-relieving intervention, even after clinical signs of pain or distress are evident
2. Application of noxious chemicals or stimuli (e.g. electrical shock) when the animal cannot avoid/escape the stimuli, and/or it is severe enough to cause pain or distress
3. Prolonged physical restraint as longer than 30 minutes.
4. Exposure to extreme environmental conditions
5. Food or water deprivation beyond that necessary for routine pre-surgical preparation or is deemed stressful to the animal
6. Euthanasia by non-AVMA approved methods

7. Any procedures for which needed analgesics, anesthetics, or tranquilizers must be withheld for justifiable purposes

References

1. USDA APHIS Policy #11