

**UNC ANIMAL USE PROTOCOL**  
**Attachment A**  
**PAINFUL & DISTRESS, ALTERNATIVES & HUMANE ENDPOINTS**

Federal regulations require that principal investigators consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals and to provide a written narrative of the methods used and sources consulted to determine availability of alternatives.

[USDA Policy #12](#) requires that searches for alternatives consider the three concepts presented in The Principles of Humane Experimental Technique by Russell and Burch. Use the following links for additional information on the “Three Rs”:

- **Replacement:** using an inanimate system as an alternative (e.g., a computer model or program, a mannequin); the replacement of sentient animals (usually vertebrates) with less sentient animals (usually invertebrates such as worms, bacteria, etc); or the use of cell and tissue cultures.
- **Reduction:** decreasing the number of animals with no loss of useful information. This may be achieved by reducing the number of variables through good experimental design, by using genetically homogeneous animals or by ensuring that the conditions of the experiment are rigorously controlled.
- **Refinement:** changing some aspect of the procedure resulting in a reduction or replacement of animals or in a reduction of any pain, stress or distress that animals may experience. An example is the establishment of early endpoints for intervention in a study that has the potential to cause pain or distress.

**ASSISTANCE: Searching for Alternatives**

**A.1.** From the table in Section III.C of the protocol, list each experiment/procedure in which animals are categorized in pain and/or distress category E, and provide a scientific justification for performing each of those procedures. Without a strong scientific justification, the IACUC will not approve a protocol in which animals will suffer more than momentary or slight pain or distress that is not alleviated by the use of anesthetics, analgesics, sedatives and/or tranquilizers.

**A.2.** Use the table below to document your search of the literature to determine that appropriate alternatives such as less sentient animal models, computer models, tissue cultures, and/or refinement of techniques are not available or appropriate for the project. A search of a minimum of two distinct databases is required.

Databases Searched	Key Words & Search Strategy <sup>1</sup>	Date Search Conducted	Years Covered by Search <sup>2</sup>	Number of Relevant References	Were Alternatives Found?
Identify below any other relevant sources you used to make your determination (scientific meetings/conferences, additional journals, consultation with experts in the procedure and species, etc.)					

<sup>1</sup> Your key words and search strategy should include your research area; the specific painful procedure for which you are seeking an alternative; the animal you are using, and the words: alternative\*, vitro, computer model\*, and animal model\*. Indicate how the keywords were combined in the search (AND, OR)

**Example:** *Note that this literature search is NOT a search for duplication of experiments.*

(liver disease) AND (laparotomy for perfusion) AND (alternative or alternatives or vitro or model or models or computer or animal model)

<sup>2</sup> Include the specific years that were searched. It is not acceptable to reference “all available”, or “1972 to present”.

**A.3.** If alternatives were found, justify why they are not being used. ☐ N/A

**A.4.a.** It is essential to establish a plan for treating animals, removing them from a study, or euthanizing them in order to minimize or alleviate unnecessary pain and/or distress. The plan should include detailed written criteria to determine at what point (humane endpoint) animals should be removed from a study or euthanized to avoid further suffering; and should clearly identify the individual(s) who can make treatment decisions including procedures to follow on weekends and holidays. ASSISTANCE for establishing humane endpoints

Clinical Signs to be Observed	When / How Often / By Whom	End Point Criteria	Intervention
1. Example: Weight Loss	5 pm weekdays by PI - weekends by (name)	20% body weight loss	Remove from study and immediately begin feeding high calorie diet.
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			

b. For each end point listed, describe in detail the intervention that will be used to alleviate or end the animals’ pain and/or distress.

1.

If there will be no intervention, provide a scientific justification for not doing so. All moribund animals must be euthanized unless there is strong scientific justification for allowing death as an endpoint.

The justification must not only explain why the study results would be compromised by intervening, but must also convince IACUC members that the importance of the study is such to justify the unrelieved suffering of the animals. The use of death as an endpoint

**A.5. ADDITIONAL NARRATIVE EXPLANATION:** Use this section to provide additional information not included above. Describe details of treatments that will be used. You should indicate if analgesic agents will be used, but do not need list specific agents and dosages here; that information should be provided in Attachment F. Include descriptions of techniques that you have refined or have adopted from other labs that are an improvement over previously employed methods. Include a description of any additional steps, monitoring procedures, methods and treatments you will employ that address the issue of improving the animal's welfare. The IACUC should have enough information to determine that you have considered a "worst-case scenario" and have adequately addressed it.

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**A.6. Attending Veterinarian Consultation**

☐ I have consulted with the AV regarding criteria and procedures for relief of animal pain and distress.