

Attachment 4**Column E Explanation Form for Regulated Species**

This form is intended as an aid to completing the Column E explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

Note: Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation.

1. **Registration Number:** 51-R-0095
2. **Number of animals used under Column E conditions in this study.** 24
3. **Species (provide common name) of animals used in this study.** Hamster
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

To test the protective efficacy of SARS-CoV-2 vaccines, animals must be exposed to (challenged) with SARS-CoV-2, and new vaccines may not provide complete protection. As a result of this exposure, all animals may experience some degree of pain/distress.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

Use of analgesics is not recommended in this vaccine testing regime due to the potential for altering the immune response

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1. **Registration Number:** 51-R-0095
2. **Number of animals used under Column E conditions in this study.** __12__
3. **Species (provide common name) of animals used in this study.** Domestic ferret
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

To test the protective efficacy of SARS-CoV-2 vaccines, animals must be exposed to (challenged) with SARS-CoV-2, and new vaccines may not provide complete protection. As a result of this exposure, all animals may experience some degree of pain/distress.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

Use of analgesics is not recommended in this vaccine testing regime due to the potential for altering the immune response.

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1. **Registration Number:** 51-R-0095
2. **Number of animals used under Column E conditions in this study.** __6__
3. **Species (provide common name) of animals used in this study.** Deer Mouse
(Peromyscus maniculatus)
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

This study examines the effects of social environment on male reproductive phenotypes in two species, using the "resident-intruder paradigm," a commonly used assay in behavioral studies. This paradigm is used to manipulate social status and competition in male mice, and likely induces chronic stress. It is under these conditions that the physiology of endocrine function and reproduction are studied. While this may not typically be considered, pain, it is considered by the UMD IACUC to be "distress."

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

The only means by which the chronic stress of the resident-intruder paradigm can be alleviated is through removal of the animal from the test conditions. Consequently, no effective means of relieving this distress exist.

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Column E Explanation Form for Regulated Species

This form is intended as an aid to completing the Column E explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. **Registration Number:** 51-R-0095
 2. **Number of animals used under Column E conditions in this study.** 6
 3. **Species (provide common name) of animals used in this study.** Oldfield Mouse
(*Peromyscus polionotus*)

1. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

This study examines the effects of social environment on male reproductive phenotypes in two species, using the "resident-intruder paradigm," a commonly used assay in behavioral studies. This paradigm is used to manipulate social status and competition in male mice, and likely induces chronic stress. It is under these conditions that the physiology of endocrine function and reproduction are studied. While this may not typically be considered, pain, it is considered by the UMD IACUC to be "distress."

2. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

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1. **Registration Number:** 51-R-0095
2. **Number of animals used under Column E conditions in this study.** 70
3. **Species (provide common name) of animals used in this study.** Guinea pig
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

Mice fail to develop tick immunity even after repeated exposure to tick bite, therefore the study uses the guinea pig model of tick feeding and *Borrelia burgdorferi* transmission that is widely used to understand immunity against tick bite and pathogen transmission.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

Although in nature infection with tick-borne pathogens may cause a mild pain and distress in infected rodents, we cannot use analgesic drugs. This is because, either opioids, steroid, or non-steroidal anti-inflammatory drugs (NSAIDs) target a variety of host peripheral and central mechanisms blocking many host enzymes, which may interfere with our planned study to assess murine immune response against infection with tick-borne pathogens. In addition, previously published studies also have not included the use of analgesia, and in order to remain consistent with those methods and studies, we cannot administer analgesics.