

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

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. **Registration Number:** 51-F-0016
2. **Number of animals used under Column E conditions in this study.** 42
3. **Species (common name) of animals used in this study.** Cavia porcellus

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.