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.

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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.

Species	Exception	Justification	Duration	Number of Animals
Deer Mice	Animal Health and Husbandry Standards 3.129, 3.131 Food/water restriction.	Appetitive motivation for behavioral study	24 hours	28
Ferrets	Animal Health and Husbandry Standards 3.129, 3.131 Food/water restriction.	Appetitive motivation for behavioral study	3-4 days/week, 24hrs/day, or 28 hours once a week	40
Gerbils	Animal Health and Husbandry Standards 3.129, 3.131 Food/water restriction.	Appetitive motivation for behavioral study	Continuously, up to 3 months	0