

**Column E Explanation**

1. **Registration Number:** 74-R-0039
2. **Number of Animals categorized as column E used in this study:** 10
3. **Species (common name) of Animals Used in this Study:** Hamster
4. **Explain the procedure producing pain and/or distress:** Animals were infected with *Clostridium difficile* spores through oral dosing, which caused diarrhea and weight loss leading to death.
5. **Provide scientific justification why pain and/or distress could not be relieved:** Prophylactic administration of analgesics or anesthetics have the potential to mask the signs of disease and alter the course of infection in a manner that would severely affect the statistical interpretation of test results. To limit pain and distress, animals were monitored three times per day by trained research staff and their clinical appearance was scored to assess disease progression and distress. Body weights were monitored at least daily and compared to animal weight pre-infection. Animals displaying clinical signs indicating morbidity were euthanized. Animals reported in the E category progressed to death too rapidly for clinical signs to be recognized and alleviated by euthanasia.