Column E Explanation

Registration Number: 74-R-0081 **Fiscal Year 2019**

1. Study One:

Number of animals used in this study: 115

Species used in this study: Hamster

Explanation of procedure producing pain and/or distress:

Animals associated with this model may experience pain or distress as a result of infection and/or treatment with a compound. Historically, animals often do not manifest any signs of pain or distress prior to death, and 75% of the animals in those studies have either remained healthy and normal or have been euthanized to prevent prolonged suffering. Signs that indicate a severe disease state as a result of Clostridium difficile infection include lethargy, acute/chronic diarrhea, hypothermia, >15% decrease in body weight, reduced water/food intake, and swollen peritoneum. In addition to the possible effects related to the infection, treatment with test articles (compounds) could also induce negative effects in an animal as well. Signs of test article related effects include acute paralysis, increased respiratory/heart rates, and a rough coat. To prevent treatment related effects, all compounds evaluated in this model are pre-screened for potential toxicity by the sponsors and selected dosing regimens will be based on what has been safely used for evaluating other test articles in this model.

All animals associated with this protocol will be monitored for severe disease every 6 to 8 hours throughout the duration of a study (up to 50 days). An clinical observation sheet for each day of a study will be maintained that will include recording observations and animal disposition, including the date (day and time) and personnel initials. Using the guidelines associated with the IACUC 'Pain and Discomfort' (document 013) and 'Humane Endpoints' (document 008) protocols, any animal that appears to be moribund will be immediately removed from the study and euthanized.

Explanation with reason(s) for why anesthetics, analgesics and tranquilizers could not be used:

Most of the test articles that will be evaluated in this model are new investigational compounds and possible drug interactions have not been fully determined. If another drug is used to relieve pain and/or distress, this may interfere with the possible therapeutic/prophylactic efficacy a test article may have in this model. For example, buprenorphine (analgesic) is metabolized by the cytochrome 450-3A4 (CYP450-3A4) pathway. This same metabolic pathway interacts with 3 different antibiotic classes that include macrolides (clarithromycin), rifamycins (rifampin), and fluoroquinolones (ciprofloxacin). Interactions of these drugs in this pathway have been shown to alter the pharmacokinetics of each drug, which can directly impact their efficacy within the host (animal or human). Additionally, several nonsteroidal anti-inflammatory drugs (NSAIDS) are metabolized by the cytochrome 450-2C9 (CYP450-2C9) pathway, which also metabolically interacts with the antibiotics metronidazole and rifampin. As is the case with buprenorphine, the interaction of NSAIDS and other drugs in the CYP450-2C9 pathway can impact the efficacy of each drug by altering their metabolism and pharmacokinetics in the host. In addition to metabolic interference, NSAID use has been shown to clinically aggravate colitis caused by intestinal infections. Given these facts about documented drug interactions within critical metabolic pathways and how these interactions can interfere with drug efficacy or disease outcome in the host (Burton ME et al., Granowitz EV et al., Hogenauer C et al.), it is imperative that non-study associated

drugs used to relieve pain and/or distress not be administered in animals associated with this model so that the therapeutic and/or prophylactic efficacy of a new investigational compound can be accurately evaluated. If a new compound is efficacious in this animal model, then these results would be critical to its development for treating life-threatening Clostridium difficile-associated diarrhea (CDAD) in humans.

References:

Burton ME, Applied Pharmacokinetics & Pharmacodynamics: Priniciples of Therapeutic Drug Monitoring. Lippincott Williams & Wilkins, 2006, ISBN 9780781744317.

Granowitz E.V., and R.B. Brown. Antibiotic Adverse Reactions and Drug Interactions. Crit Care Clin, 2008 Apr;24: 421-442.