Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. 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:
   74-R-0039

2. Number of Animals Used in this Study:
   83

3. Species (common name) of Animals Used in this Study:
   Gold Syrian Hamsters

4. Explain the procedure producing pain and/or distress.

   The protocol is designed to evaluate the effectiveness of antibiotic treatments for *Clostridium difficile* infections (CDI). Each year in the United States about half a million people contract CDI, usually in hospitals or in long-term care facilities. Infections can be caused by antibiotic treatment, so new antibiotics need to be developed so that patients can be effectively treated.

   In these studies, we want to determine an effective compound against CDI. *C. difficile* is highly infectious to hamsters, and they are the gold standard model for the infection and treatments against CDI.

   Control Animal Group: In these experiments, the Control group is defined as subjects receiving the infectious dose of *C. difficile* and is not given the test drugs or analgesics. The control group is critical to the interpretation of the test data, i.e. the statistical evaluation of the test data relies solely on what happens in the control group. To ensure the validity of the data, both the test groups and control groups are similarly treated, except for the use of test drug in the experimental group. Therefore, since analgesics are not given to the test group, analgesics are also not given to the control group; this ensures that both the test group and control group are evaluated under the same conditions with the only variable being the test antibiotic used in the experimental group.

   In this model, pain and/or distress results from the establishment of a gastrointestinal infection in the hamster, after administration of an infectious dose of *C. difficile* to the animal.

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.

   Justification for not administering analgesics: Administration of analgesics may alter the course of infection leading to an increase in survival of hamsters in the control group, or conversely cause a decrease in survival; which would severely affect the statistical interpretation of test results, since the quality of control group data is critical to evaluating the results of the test group as mentioned above (*1-5*). The effect of analgesics may occur through a delay of gastric clearance, which could retain and increase the toxin load in gut, thereby increasing disease severity and likely producing a faster time to death (*6-10*). According to White et al. (*6*) pain management is challenging in patients with toxic megacolon because non-steroidal anti-inflammatory drugs may exacerbate bleeding; further, opioids may

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also adversely affect bowel peristalsis causing an increased risk of colonic perforation. Therefore, the use of analgesics to relieve pain may adversely affect these experiments. Although, we have not found there to be use of analgesics in the hamster model of CDI, from the literature (Jan 11th 2012; Ref. 1-5 as examples), the main reasons for not using analgesics in our experiments are described above. Should we use analgesics in the control group, it would also be scientifically required that they also be used in the test group to mitigate differences between the two groups. There is a potential for analgesics to affect the activity or pharmacokinetic (PK) properties of test drugs administered, which will affect the outcome of the experiment and generate false data.

References:

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3. Anton, P.M., *et al.* Rifalazil treats and prevents relapse of clostridium difficile-associated diarrhea in hamsters. *Antimicrob Agents Chemother* **48**, 3975-3979 (2004).
4. McVay, C.S. & Rolfe, R.D. In vitro and in vivo activities of nitazoxanide against Clostridium difficile. *Antimicrob Agents Chemother* **44**, 2254-2258 (2000).
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6. White M et al. Pain management in fulminating ulcerative colitis. Paediatr Anaesth. 2006 Nov;16(11):1148-52.
7. Herbert MK et al. Peristalsis in the Guinea pig small intestine in vitro is impaired by acetaminophen but not aspirin and dipyrone. Anesth Analg. 2005 Jan;100(1):120-7.
8. Fekety R. Guidelines for the diagnosis and management of *Clostridium difficile* associated diarrhea and colitis. American College of Gastroenetrology, Practice Parameters Committee. *Am J Gastroenterol.* 1997;92:739-50.
9. Walley T, Milson D. Loperamide-related toxic megacolon in *Clostridium difficile* colitis. *Postgrad Med J.* 1990;66:582.
10. Vinagre, AM. Effect of 4-aminoantipyrine on gastric compliance and liquid emptying in rats. Braz J Med Biol Res. 2007 Jul;40(7):903-9.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

N/A

Agency_____ CFR_____