USDA, APHIS Form 7023 (FY21)
Centers for Disease Control and Prevention Registration Number: 57-F-0004

Attachment A: Category E Explanations

Protocol: B
Species (common name): Hamster
Number: 30

Explanation of procedure producing pain and/or distress:
Currently there are no approved antiviral therapies available to treat (b) (3) (A) disease in humans. The aim of these studies is to investigate the use of defective interfering particles (DIs; non-spreading, non-replicating particles) as therapeutics for (b) (3) (A) disease. Hamsters are an established disease model of (b) (3) (A) and show similar pathogenesis and disease as humans. (b) (3) (A) infection in hamsters can produce a respiratory disease phenotype that can rapidly progress from absent or mild clinical signs to severe disease and death with very little time for intervention in certain animals.

Justification why pain and/or distress could not be relieved:
Analgesics have been proven to interfere with immunologic responses and can exacerbate liver damage. It is also known that inflammation and inflammatory mediators may play a major role in the pathogenesis of (b) (3) (A) nfection. Based on these factors, analgesics could not be used since they could affect the clinical outcome of the disease and interfere with assessment of therapeutics, and thus alter the theoretical basis of the experiment. All efforts were made to ensure that the animals experienced the least amount of pain and distress necessary to accomplish the goals of the experiment. All animals were monitored daily by an experienced animal care technician or the PI for signs of clinical illness; a pain/euthanasia scale that takes into account the total health parameters of each individual animal was utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Animals scored at 8-9 points were monitored two times per day. Animals scored at 10 total points or above were humanely euthanized, and euthanasia was performed prior to endpoint criteria being reached whenever possible. In this case, despite all attempts made to prevent it, a subset of animals was not able to be euthanized prior to succumbing to infection.

Protocol: C
Species (common name): Hamster
Number: 4

Explanation of procedure producing pain and/or distress:
Development and evaluation of new cost-effective rabies biologics is critical in continuing to prevent and reduce disease. Griffithsin, a protein with antiviral properties, has been widely studied lately, and tested against several other RNA viruses that, like Rabies virus, use the glycoprotein to infect the host cells. Hamsters are commonly used as model systems to study the effects of antiviral compounds on the progression of rabies virus infection. Those antiviral compounds that inhibit rabies virus *in vitro* need to be tested in an animal model to assess their efficacy *in vivo*. Syrian hamsters are well established in the laboratory and in the literature for rabies pathogenesis studies and the evaluation of new biologics. The development of clinical rabies infection is expected to occur in a subset of the infected animals. There are occasionally animals that progress to a terminal state before they can be humanely euthanized.

Justification why pain and/or distress could not be relieved:

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Attachment A: Category E Explanations

Protocol: A
Species (common name): Guinea Pig
Number: 2

Explanation of procedure producing pain and/or distress:
Guinea pigs are known to be susceptible or immunogenic to many human pathogens; for that reason, this species has been used for identification, isolation, and even differentiation of *rickettsiae*. CDC studies have demonstrated that Guinea pigs have been found susceptible to *Rickettsia rickettsii*, *R. parkeri*, *R. conorii*, and *R. slovaca* with noticeable variations between pathogens in severity and dynamics of clinical signs as well as in necropsy results. Infestation of guinea pigs with infected ticks of these species reproducibly resulted in typical clinical signs of infection. The more virulent isolates of *R. rickettsii* can cause rapidly progressive illness in some of the guinea pigs. There may occasionally be animals that progress to a terminal state before they can be humanely euthanized.

Justification why pain and/or distress could not be relieved:
Lidocaine or prilocaine topical analgesic cream was used to alleviate discomfort associated with ear biopsies. Analgesia could not be used for alleviation of pain or distress due to rickettsial infection because use of analgesia could interfere with immune responses to rickettsial infection and mask clinical signs necessary to study pathogenesis. In addition, some animals progressed rapidly from an apparently healthy status to a terminal state overnight or between routine check periods. All infected animals were monitored at least twice per day and any animal whose condition was likely to reach the humane endpoint criteria prior to the next health check was euthanized. All investigators and staff were trained to evaluate and assess animals according to the humane endpoints scale, and any animal showing >25% weight loss or a total score of 10 on the scale was humanely euthanized. However, a small percentage of infected animals progressed to a terminal state very abruptly, within 2 to 4 hours. Any animals that died prior to reaching humane euthanasia endpoint criteria were reported in category E.