Attachment A: Category E Explanations

Explanations listed in this attachment refer to protocol activities that have been approved through the Full Committee Reviews (FCRs) by CDC Atlanta IACUC.

Protocol: A
Species (common name): Ferret
Number: 1

Explanation of procedure producing pain and/or distress:
Ferrets are naturally susceptible to influenza viruses, including many non-seasonal subtypes of animal-origin. Because influenza virus infection in the ferret model is known to mirror that of a human infection they are used to produce high titers of strain-specific antibodies. Although not all strains of highly pathogenic avian influenza A (H5N1) virus will cause illness in ferrets, some of the influenza viruses studied under this protocol are highly virulent for ferrets and may cause severe morbidity and potentially mortality.

Justification why pain and/or distress could not be relieved:
Analgesics have been proven to interfere with immunologic responses including antibody production. Consequently, 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. Therefore, humane end points were used to increase monitoring when clinical signs were present and euthanasia was conducted when the euthanasia criteria were met to minimize the pain or distress that the animals experienced. However, due to the acute onset of severe disease, one animal succumbed to infection prior to euthanasia. The most common clinical signs observed included nasal discharge and weight loss.

Protocol: B
Species (common name): Fruit Bat
Number: 1

Explanation of procedure producing pain and/or distress:
R. aegyptiacus are the only known natural reservoir for Marburg virus. The objective of this study is to investigate the effect of chemical immunosuppression with Dexamethasone (Dex) in Marburg virus-infected *Rousettus aegyptiacus* on virus persistence, clearance and shedding. In non-immune compromised bats, Marburg virus causes little to no disease. However, due to the immune suppressive treatment of these animals, bats infected by virus could potentially develop symptomatic, severe illness.

Justification why pain and/or distress could not be relieved:
Because the suppression of the bat immune system has never been done before, it is difficult to know the subsequent health outcome. Since the objective of this project is to study immune responses and pathogenesis of the virus, analgesia could not be used, as it might alter the immune response to viral infection and could mask clinical signs necessary to study the

pathogenesis of the disease. Consequently, 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. Therefore, humane end points were used to increase monitoring when clinical signs were present, and euthanasia was conducted when the euthanasia criteria were met to minimize the pain or distress that the animals experienced. However, due to the acute onset of severe disease, one animal succumbed to infection prior to euthanasia. The most common clinical signs observed included lethargy and weight loss.

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

Explanation of procedure producing pain and/or distress:
Currently there are no approved antiviral therapies available to treat Nipah virus 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 Nipah virus disease. Hamsters are an established disease model of Nipah viruses and show similar pathogenesis and disease as humans. Nipah virus causes severe encephalitis and respiratory disease in humans and similar symptoms may present in hamsters.

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 Nipah virus infection diseases. Based on these factors, analgesics were not 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. Consequently, 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. Therefore, humane end points were used to increase monitoring when clinical signs were present, and euthanasia was conducted when the euthanasia criteria were met to minimize the pain or distress that the animals experienced. However, due to the acute onset of severe disease, thirty animals succumbed to infection prior to euthanasia. The most common clinical signs observed included neurological signs and weight loss.