Column E Explanation

02 JAN 2018

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: 10-F-0002

2. Number of animals used in this study: 170

3. Species (common name) of animals used in the study: **Guinea Pig**

4. Explain the procedure (the cause of the pain) producing pain and/or distress:

The pain is a result of an infection in the eye or colon after inoculation with Shigella species. The infection causes a mild to severe keratoconjunctivitis (eye) or colitis.

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 would interfere with test results. (For Federally mandated testing, see item 6 below):

The development of an efficacious vaccine against shigellae requires an accurate evaluation of the immune response raised by vaccine candidates. The use of analgesics results in immunosuppression (Pruett, 1992), which would confound or even invalidate immunological analyses as well as any assessment of vaccine-induced protection. Drug-induced immunosuppression would also increase the severity of the experimental eye infection, since immunized animals are frequently protected from experimental disease. In particular, the use of anti-inflammatory analgesics such as aspirin or ibuprofen would invalidate the disease model, since a key aspect of the experimental disease is the inflammation caused by shigella invasion into the corneal epithelium.

Buprenorphine and butorphenol have been previously tested by others for their effects on the Sereny disease model for shigellae (Swearengen et al., 1993; Hanson et al., 2001). These studies showed that the use of analgesics increases the purulence and crustiness of Shigella-infected eyes, most likely due to the attendant lethargy and abnormal grooming habits. Since purulent discharge is a key component of the disease scoring (Hartman et al. 1991), excessive purulence due to analgesics would confound the interpretation of the disease. Specifically, a drug-related increase in purulent discharge would lead to artificially high disease scores and a misinterpretation of the virulence of a wild-type Shigella strain (false positive control), a false negative for vaccine safety, and ultimately a false negative for vaccine