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: 10-F-0002

2. Number of animals used in this study: 186

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 after inoculation with Shigella species. The infection causes a mild to severe keratoconjuctivitis (eye).

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 study of the immune response to and protective efficacy of vaccine candidates directed against shigellae, which are the primary goal of the MRMC, requires an accurate evaluation of the immune response raised by the administration of these vaccines. The use of analgesics, particularly opiates or narcotics, result in the immunosupression (see Einstein et el, 1993, Pruett, 1992, and Einstein 1998), which would invalidate the results of experiments testing immune responses as well as increasing the severity of the possible eye infection, since immunized animals frequently develop either mild infection or no infection at all. Analgesics such as aspirin or ibuprofen are anti-inflammatory, and since the keratoconjunctivits is largely a result of epithelial cell inflammation due to bacterial invasion, the use of such anti-inflammatory agents would also invalidate the model.

Two additional publications (Swearengen et al, 1993; Hanson et al, 2001) have studied the effects of analgesics (buprenophrine) on the Sereny test. These studies have shown that the use of analgesics increases the purulence and crustiness of Shigella-infected eyes, probably due to the lethargy of the animals that prevented normal grooming habits (Swearengen et al, 1993). Scoring of eyes crusted shut is very difficult because the eyes are glued shut by exudates, a problem not normally encountered. This problem complicates the interpretation of the reaction and therefore assessment of the virulence of shigellae. As the discharge is part of the Hartman scoring system (Hartman et al, 1991), which we have used for over 18 years, an increase in

discharge will lead to artificially high scores that may lead to a misinterpretation of the virulence of a wild-type Shigella strain, the misinterpretation of the safety (lack of reactogenicity) of a live-attenuated vaccine strain, and finally the misinterpretation of a vaccine's efficacy. The possible enhanced immune response in buprenophrine-treated animals could lead to misinterpretation of the immunogenicity and efficacy of experimental vaccines.

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):

Agency: _____ CFR: _____

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1. Registration Number: 10-F-0002

2. Number of animals used in this study: 192

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 keratoconjuctivitis (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 study of the immune response to and protective efficacy of vaccine candidates directed against shigellae, which are the primary goal of the MRMC, requires an accurate evaluation of the immune response raised by the administration of these vaccines. The use of analgesics, particularly opiates or narcotics, result in the immunosupression (see Einstein et el, 1993, Pruett, 1992, and Einstein 1998), which would invalidate the results of experiments testing immune responses as well as increasing the severity of the possible eye infection, since immunized animals frequently develop either mild infection or no infection at all. Analgesics such as aspirin or ibuprofen are anti-inflammatory, and since the keratoconjunctivits is largely a result of epithelial cell inflammation due to bacterial invasion, the use of such anti-inflammatory agents would also invalidate the model.

Two additional publications (Swearengen et al, 1993; Hanson et al, 2001) have studied the effects of analgesics (buprenophrine) on the Sereny test. These studies have shown that the use of analgesics increases the purulence and crustiness of Shigella-infected eyes, probably due to the lethargy of the animals that prevented normal grooming habits (Swearengen et al, 1993). Scoring of eyes crusted shut is very difficult because the eyes are glued shut by exudates, a problem not normally encountered. This problem complicates the interpretation of the reaction and therefore assessment of the virulence of shigellae. As the discharge is part of the Hartman scoring system (Hartman et al, 1991), which we have used for over 18 years, an increase in

discharge will lead to artificially high scores that may lead to a misinterpretation of the virulence of a wild-type Shigella strain, the misinterpretation of the safety (lack of reactogenicity) of a live-attenuated vaccine strain, and finally the misinterpretation of a vaccine's efficacy. The possible enhanced immune response in buprenophrine-treated animals could lead to misinterpretation of the immunogenicity and efficacy of experimental vaccines.

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Agency: _____ CFR: _____