Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 18

3. Species (common name) of animals used in this study: Swine

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

   Chemical agent administration. The dosing procedure involved inhalation administration under anesthesia which did not cause more than momentary pain or distress. The resultant intoxication may have caused pain and/or distress. The resultant intoxication may have caused pain and/or distress including tremors, gasping, forced abdominal respirations, convulsions, and vomiting.

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.

   Analgesics, analgesics or tranquilizers would be expected to mask the clinical appearance or potentiate the effects and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate a model and develop data necessary for definitive studies [required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 13

3. Species (common name) of animals used in this study: Rhesus macaque

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

   The dosing procedure involved intramuscular administration of nerve agent and treatments which did not cause more than momentary pain or distress; however the resultant intoxication with nerve agent may have caused pain and/or distress including respiratory distress and fasciculations. This work was conducted to determine the efficacy of scopolamine in the nonhuman primate following nerve agent exposure.

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.

   Analgesics, analgesics or tranquilizers would be expected to mask the clinical appearance or potentiate the effects and thus confound results or invalidate the study. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. The development of scientifically robust data was critical to the development of human safety data for eventual FDA licensure of scopolamine.

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:

   This work was conducted to validate or qualify a model and/or develop data necessary for definitive studies [that are required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 18

3. Species (common name) of animals used in this study: Rhesus macaque

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

   Aerosol challenge with ricin toxin. The challenge was performed under injectable anesthesia using a head-only exposure chamber. The challenge procedure with select agent itself is not painful but resultant toxicity may have caused pain and/or distress including lethargy, respiratory distress, moribundity and other abnormal clinical observations. This work was conducted to test the effectiveness of an antibiotic treatment.

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting intoxication process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to qualify a model and develop data necessary for definitive studies [required by the federal government under 21CFR314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 32

3. Species (common name) of animals used in this study: Cynomolgus macaque

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

   Aerosol administration of infectious bacteria. The challenge was performed under anesthesia. The challenge procedure with select agent did not cause more than momentary pain or distress but resultant infection may have caused more than momentary pain and/or distress including hunched posture, coughing, labored breathing and lethargy. This work was conducted to evaluate the effectiveness of vaccination (proprietary).

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   Agency: FDA

   CFR: As appropriate, study conducted under Title 21, Food and Drugs, specifically, 21CFR314.610, approval based on evidence of effectiveness from studies in animals (under subpart I -approval of new drugs when human efficacy studies are not ethical or feasible), and 21CFR601.91, approval based on evidence of effectiveness from studies in animals (under subpart H -approval of biological products when human efficacy studies are not ethical or feasible).

Column E Explanation Form

2. Registration Number: 31-R-0021

2. Number of animals used: 8

3. Species (common name) of animals used in this study: Cynomolgus macaque

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

   Aerosol administration of infectious bacteria. The challenge was performed under anesthesia. The challenge procedure with select agent did not cause more than momentary pain or distress but resultant infection may have caused more than momentary pain and/or distress including hunched posture, coughing, labored breathing

Summary of Exceptions to the Regulations or Standards

Note: Exceptions were IACUC-reviewed and approved.

A. Sanitization of Primary Enclosures

1. Variances were granted to the cage change requirement for rabbits. The pan liners were changed per SOP.

    a. Variance was granted to avoid additional handling of the animals and reduce stress prior to end of study events. 18 rabbits for 1 day and 19 rabbits for 2 days.

2. Variances were granted to the cage change requirement for primates. The cages were cleaned per SOP.

    a. Variance was granted to avoid additional handling and reduce stress prior to end of study events. 10 rhesus macaques for 1 day.
    b. Variance was granted to avoid additional handling and reduce stress prior to internal transport or end of study events. Up to 45 cynomolgus macaques for 1 or 2 days each.

and lethargy. This work was conducted to generate serum and plasma for development of early disease detection assays.

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate or qualify a model and develop data necessary for definitive studies [that are required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 11

3. Species (common name) of animals used in this study: Cynomolgus macaque

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

   Aerosol challenge with infectious bacteria. The challenge was performed under anesthesia using a head-only exposure chamber. The challenge procedure with select agent itself is not painful but resultant bacterial infection may have caused pain and/or distress including anorexia, fever, lethargy, weakness and respiratory distress. This work was conducted to evaluate the effectiveness of antibiotic treatment (proprietary).

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   Agency: FDA

   CFR: As appropriate, study conducted under Title 21, Food and Drugs, specifically, 21CFR314.610, approval based on evidence of effectiveness from studies in animals (under subpart I -approval of new drugs when human efficacy studies are not ethical or feasible), and 21CFR601.91, approval based on evidence of

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 68

3. Species (common name) of animals used in this study: Ferret

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

   The ferrets were subjected to an intravenous (IV) exposure of classified compounds of interest. The IV exposure did cause more than momentary distress in animals exposed to large doses. Animals exhibited signs of distress including ataxia, respiratory distress in some animals and occasionally tremors.

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.

   Analgesics, analgesics or tranquilizers would be expected to mask the clinical appearance or potentiate the effects and thus confound results or invalidate the study. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate a model and develop data necessary for definitive studies [required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

1. Number of animals used: 349

2. Species (common name) of animals used in this study: Guinea pig

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

   Intradermal injection with bacterial spores. The dosing procedure involved an injection with select agent which did not cause more than momentary pain or distress. The resultant bacterial infection may have caused pain and/or distress including lethargy and /or respiratory distress in some animals. Some animals show no signs prior to being found dead. This work was conducted to determine relative potency of different vaccine lots.

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:
   This work was conducted to qualify a model and develop data necessary for definitive studies [required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 268

3. Species (common name) of animals used in this study: Guinea pig

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

   Aerosol challenge with bacterial toxin. The challenge was performed using a muzzle-only exposure chamber. The challenge procedure with select agent itself is not painful but resultant intoxication may have caused pain and/or distress including lethargy and/or respiratory distress in some animals. This work was conducted to evaluate the effectiveness of monoclonal antibodies (proprietary).

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.

   Bacterial toxins, by the nature of their mechanism of action, are considered paralytic agents. As the cause of death is respiratory failure, anesthetics cannot be used to alleviate distress as they also act as respiratory depressants that could potentiate the toxic action of the biological agent. Opioid analgesics and barbiturate sedative-hypnotics both cause respiratory depression. Benzodiazepines, while having fewer effects on respiration, have been shown to have substantial respiratory interactions when used in combination with other neuroleptic agents. Because of these side effects, anesthetics, analgesics, and sedatives could potentiate the toxicologic effects of the toxin.

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:

   This work was conducted to validate or qualify a model and develop data necessary for definitive studies [that are required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 79

3. Species (common name) of animals used in this study: New Zealand White rabbit

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

   Aerosol challenges with bacterial spores. The challenge procedure itself is not painful but resultant bacterial infection with select agent may have caused pain and/or distress including lethargy, respiratory distress in some animals and occasionally seizures. Some animals show no signs prior to being found dead. This work was conducted to evaluate the effectiveness of vaccination (proprietary).

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate or qualify a model and develop data necessary for definitive studies [that are required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 41

3. Species (common name) of animals used in this study: New Zealand White rabbit

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

   Intradermal challenge with virus. The challenge procedure itself is not painful but resultant viral infection may have caused pain and/or distress including lethargy and respiratory distress in some. This work was conducted to evaluate the effectiveness of antiviral treatment (proprietary).

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   Agency: FDA

   CFR: As appropriate, study conducted under Title 21, Food and Drugs, specifically, 21CFR314.610, approval based on evidence of effectiveness from studies in animals (under subpart I -approval of new drugs when human efficacy studies are not ethical or feasible), and 21CFR601.91, approval based on evidence of effectiveness from studies in animals (under subpart H -approval of biological products when human efficacy studies are not ethical or feasible).

Column E Explanation Form

1. Registration Number: 31-R-0021

2. Number of animals used: 7

3. Species (common name) of animals used in this study: New Zealand White rabbit

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

   Aerosol challenges with bacterial spores. The challenge procedure itself is not painful but resultant bacterial infection with select agent may have caused pain and/or distress including lethargy, respiratory distress in some animals and occasionally seizures. Some animals show no signs prior to being found dead. This work was conducted to evaluate the effectiveness of antibiotic treatment (proprietary).

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.

   Anesthetics, analgesics or tranquilizers would be expected to alter the pathogenesis, signs/symptoms and/or progression of any resulting infectious process and thus confound results or invalidate the study. There are no known characterized, surrogate markers to predict mortality. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. Animals exhibiting clinical signs meeting euthanasia criteria per protocol or those which were moribund were euthanized to alleviate individual animal specific pain and distress. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate a model and develop data necessary for definitive studies [required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021
2. Number of animals used: 31
3. Species (common name) of animals used in this study: Guinea pig
4. Explain the procedure producing pain and/or distress:

   The guinea pigs were subjected to a subcutaneous (SC) exposure of physostigmine. The SC exposure did cause more than momentary distress in some but not all animals exposed to physostigmine (n=28). Animals exhibiting mild-to-moderate clinical signs (ataxia, fasciculations, tremors) often recovered without signs of distress by the 120-minute post-challenge endpoint. During the Phase I challenge level dose-ranging study, three (3) animals receiving the highest doses of physostigmine succumbed to challenge. Challenge at the higher doses of physostigmine was stopped per the direction of the Study Director and these challenge doses were not used for follow-on work.

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.

   Analgesics, analgesics or tranquilizers would be expected to mask the clinical appearance or potentiate the effects and thus confound results or invalidate the study. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate a model and develop data necessary for definitive studies [required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

Column E Explanation Form

1. Registration Number: 31-R-0021
2. Number of animals used: 48
3. Species (common name) of animals used in this study: Guinea pig
4. Explain the procedure producing pain and/or distress:

   The guinea pigs were subjected to an intravenous (IV) exposure of classified compounds of interest. The IV exposure did cause more than momentary distress in animals exposed to large doses. Animals exhibiting mild clinical signs often recovered without signs of distress and those with signs of distress displayed prostration, respiratory distress and ataxia. Some animals showed no signs prior to being found dead.

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.

Analgesics, analgesics or tranquilizers would be expected to mask the clinical appearance or potentiate the effects and thus confound results or invalidate the study. Experienced staff veterinarians and animal technicians were on site to monitor the animal status and health during study conduct, and any potential animal welfare issues. The development of scientifically robust data was critical to the development of human safety data.

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:

   This work was conducted to validate a model and develop data necessary for definitive studies [required by the federal government under 21CFR.314.610 - subpart I -approval of new drugs when human efficacy studies are not ethical or feasible, and/or 21CFR601.91 - subpart H -approval of biological products when human efficacy studies are not ethical or feasible].

effectiveness from studies in animals (under subpart H -approval of biological products when human efficacy studies are not ethical or feasible).