This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _50 (40 in Cat E)_____of animals in this study.

3. Species (common name)__Sheep_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

A small area (less than 1.5-2 mm) above the heel bulb on each claw of both hind feet will be scarified (epidermis partially removed) using one of several methods including rotary tool with abrasive bits, tape stripping, or brush. Cotton padding soaked in sterile phosphate buffered saline (50%), Difco Nutrient Broth (25%), and bovine rumen fluid (25%) will be placed over the area, secured with a layer of loose cling gauze. The entire foot will be then wrapped with plastic film, secured with a layer of VetWrap and held in place with tape. Animals may experience altered gait for the first day or two as they adjust to the "boots". No long-term adverse effects are expected as a result of just preparing and wrapping the feet.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Scarification/abrasion of the skin could be considered a painful procedure. Topical analgesic creams have been shown to have strong antimicrobial activity which will inhibit the normal flora and inoculated organisms which form the lesions, having an adverse effect on lesion outcome. Pain relieving medications will be given to any animals showing lameness after inoculation.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _23 (1 in Cat E)_____of animals in this study.

3. Species (common name)__Sheep_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal, oronasal or intracranial inoculation with Bovine Spongiform Encephalopathy positive inoculum. Clinical (neurologic) signs may occur as a result of experimental inoculations between 6 months and 7 years after the procedure which may include ataxia, tremor, prolonged recumbency, scratching/loss of wool, or unawareness of surroundings.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Drugs will be used to alleviate pain and distress associated with procedures or intercurrent disease. Animals are requested in category E since they may develop clinical signs of prion disease after inoculation. There are no known treatments for prion disease. Animals will be euthanized as soon as possible after unequivocal signs of prion disease are noted.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number: 42-G-0001

2. Number _112 (7 in Cat E)_____of animals in this study.

3. Species (common name) Sheep of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Oral, oronasal, or intracerebral inoculation of transmissible spongiform encephalopathy (TSE). Clinical (neurologic) signs may occur as a result of experimental inoculations months to years after the procedure which may include ataxia, tremor, prolonged recumbency, scratching/loss of wool, or unawareness of surroundings.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Drugs will be used to alleviate pain and distress that occurs during procedures or due to intercurrent disease. Animals are requested in category E due to the potential that they will exhibit clinical signs as a result of being inoculated with a prion disease agent. Currently, there are no known treatments for prion disease.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _215 (170 in Cat E)_____of animals in this study.

3. Species (common name)__Mouse_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intraperitoneal challenge of bovine respiratory disease complex causing bacteria and intravenous or intraperitoneal administration of NK-lysin-derived peptides. Mice are expected to develop septicemia following H. somni challenge along with severe clinical signs such as ruffled fur, hunched, eyes closed, reduced activity, cold ears, reluctant to move when prodded and moribund.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Typical treatment to control BRDC would include antibiotics such as Excenel, Nuflor, and Baytril. However, treatment of mice with these antibiotics will affect the efficacy of NK-lysin peptides on BRDC causing bacterial pathogens. Therefore any antibiotic cannot be administered and mice will be euthanized immediately when they show severe clinical signs of septicemia.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _3395 (380 in Cat E)_____of animals in this study.

3. Species (common name)__Mouse_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which

may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intracranial or intraperitoneal inoculation of transmissible spongiform encephalopathy (TSE). Lack of hygienic behavior (urine stained or roughened haricot), ataxia, circling, or inability to rise are suggestive of clinical signs.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Drugs can be used to alleviate pain and distress associated with treatments or concurrent illness. Some mice are expected to develop clinical signs of prion disease for which there is no treatment, thus all mice in the protocol are listed as category E.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _40 (33 in Cat E)_____of animals in this study.

3. Species (common name)__Ferret_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation of Influenza A virus. Only mild clinical signs are anticipated following infection with IAV such as lethargy, inappetance, fever, nasal and ocular discharge, coughing.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

We anticipate only mild clinical signs following infection with IAV. Since the use of drugs would mask the clinical signs of influenza illness, evaluation of clinical signs without the use of drugs is required to meet the project objectives of IAV pathogenesis, transmission, and host response in ferrets.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number: 42-G-0001

2. Number _15 (6 in Cat E)_____of animals in this study.

3. Species (common name) White-tailed Deer of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Oronasal inoculation of Transmissible Spongiform Encephalopathy (TSE). No immediate clinical effects or changes are expected from this procedure. Clinical signs of weight loss, altered gait, tremor, or behavioral change could occur 18-60 months post inoculation.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Drugs will be used to alleviate pain and distress that occurs during procedures or due to intercurrent disease. Animals are requested in category E due to the potential that they will exhibit clinical signs as a result of being inoculated with a prion disease agent. Currently, there are no known treatments for prion disease.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number: 42-G-0001

2. Number _ 276 (25 in Cat E) of animals in this study.

3. Species (common name) Turkeys of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Oral gavage into the crop of a live recombinant attenuated Salmonella vaccine with or without a Campylobacter antigen in phosphate buffered saline, and oral gavage with Campylobacter jejuni, Campylobacter coli and/or Salmonella enterica. In very young poults (up to two weeks old), Salmonella can cause disease (and potentially death). The disease symptoms may vary and include weakness, loss of appetite and poor growth. The animals may crowd close to heat sources and sit with drooping wings and their eyes closed. Watery diarrhea may also occur as a result of Salmonella inoculation.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The aim of this study is to evaluate the efficacy of a novel vaccine and whether if affects colonization by Salmonella enterica or Campylobacter, or a combination of both. The use of antimicrobials to treat any clinical signs detected may directly confound interpreting the efficacy results against the different inocula. Campylobacter colonization does not cause morbidity or mortality in turkeys. However, it is possible that other opportunistic pathogens present in the distal intestinal tract may take advantage of intestinal dysbiosis created by Campylobacter or Salmonella enterica inoculations, causing morbidity (e.g., depression, anorexia or watery diarrhea) severe enough to warrant euthanasia. Although the development of clinical disease after the inoculation by Campylobacter or Salmonella enterica is unlikely, we are uncertain whether poults co-inoculated with Campylobacter and Salmonella will produce clinical disease. As a contingency, all Salmonella and Campylobacter co-colonized poults have been placed in category E.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _12 (1 in Cat E)_____of animals in this study.

3. Species (common name)__Raccoons_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intracranial or oranasal inoculation of transmissible spongiform encephalopathy (TSE). Signs suggestive of prion disease (that may occur 6 months or longer post-inoculation) include hyperexcitability, incoordination (usually noted by inability to climb in the cage), trembling, recumbency, poor awareness of surrounding, non-responsiveness, or self-mutilation.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Drugs will be used to relieve pain and distress that results from procedures and intercurrent disease. Category E animals are requested because raccoons are expected to develop clinical signs due to the experimental inoculation with prion agents. There are no effective treatments for prion diseases.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _208 (105 in Cat E) _____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation with Porcine Reproductive and Respiratory Syndrome Virus; or a single porcine respiratory disease complex (PRDC) pathogen or their deletion mutants. Following challenge, it is anticipated that some pigs will develop fever, anorexia, lethargy, lameness, and/or sneezing/coughing. Some of the bacteria can cause severe systemic disease including septicemia and/or meningitis.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

To evaluate the pathogenicity of a virus or bacterial isolate/mutant, or the efficacy of intervention strategies such as vaccination, we will need to evaluate host response which precludes the use of drugs that might alleviate clinical signs thus masking the true pathogenic nature of the inoculum. If severe illness occurs animals will be euthanized.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _221 (160 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which

may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation with influenza A viruses will use physical restraint of the pig and inoculum will be dripped into each nostril. Intratracheal inoculation with influenza A viruses will require anesthetizing the pig. Inoculum will be passed into the trachea via a small plastic tube. Only mild clinical signs are anticipated following infection with IAV such as lethargy, inappetence, fever, nasal and ocular discharge, coughing.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Only mild clinical signs are anticipated following infection with influenza A viruses. Since the use of drugs would mask the clinical signs of influenza illness, evaluation of clinical signs without the use of drugs is required to meet the project objectives of IAV pathogenesis, transmission, and host response in swine.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _248 (248 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Phosphate buffered saline containing Salmonella typhimurium strain, Salmonella vaccine, or Salmonela enterica strain will be administered intranasally. Clinical signs to be observed could be a transient febrile response (less than or equal to 106 F, normal is 102-103 F) with enterocolitis, increased respiration and depression lasting up to 4 days.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The validity of the experimental results requires that the infectious disease induced by Salmonella enterica be allowed to manifest itself without the use of therapeutic drugs. Otherwise, the altered state of the host due to the use of antimicrobials may change the clinical response, potentially modifying the observed pathogenicity of the Salmonella strains and the usefulness of the prospective vaccine strain.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number: 42-G-0001

2. Number _148 (148 in Cat E)_____of animals in this study.

3. Species (common name) Pigs of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal or oral challenge of swine enteric coronavirus (SECV). Challenge may induce mild to moderate diarrhea that would be recognized as a loose/liquid stool for several days. In pigs less than 10 lbs, challenge will induce a severe diarrhea (watery) and vomiting which would result in death for the most affected pigs.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Some of the proposed studies require evaluation of protective immunity. If efficacious pain relieving drugs or other ameliorative treatments were used, they would mask clinical signs which would obscure the evaluation of protective immunity. Some of the proposed studies require the evaluation of the pathogenesis of the disease which requires the disease to progress naturally, thus unaffected by drugs.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number: 42-G-0001

2. Number _30 (10 in Cat E)_____of animals in this study.

3. Species (common name) Pigs of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which

may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation of pseudorabies virus (PRV). Anticipated clinical signs would be pyrexia, anorexia, dyspnea, listlessness, and possible central nervous system (CNS) signs recognized as ataxia, convulsions, and general unresponsiveness to people.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

It is necessary to have a wild-type challenge group that may get sick, for which no ameliorative treatments may exist to produce a natural immune response to PRV infection. This allows the development of antibodies in a normal fashion which will produce samples that will be used to evaluate a diagnostic assay that will be applied for use in the field to test animals naturally infected with PRV.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _60 (27 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal challenge of pigs with Senecavirus A (SVA). Experimental infections with Senecavirus A have induced only skin lesions limited to the snout and coronary band with minimal clinical effect.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Three part answer: 1) To study the pathogenesis of the SVA infection in swine, the host response has to be "normal," no alterations from extraneous treatments. 2) If drugs would be beneficial in reducing pain/distress, then they would have some effect on the pig's response which alters the pathogenesis of the virus. 3) To evaluate the potential efficacy of a vaccine or treatment, the natural expression of the disease is necessary.

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _36 (8 in Cat E)_____of animals in this study.

3. Species (common name)__Hamsters_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Hamsters may be inoculated with live leptospires using urine, concentrated urine, culture medium, liquid culture, or tissue homogenate in transport medium administered intraperitoneally. Hamsters inoculated with leptospira can develop outward clinical signs (observable hemorrhage, ruffled hair coat, weight loss, and isolation).

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The observation of clinical signs and their stage of advancement will be used to indicate euthanasia. Alleviating or relieving these signs will interfere with assessment. No alternatives to the painful procedure were identified which will allow evaluation of leptospiral virulence. Animals will be weighed once daily beginning on day of inoculation, weight loss of >20% or development of outward clinical signs (observable hemorrhage, ruffled hair coat and isolation, loss of interest in food and water) will warrant euthanasia. (Haake. Current Protocols in Microbiology, 2006 Supp. 2, 12E.2.1)

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _4 (4 in Cat E)_____of animals in this study.

3. Species (common name)__Guinea Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Guinea pigs may be inoculated with leptospira using urine, concentrated urine,culture medium, liquid culture, or tissue homogenate in transport medium administered intraperitoneally. Guinea pigs inoculated with leptospira can develop outward clinical signs (observable hemorrhage, ruffled hair coat, weight loss, and isolation).

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The observation of clinical signs and their stage of advancement will be used to indicate euthanasia. Alleviating or relieving these signs will interfere with assessment. No alternatives to the painful procedure were identified which will allow evaluation of leptospiral virulence. Animals will be weighed once daily beginning on day of inoculation, weight loss of >20% or development of outward clinical signs (observable hemorrhage, ruffled hair coat and isolation, loss of interest in food and water) will warrant euthanasia. (Haake. Current Protocols in Microbiology, 2006 Supp. 2, 12E.2.1)

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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None