ATTACHMENT to APHIS FORM 7023 (93-R-0440)

3. The following are the locations where regulated animals were housed or used during the year [Section 2.36(b)(4)]:

Column E:

The University of California at San Francisco is committed to using laboratory animals in such a way as to minimize pain or discomfort. The Committee reviews each project and many protocols have been redesigned to meet this goal. Attached are the explanations of the procedures producing pain or distress in the animals covered by Subchapter A - Animal Welfare and reported in column E during the period 10/1/09 through 9/30/10 and the reasons anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretations of the research. Separate Optional Column E form (1) is attached.

ATTACHMENT to APHIS FORM 7023, Federal Fiscal Year 2009/2010 (93-R-0440).

Column E Explanation

1. Registration Number: 93-R-0440

2. Number of animals used in this study 202

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

New Zealand White Rabbit

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

Bacterial infection, either endocarditis or bacteremia with *S. aureus* or streptococci, is established by intravenous injection of bacteria. Bacterial pneumonia with S. aureus is also established by endotracheal instillation of bacteria. The infection, as it is the condition under study, is not treated with specific therapy. The systemic inflammatory response that accompanies infection may or may produce distress or discomfort, depending on the clinical course of disease, which is not predictable in any individual animal. Infection accompanied by hypotension and CNS depression preclude access to food and water, which is monitored by following weight and activity level and treated with fluid administration, as needed.

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. (For Federally mandated testing, see Item 6 below).

It is debatable whether any of these rabbits actually experienced pain or distress from the infection itself, as in humans the studied condition is not reported as painful. Once infection is established administration of narcotics or anti-inflammatory agents is not feasible as these may either hasten death or alter the course of the condition under study. Monitoring parameters such as fever do not accurately predict outcome. Approximately 20% of animals with bacteremia survive with no apparent ill effects other than fever and modest weight loss. About half of the rabbits manifest sufficient weight loss, are moribund or otherwise unable to access food and water and may be euthanized. Up to a quarter of rabbits die. Due to the inability to predict death

in individual animals, all animals are potentially at risk for unrelieved distress and death. Accordingly, all infected animals have been classified 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):

NA

1. Registration Number: 93-R-0440

2. Number of animals used in this study 124

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

New Zealand White Rabbit

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

Staphylococcus aureus, particularly community-associated methicillin-resistant Staphylococcus aureus, causes severe infections and high mortality rates, particularly in patients with pneumonia. Rabbit models reproduce many of the important features of staphylococcal pneumonia as they occur in humans. Rabbits also exhibit pathogen-host specificities that are similar to humans. Our goal is to use the rabbit pneumonia model to further define bacterial factors that are important in virulence and pathogenesis of pneumonia caused by *Staphylococcus aureus* and to test various therapeutic approaches (antibodies-based therapies, vaccination, antimicrobials) for treatment of these severe infections. To establish bacterial infection, S. aureus inoculum is instilled endobronchially through a soft catheter positioned via an endotracheal tube in an anesthetized animal.

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. (For Federally mandated testing, see Item 6 below).

These infection models produce few, if any, early indicators of distress or discomfort that can be used to guide interventions to relieve either condition. These are also particularly severe infections, both in rabbits and in humans, the latter suffering a 25-40% mortality rate even when appropriate therapy is administered. The rapidity with which the infection can advance in some animals also defies prediction of distress or discomfort. Other than administration of fluids, interventions short of euthanasia (e.g., administration of antibiotics or analgesia) would either invalidate the model by altering the natural progression of the disease which is under study, alter the pathophysiology or host response, or hasten death. Deaths are possible in these infection models and they cannot be accurately or reliably predicted in the individual animal. Monitoring parameters such as fever do not accurately predict outcome. Approximately 20% of animals with bacteremia, for example, survive with no apparent ill effects other than fever and modest weight loss. About half of the rabbits manifest sufficient weight loss, are moribund or otherwise unable to access food and water. These appear to correlate with extent of disease and can be useful endpoints in competition experiments in particular. Animals with these findings are removed from the study. Yet, up to half or more of rabbits die and they do so without manifesting findings of severe disease. There are no early indicators short of death or moribund condition that can be used as a clinical endpoint that would allow us to define the outcome of interest: whether the host clears the organism or not from the target tissue or tissues under study. Due to the inability to predict death in individual animals, all animals are potentially at risk for unrelieved distress and death. Accordingly, all infected animals will be classified 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):

NA

1. Registration Number: 93-R-0440

2. Number of animals used in this study 1

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

Cynomolgus macaque

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

One animal experienced an acute onset of hind limb paresis following an MRI guided infusion. This condition was only partially responsive to extensive veterinary treatment and was still present three months following onset at the time of necropsy. The animal lost approximately 15% body weight and we believe the weight loss was a reflection of this condition and retrospectively reclassify this animal in Column E.

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. (For Federally mandated testing, see Item 6 below).

Animal received extensive veterinary care to treat this condition. There was limited success in therapies that were utilized.

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):

NA