COLUMN E EXPLANATION

1. REGISTRATION NUMBER: 15-V-0001

2. Number of animals used in the study: 89

3. Species: Mouse

4. Explain procedure producing pain/distress:

Experiments will assess the effects of lipopolysaccharide (also known as LPS or endotoxin) on lung edema in the PKCδ transgenic mice. The conscious mice will be injected with ~100µl vehicle or 2.5-10mg/ kg body weight of LPS intraperitoneally (IP) and experimented upon at 6h and 24h post-injection. Alternatively, the LPS would be introduced in a sedated mouse intratracheally (IT) at a dose of 2.5µg/ gm body weight (~50µl) via 20g needle and experimented upon at 1 or 2h following exposure. Concentrations of LPS are based upon published work (JCI (2006) 116:2333; AJP (2004) 288:L1026). The mice will then be used to assess Evans Blue Dye (EBD) extravasation, wet to dry lung weights, and bronchoalveolar lavage (BAL) fluid content in the intact mouse or capillary coefficient in isolated perfused lungs.

In addition, experiments will determine the survival times of mice following intratracheal (IT) administration of lipopolysaccharide (LPS). Initial experiments will use the dose 2.5µg/ gm body weight of LPS. However, there is a chance that this lower dose of LPS is not sufficient to delineate the differential susceptibility to death. Thus, should we not note significant degree of death with the 2.5µg/ gm body weight of LPS dose in the mice, we would then increase the dose 2X, 5X, or 10X until an optimal dose is found (These doses are based on many peer-reviewed papers, using a variety of mouse strains). The mice will be monitored twice a day for 7 days. Should the animals display the following signs of morbidity or moribund (http://www.bu.edu/research/compliance/oversight-committee/iacuc/policies/policy-for-humane-endpoints.shtml), then the animals will be euthanized by an overdose of pentobarbital (120mg/kg) given intraperitoneally.

- Clinical Signs of Moribund Condition in Animals:
  - Impaired mobility (the complete inability to reach food and water)
  - Inability to remain upright
  - Hunched posture for more than 48 hours
  - Labored breathing and cyanosis
  - Clinical dehydration and/or prolonged decreased food intake (more than 48 hours)
  - Muscle atrophy and signs of lethargy and lack of physical activity
  - Severe, rapid weight loss and emaciation
  - Chronic diarrhea or constipation for more than 48 hours

- Bleeding noted from any orifice on 2 consecutive occassions
- Self-mutilation
- No response to external stimuli

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.**

Literature demonstrates that the survival time for these animals, following the LPS exposure at these doses, is 5-7days. We plan to isolate the lungs/ hearts at <24h post-injection, thus anticipate that the animals will have experienced minimal pain and distress. To provide analgesia would interfere with the anticipated disease process by which the LPS is mediating its effect (Chuang et al. (1987) Archives of Surgery 122:940; Rinaldo and Dauber (1985) Circulatory Shock 16:195). If animals are experiencing excessive distress or pain, they will be humanely euthanized.