## 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: 33 - R - 00 2 √ of animals used in this study. 2. Number 3. Species (common name) Guinea Pig\_of animals used in the study.

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

Asthma was induced by a series of three intraperitoneal (i.p.) injections of ovalbumin (OVA 100 µg and aluminium hydroxide 100 mg in 1 mL saline) on days 1, 3 and 5. After a subsequent 2-week sensitization period (during which the animals were returned to their cages without any additional treatment), the animals were placed in a cylindrical Plexiglas chamber where they were observed for at least 1 minute and then exposed to a nebulized solution of ovalbumin (5 mg/ml in saline) for 30 minutes using a commercial nebulizer with a constant pressure at ~0.3 ml/min. This protocol was be repeated at one day intervals for a total of 3 trials and induced asthmatic symptoms in the ovalbumin-sensitized guinea pigs. The sequence of respiratory signs observed in this model include rapid breathing, restlessness, chewing, ruffling of the fur, then labored breathing with the use of accessory muscles of respiration, prolonged expiration, gasping, coughing, dilation of alae nasi, and finally cyanosis and convulsion.

 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)

Animals were not given analgesic or anesthetics to relieve these symptoms, because such treatment would reduce or prevent the asthmatic symptoms, which are required for assessing the severity of responses. Anesthetics often affect ion channels and might also therefore interfere with parameters measured in airway smooth muscle cells post euthanasia. A search of PubMed (1998 – 2013) was used to determine that anesthetics and analgesics would reduce or prevent asthmatic symptoms, which are required to assess the severity of the response.

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):

CFR N/A Agency