

Column E Explanation

1. Registration Number: 33-V-0004/554
2. Number 6 (Category E) of animals used in this study.
3. Species (common name) Guinea pig of animals used in the study.
4. Explain the procedure producing pain and/or distress.

Animals are exposed for 30 min to a treatment to induce an asthma attack.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means use to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below).

To induce asthmatic attacks in guinea pigs, we will administer a series of three i.p. injections of ovalbumin (OVA 100 µg and aluminium hydroxide 100 mg in 1 mL saline) over a week, followed by a two week sensitization period and then exposure to a fine mist of aerosolized ovalbumin (5 mg/ml in saline) solution for 30 min. The latter procedure will be repeated for three trials with a one day interval between each trial. Aerosolized ovalbumin produces symptoms that may include rapid breathing, restlessness, chewing, ruffling of the fur, labored breathing with the use of accessory muscles of respiration, prolonged expiration, gasping, coughing, dilation of alae nasi, cyanosis and convulsion. To minimize pain and distress, animals will be removed from the test if they display signs of cyanosis or convulsion, monitored until they recover, and returned to their cage until the next scheduled treatment. If cyanosis and convulsions are not relieved by immediate removal from the exposure chamber, the animal will be humanely euthanized. Animals will not be 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 (Zhou, C., Liu, J., & Chen, X.-D. (2012). General anesthesia mediated by effects on ion channels. *World Journal of Critical Care Medicine*, 1: 80–93) and might also therefore interfere with parameters (e.g. Kv currents) measured in ASMCs post euthanasia.