Humane Endpoints Checklist

**ITEM 1. Describe whether humane endpoints\* were used for all animals involved in the study.**

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| --- | --- | --- |
|  | **Recommendation** | **Section/Paragraph** |
| **If humane endpoints\* were used, report the following:** |
| **1** | **The specific criteria used to determine when animals should be euthanized** | **The rats were euthanized when they need to performed a cesarean section.** |
| **2** | **Once animals reached endpoint criteria, the amount of time elapsed before euthanasia** | **About one hour.** |
| **3** | **Whether any animals died before meeting criteria for euthanasia** | **No** |
| **If humane endpoints\* were not used, report the following:** |
| **1** | **A scientific and ethical justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered but could not be used** |  |
| **2** | **Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design** |  |

**ITEM 2. Include the following details of the study design and outcomes.**

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|  | **Recommendation** | **Section/Paragraph** |
| **1** | **The duration of the experiment** | **About one year** |
| **2** | **The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals** | **60 rats were used and euthanized and no rat was dead** |
| **3** | **How frequently animal health and behavior were monitored** | **Once every two days** |
| **4** | **All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions** | **Rats were housed in a temperature-controlled room (23±1℃) and kept under a 12:12-h light/dark cycle with food and water ad** libitium. **Rats were anesthetized with isoflurane (5% in 100%O2) inhalation and maintained with a gas mask (2% isoflurane)** |
| **5** | **Any special training in animal care or handling provided for research staff** | **The special training include understanding mechanisms of various euthanasia, relieving animals of pain or fear and confirming their death.** |