

### Category E Explanation

Study Title: Assessment of Efficacy of "X company" Test Article in Preventing Ototoxicity After Cisplatin and Aminoglycoside Exposure in Guinea Pigs

Study Design: Animals in this study received one intraperitoneal injection of 12mg/kg (high dose) of Cisplatin. Cisplatin is a chemotherapy drug and is known to be cytotoxic, nephrotoxic, and neurotoxic.

Animal Welfare Plan for animals receiving Cisplatin:

Animal welfare assessments:

- Health observations will be performed and documented:
  - Day 0-15: Once per day for all study animals
  - Day 1-8: Three times per day for Cisplatin animals
  - Day 9-15: Twice per day for Cisplatin animals
- Cages with animals that received cisplatin will be kept on warming pads to maintain normal body temperature
- All animals receiving cisplatin will be hydrated by the subcutaneous administration of sterile Saline (0.9% NaCl solution) to prevent dehydration. Sterile 0.9% Saline (amount to be determined by veterinarian) will be given the same day as cisplatin administration (Day 1) and for the following 7 days (days 1-8).
- Body weights will be taken, at least:
  - Once during acclimation
  - Day of baseline ABR
  - Once daily, Days 0-14
  - Day of endpoint ABR
- Pain and Distress/Humane endpoints: Animals will be removed from the study or humanely euthanized for the following conditions and according to Turner Scientific SOPs:
  - 20% weight loss compared to the pre-injection weight
  - Dehydration >24 hours, following SQ saline injections for supportive care.  
(Noting that thick sticky saliva, crusty eyes, poor appetite, small amounts of dark urine, or hard dry fecal pellets are signs of dehydration in guinea pigs)
  - Lethargy (at discretion of AV)
  - Reluctance to move (at discretion of AV)
  - Diarrhea (at discretion of AV)

Animals removed from study will be replaced, taking into consideration the scientific aims and objectives as well as anticipated pain and distress conditions. The PI, AV, and study sponsor will discuss additional animals placed in study.

Evaluation of Pain and Distress prior to study start:

Although a plan was created to help support the animal's health and well-being, it is well known that animals and humans receiving chemotherapy treatments suffer pain and distress as a result of these treatments. Due to assessing the sponsor's test article effectiveness, pain medications were not allowed for this study.