Annual Report of Research Facility
Column E Explanation
Fiscal Year: October 1, 2021 – September 30, 2022

Registration Number: 41-R-0061

Research Facility Headquarters Address: 2540 Executive Drive, St Paul, MN 55123

Number of animals used in the study: 29

Species: Guinea Pig

Explanation of the procedure producing pain and distress:

The Guinea Pig Maximization Sensitization Test is required to assess the potential of medical devices to cause skin sensitization reactions in patients.

For each study, 11 test animals and 6 control animals are used, and undergo 3 phases: Induction 1, Induction 2, and Challenge.

The Induction 1 consists of three pairs of intradermal injections per animal. The animals are injected with: two injections (0.1 mL each) of 0.9% Sterile Saline or Oil mixed with Freund's Complete Adjuvant (FCA); two injections (0.1 mL each) of the test article or control vehicle; and two injections (0.1 mL each) of the test article or control vehicle in a 1:1 ratio with 0.9% Sterile Saline/FCA (1:1).

6 days later, 0.5-1.0 mL of a topical application of 10% sodium lauryl sulfate (SLS) in mineral oil is applied to enhance the transport of the test solution across the skin barrier by causing a mild inflammatory reaction. After approximately 24 hours, the SLS is removed by gently wiping with gauze, and Induction 2 is initiated by applying a patch of the test article or control directly to the Induction 1 sites. The animals are wrapped with elastic bandage and hypoallergenic tape to secure the patches. The patches are removed after approximately 48 hours.

14 days later, the challenge phase is initiated by applying a patch of the test article or control to naive skin sites. The animals are wrapped with elastic bandage and hypoallergenic tape to secure the patches. The patches are removed after approximately 24 hours, and the sites are examined over 48 hours for signs of erythema and/or edema.

A positive control test using well-known contact allergens (e.g. 2,4-dinitrochlorobenzene (DNCB)), is also performed once every three months to validate the system.

Animals in this study are initially categorized under Pain and Distress Category C since the procedures are not expected to cause more than momentary pain or distress. Guinea pigs are recategorized retrospectively, based on the pain and distress that the individual animal experienced during the study. During the 2022 fiscal year, the following guinea pigs were recategorized:

- 2 guinea pigs used for this study were found dead following a wrap period. A necropsy indicated that the livers were misshapen, and the stomachs were firm, most likely due to wraps that were too tight. These animals were recategorized into Pain and Distress Category E.
- 15 guinea pigs used for the study were found dead at some point during the study. Necropsies indicated that the cause of death was typhlitis and/or colitis secondary to bacterial overgrowth. The infections were likely exacerbated by the stress of testing and handling. These animals were recategorized into Pain and Distress Category E.
- 8 guinea pigs used for the study displayed clinical signs including lethargy, labored breathing, hunched posture, and/or cyanosis. Upon identification of the clinical signs, the veterinarians were contacted. In some cases, supportive care was provided, but all cases resulted in the

humane euthanasia of the animals. Necropsies indicated that the animals had typhlitis and/or colitis secondary to bacterial overgrowth. The infections were likely exacerbated by the stress of testing and handling. These animals were recategorized into Pain and Distress Category E.

- 2 guinea pigs used for the study were found dead at some point during the study. Necropsies indicated that the cause of death was pneumonia due to an infection with guinea pig adenovirus. The infections were likely exacerbated by the stress of testing and handling. These animals were recategorized into Pain and Distress Category E.
- 1 guinea pig used for the study displayed clinical signs including abnormal respiration, during the challenge wrap phase. Upon identification of the clinical signs, the veterinarians were contacted, and the animal was humanely euthanized. A necropsy indicated that the cause of the clinical signs was pneumonia due to an infection with guinea pig adenovirus. The infection was likely exacerbated by the stress of testing and handling. This animal was recategorized into Pain and Distress Category E.
- 1 guinea pig used for the study displayed clinical signs including dyspnea and epistaxis while being wrapped. The veterinarians were contacted, and the animal was humanely euthanized. A necropsy indicated that the cause of the clinical signs was hemorrhages within the alveoli and bronchioles, suggestive of peracute pulmonary hypertension. The hypertension was likely caused by the stress of handling the animal during the wrapping procedure. This animal was recategorized into Pain and Distress Category E.

Scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight:

Animals are initially categorized under Pain and Distress Category C since the procedures are not expected to cause more than momentary pain or distress. However, the animals are closely monitored for signs of pain and distress throughout the study. If any animal displays clinical signs of pain and distress, the veterinarians may prescribe analgesics or supportive care. Non-steroidal anti-inflammatory drugs (NSAIDs) are typically avoided due to the anti-inflammatory effects which may mask erythema and/or edema induced by the test articles, resulting in false negatives. Humane endpoints are also used to identify the point in which an animal should be humanely euthanized:

- Weight loss greater than 20% of the initial weight with clinical signs of illness and/or distress.
- Prolonged durations of anorexia, greater than three days with veterinary consultation.
- The presence of health problems refractory to medical intervention.

What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number:

40 CFR § 798.4100

ISO 10993-10

ASTM-F720

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Registration Number: 41-R-0061

Research Facility Headquarters Address: 2540 Executive Drive, St Paul, MN 55123

Number of animals used in the study: 6

Species: Rabbit

Explanation of the procedure producing pain and distress:

Rabbit pyrogen testing is required to assess the potential of medical devices to cause a fever in patients.

Prior to experimental testing, all animals must pass both an acclimation and SHAM tests. The acclimation test requires the animals to be restrained in a loose-fitting neck stock for 0.5 hours and 1.0 hours on separate days. The animals are closely monitored while in the stock, and any above average restlessness, kicking, struggling, or vocalization will define a failure of the acclimation test. If an animal fails an acclimation test, they will be removed from study.

After passing the acclimation tests, the rabbits will undergo a SHAM test, in which the animals are placed in the stock, and a lubricated thermocouple (probe) will be placed approximately 7.5 cm into the rectum to monitor the animal's body temperature. A baseline temperature will be collected, then 3-10mL/kg of saline will be injected into the marginal ear vein. After injection, the body temperature of each rabbit will be monitored and collected over a 3-hour period.

For the experimental test, 3 rabbits will be restrained and probed in the same manner as the SHAM test. A baseline temperature will be collected just prior to injection of the test article solution (3-10mL/kg) into the marginal ear vein. After injection the body temperature of each rabbit will be monitored over a 3-hour period. The temperatures will be evaluated to determine the pyrogenicity of the test articles.

Animals are initially categorized under Pain and Distress Category C since the procedures are not expected to cause more than momentary pain or distress. Rabbits are recategorized retrospectively, based on the pain and distress that the individual animal experienced during the study. During the 2022 fiscal year, the following rabbits were recategorized:

- 3 rabbits used for pyrogen testing experienced unanticipated spinal fractures while being restrained in the stock for either the sham or experimental test. In all cases, the technician performing the procedures noted that the animals had abnormal anal tone and weakness of the hindlimbs. The veterinarians were immediately contacted, diagnostic tests performed (neurological exams and/or radiographs), and the animals were humanely euthanized. Due to the nature of the injuries, the animals were recategorized into Pain and Distress Category E.
- 1 rabbit intended for pyrogen testing was found dead one day after a sham test was performed. A necropsy indicated a perforation of the colon and peritonitis. The perforation likely occurred from the temperature probe placed in the rectum to measure body temperature throughout the sham test. This animal was recategorized into Pain and Distress Category E.
- 1 rabbit intended for pyrogen testing was noted as having blood in the mouth and nose following the sham test. The animal also appeared cyanotic. The veterinarians were immediately contacted, and the animal was humanely euthanized. The necropsy indicated that the animal likely experienced unanticipated pulmonary trauma related to the saline dose. This animal was recategorized into Pain and Distress Category E.
- 1 rabbit used for pyrogen testing experienced convulsions and respiratory failure during dosing of the test article. The animal was immediately provided with oxygen support, and the

veterinarians were contacted. However, the animal did not recover. The event was attributed to the test article itself. This animal was recategorized into Pain and Distress Category E.

Scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight:

Animals are initially categorized under Pain and Distress Category C since the procedures are not expected to cause more than momentary pain or distress. However, the animals are closely monitored for signs of pain and distress throughout the study. If any animal displays clinical signs of pain and distress, the veterinarians may prescribe analgesics or supportive care. Non-steroidal anti-inflammatory drugs (NSAIDs) are typically avoided due to the antipyretic effects which may mask fevers induced by the test articles, resulting in false negatives. Humane endpoints are also used to identify the point in which an animal should be humanely euthanized:

- Weight loss greater than 20% of the initial weight with clinical signs of illness and/or distress.
- Prolonged durations of anorexia, greater than three days with veterinary consultation.
- The presence of health problems refractory to medical intervention.

What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number:

21 CFR § 610.13.b.

ISO 10993-11

USP 151

USP 1041

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Research Facility Headquarters Address: 2540 Executive Drive, St Paul, MN 55123

Number of animals used in the study: 1

Species: Rabbit

Explanation of the procedure producing pain and distress:

Primary Skin Irritation (PSI) studies are required to assess the potential of medical devices to cause dermal irritation in patients.

For each study, 3 rabbits are used, and the test article will be administered in two topical doses, one on each side of the vertebral column. A dose may consist of 0.5 mL of a liquid, 0.5g of a powder, 0.5 mL of a gel, or 1 square inch representative sample of a solid material. Liquids or semi-solids will be applied to a 1 square inch porous gauze patch. Also, negative control patches consisting of gauze (if test article is a solid) or tap water placed onto gauze (if test article is a liquid, gel, or powder) are placed next to the test patches, one patch on each side of the vertebral column. The patches will be held in contact with the skin with hypoallergenic dermal tape and the animal will be wrapped with an elastic bandage for a minimum of 4 hours or a minimum of 24 hours. As part of the application, all wraps are manually checked by the technicians to ensure that the wraps do not impede normal breathing.

After the exposure period, the dressing and patches are removed and the sites are observed and scored for erythema and edema over a 72-hour period, after which the animals are humanely euthanized. After the 72-hour observation and scoring period, the animals are humanely euthanized.

Animals in this study are initially categorized under Pain and Distress Category C since the procedures are not expected to cause more than momentary pain or distress. Rabbits are recategorized retrospectively, based on the pain and distress that the individual animal experienced during the study. During the 2022 fiscal year, the following rabbits were recategorized:

- 1 rabbit used for this study experienced an unanticipated spinal fracture following a 24-hour wrap period. Upon identification of hindlimb paralysis, the veterinarians were immediately contacted, diagnostic tests performed (neurological exams and/or radiographs), and the animal was humanely euthanized. Due to the nature of the injury, the animal was recategorized into Pain and Distress Category E.

Scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight:

Animals are initially categorized under Pain and Distress Category C since the procedures are not expected to cause more than momentary pain or distress. However, the animals are closely monitored for signs of pain and distress throughout the study. If any animal displays clinical signs of pain and distress, the veterinarians may prescribe analgesics or supportive care. Non-steroidal anti-inflammatory drugs (NSAIDs) are typically avoided due to the anti-inflammatory effects which may mask erythema and/or edema induced by the test articles, resulting in false negatives. Humane endpoints are also used to identify the point in which an animal should be humanely euthanized:

- Weight loss greater than 20% of the initial weight with clinical signs of illness and/or distress.

- Prolonged durations of anorexia, greater than three days with veterinary consultation.
- The presence of health problems refractory to medical intervention.

What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number:

16 CFR § 1500.41

ISO 10993-10