Procedure Type: Diabetes Induction

Procedure Title: Diabetes Induction & Monitoring in Mice
Species: Mouse
Pain/Distress Category: D

Background Information:

Husbandry Requirements:

You must notify the OLAC Facility Supervisor via a Request for Special Services a minimum of 1 week before initiation of use to ensure proper procedures are in place for handling of contaminated disposable cages and bedding (may be present in excreta/bedding). A hazard label indicating the following must be affixed to the Standard Cage Card: hazard type, date of administration, dosage, agent, administration route, and initials of contact person. Cage must be labeled for 72 hours after last dosing AND until contaminated bedding is changed, unless longer time frames are required as identified in the risk assessment during the planning phase. Disposable animal cages should be nested and lidded with bedding left inside and disposed of in yellow trace chemotherapy barrels in the animal facility. All spills and surfaces should be cleaned first with 10% bleach, followed by soap and water; there is no validated method reported for chemical inactivation.

Safe Handling & Disposal:

Personnel working with streptozotocin/alloxan or animals being treated with streptozotocin/alloxan should wear safety eyewear, chemical resistant gloves, and a lab coat + appropriate street clothing (long pants, closed toe shoes); powder should only be handled in a chemical fume hood or other appropriate engineering control. Cage changes should occur only within a chemical fume hood or biosafety cabinet. All spills and surfaces should be cleaned first with 10% bleach, followed by soap and water. NOTE: There is no validated method for chemical inactivation.

An approved SOP for these procedures must be kept on file with EH&S before the start of work. Contact EH&S for safe handling guidelines if using dosages or treating for durations outside what is specified in this procedure, as additional controls may be warranted to avoid potential exposures to personnel or hazardous waste management.

General compound administration guidelines: Doses will comply with ACUC Guidelines for “Dosing Techniques and Limits.” If doses will not comply with ACUC Guidelines, insert variation with justification below in the section: "How does this procedure fit into or address your overall research goals?"

Maintenance of Chemically induced Diabetes:

1. Two to five days after the development of diabetes (as indicated by blood glucose levels) mice will receive an insulin-secreting pellet if further long-term study is necessary.

2. Insulin pellets, releasing approximately 0.1 U /24 hr for >30 days, are implanted subcutaneously (please add eProtocol Procedure describing implantation of these pellets. If making an incision to implant, the pre-filled Procedure "Osmotic Pump Implantation in Mice," may be used, substituting the pump with the insulin pellet).

3. Mice weighing less than 25 grams only require one insulin pellet; larger mice whose blood glucose is not controlled within a normal range (70-150 mg/dL) will receive two insulin pellets. Administer the number of pellets per manufacturer instructions, and include the dose in the Other Agents Utilized Tab.

Procedure Description Tab:

Procedure Description: (select all that apply):

□	Chemical Induction of Diabetes in Mice	**Diabetes Induction in Mice:** This procedure involves chemically inducing diabetes in mice. <u>Procedural Steps:</u> 1. Following the steps outlined below, inject mice (aged 6 weeks or older) IV or IP with alloxan (50-150 mg/kg once or divided into multiple doses over 3-5 days. Dosage may vary with rodent strain and specific model). Alternatively, inject mice IP with streptozotocin (STZ; 150-200 mg/kg once or divided into multiple doses over 3-5 days. Dosage may vary with rodent strain and specific model). *<u>IV Procedural Steps:</u>* a. Restrain the mouse in a rodent restrainer so that the tail vein is accessible. The animal will be monitored throughout the duration of restraint and will be removed if signs of pain or distress are observed. Restraint will be < 5 minutes. b. Stimulate dilation of the tail veins by exposing to direct heat source (e.g., heat lamp, warm water blanket, warm compress) or by placing the tail in warm water (30-35°C) for 5-10 minutes. Use extreme care not to burn or overheat the mouse. c. Prep the venipuncture site with an alcohol swab. d. The injection is made into the lateral tail vein using a 24-27 gauge needle. Gently grasp the tail and insert the needle into the vein, bevel up, keeping the needle and syringe as parallel to the tail as possible, at a distance approximately 1/2-2/3 the way from the base of the tail. e. Once proper placement is confirmed, inject slowly and steadily so as not to inject the solution outside the vein. The vein is very superficial and there should not be any resistance upon injection. If resistance is encountered, remove the needle and reinsert above (proximal to) the first site. f. Once the needle is removed, apply gentle pressure to the site with a gauze pad until bleeding has stopped. g. Return mouse to its cage. *<u>IP Procedural Steps:</u>*

		a. Animals will be restrained manually or placed in a plastic decapicone bag to facilitate restraint. Restraint will be < 5 minutes. b. The injection is made using a 22-27 gauge needle in the left or right lower quadrant of the abdominal cavity, in between the midline and medial side of the hind leg to avoid the liver and the bladder. The needle should be angled 20-30 degrees relative to the animal to avoid penetration of the abdominal organs. Slowly inject solution. d. If bleeding occurs at the injection site, pressure will be applied until hemostasis is achieved. e. Return mouse to its cage. *Note: To reduce discomfort, use a new needle for each animal, bring solutions for injection to 37°C, and avoid injecting material with a high or low pH.* 2. Beginning on day 1 following alloxan or STZ administration, check blood glucose levels daily for 5-7 days (see Blood Glucose Monitoring in Mice procedure below), then monitor weekly thereafter. Record all results in study records. 3. Monitor mice (e.g., Body Condition scoring index, body weight) at least 2 times per week once they develop diabetes. They may/will require more frequent cage changes due to increased urine output. **Potential Adverse Events:** Seizure, ataxia, severe weight loss, dehydration, hypoglycemia, tissue irritation, dermatitis, perforated bowel or bladder, peritonitis, damage to internal organs.

□	Blood Glucose Monitoring in Mice	**Blood Glucose Monitoring in Mice:** This procedure describes the steps for chronic monitoring of glucose using blood samples taken from the chemically-induced diabetic mice. Procedural Steps: 1. Following the steps outlined below, collect approximately 25 µl blood. Note: Preferred blood collection methods include the submandibular, submental, lateral saphenous, or tail vein since the amount of blood required is small and these methods do not require anesthesia. Add the applicable pre-filled blood collection procedure. *Lateral Tail Vein or Tail Artery Procedural Steps:* a. Restrain mouse in rodent restraint apparatus. b. Warm tail to dilate vessels (heat lamp, warm water, or warm compress). c. Moisten venipuncture site with alcohol. d. Using a 25-27g needle on a 0.5 -1cc syringe, insert the needle, bevel facing up into vessel. Gently pull back on the plunger to avoid collapsing the blood vessel. e. Alternatively, puncture the blood vessel with the needle and allow the blood to drip into a microcentrifuge tube or be collected by capillary action into a blood collection tube. f. Remove needle if utilized and apply pressure to puncture site with a gauze pad until bleeding stops. *Facial Vein (Submandibular) Procedural Steps:* a. Scruff mouse by grasping loose skin over the shoulders between thumb and index finger of non-dominant hand. b. Puncture facial vein, located slightly behind the mandible, but in front of the ear canal near the bald spot or "dimple," in a swift, lancing motion with a 4.0-5.5mm lancet or tip of a 19-25g needle; blood will flow immediately if in the correct location. c. Collect sample into a pipette via capillary action or allow blood to drip into a microcentrifuge or blood collection tube. d. Apply pressure with a gauze pad until bleeding stops. **Potential Adverse Events:** Depth of the puncture must be controlled or excessive bleeding, entry into the ear

		canal, entry into the oral cavity, hematoma formation, trauma to the underlying muscles or infection can occur. *Note: Hemostasis may take longer than other methods of blood collection.* *<u>Lateral Saphenous Procedural Steps:</u>* a. Place mouse head first in restraint tube (a 50 ml plastic conical test tube or syringe casing works well). b. Extend hind limb over top edge of the tube, applying gentle pressure above the knee joint or use a small tourniquet to hold off the vessel. c. Apply sterile ophthalmic ointment to allow the blood to pool at the site, and part hair to visualize vessel. d. Puncture vessel with 25g needle in a swift, lancing motion; blood will flow from site and pool on the ointment. e. Collect sample into a pipette via capillary action or allow blood to drop into a microcentrifuge or blood collection tube. f. Release downward pressure on leg and apply gentle pressure to venipuncture site with a gauze pad until bleeding stops. g. Removal of the scab will enable serial sampling. 2. Place a droplet on a glucose test strip; read and record glucometer results. 3. Blood glucose monitoring should be performed daily for the first 7 days following chemical induction of diabetes, then once weekly once insulin pellets are implanted. **Potential Adverse Events:** Excessive bleeding, hematoma formation, tissue trauma, or infection. Submandibular blood collection method only: Depth of the puncture must be controlled or excessive bleeding, entry into the ear canal, entry into the oral cavity, hematoma formation, trauma to the underlying muscles or infection can occur.

□	Urine Glucose Monitoring in Mice	**Urine Glucose Monitoring in Mice:** This procedure describes the steps for chronic monitoring of glucose using urine samples taken from the chemically-induced diabetic mice. <u>Procedural Steps:</u> 1. Place a urine test strip on a clean surface. 2. Restrain the mouse over the urine test strip and gently depress the abdomen until the animal urinates. It should only take 1 drop of urine for the test strip. Alternatively, place the animal in a clean cage without bedding and observe for urine production for several minutes, dipping the test strip in the droplet that is produced. 3. Compare the color change on the test strip to the color index on the test strip container to determine the concentration of urine glucose 4. Urine glucose monitoring can be performed in lieu of, or in addition to, blood glucose monitoring for the first 7 days following chemical induction of diabetes, then once weekly once insulin pellets are implanted. **Potential Adverse Events:** Bladder rupture.

How does this procedure fit into or address your overall research goals? (Insert protocol-specific rationale here.)

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.
Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.
Mice will be examined immediately following diabetes induction, as well as the following day, for the above-mentioned adverse events, as well as general appearance, activity level, appetite, weight loss, and Body Condition Score.
The endpoint for mice will be successful induction of diabetes, followed by a protocol-described duration, at which time mice will be euthanized and various tissues will be harvested for analysis. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?
If the mouse has a glucose level of >400 mg/dl, if the animal is moribund as defined by the ACUC "Guidelines for Humane Endpoints in Animal Studies", if adverse effects are noted the animal will be euthanized. If appears moribund/lethargic, has a BSC < 2, or if adverse effects any other abnormal signs are noted the mouse will be euthanized immediately.

Peri-Procedure Care/Analgesics Tab:

Recovery Location Building Name: (Insert protocol-specific information here.) **Room Number:** (Insert protocol-specific information here.)
Responsible Personnel: (Insert protocol-specific information here.)

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.) Mice will be monitored following diabetes induction for general appearance, activity level, weight loss, or signs of infection.

Monitoring Duration
Mice will be examined immediately following injection(s) as well as the following day, for general appearance and activity level, as well as potential adverse events based on the method of diabetes induction and monitoring (see Procedure Description).

Monitoring Frequency
Mice will be monitored daily for the first week and once a week thereafter to ensure that they are euthanized according to established endpoints.

Describe what actions will be taken if parameters monitored fall outside normal ranges: OLAC veterinary staff will be consulted or the mouse will be euthanized.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened food may be provided on the cage floor.

Describe record keeping/documentation methods for post-procedure monitoring: A record of the compound(s) administered, samples collected, the date, and the animal's ID will be kept in the laboratory notebook.

Other Agents Utilized Tab:

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency, and duration of administration

Streptozotocin	150-200 mg/kg	Intraperitoneal (IP)	Mice will be injected intraperitoneally once or the total dose will be divided into multiple doses over 3-5 days. (Indicate any variations in dosage due to rodent strain and specific model here.)
Alloxan	50-150 mg/kg	Intravenous (IV)	Mice will be injected intravenously once or the total dose will be divided into multiple doses over 3-5 days. (Indicate any variations in dosage due to rodent strain and specific model here.)
Alloxan	50-150 mg/kg	Intraperitoneal (IP)	Mice will be injected intraperitoneally once or the total dose will be divided into multiple doses over 3-5 days. (Indicate any variations in dosage due to rodent strain and specific model here.)
Insulin pellets	Dosage per manufacturer instructions	Subcutaneous (SC)	[Describe the number of times pellets need to be placed depending on the length of the study]

Literature Search for Alternatives:

Suggested Keywords
Diabetes, alloxan, streptozotocin, urine and blood glucose monitoring, mouse, alternatives, refinement

Updated/ACUC approved:
Jan. 2022