

## ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

### STREPTOZOTOCIN USE IN MICE AND RATS

*This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.*

<b>V1 Date of Approval:</b>	4 April 2016
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#### 1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- Blood collection
- Handling and restraint of mice and rats
- Injections

#### 2. SUMMARY

Streptozotocin is a chemical that can be administered to rodents for inducing diabetes mellitus by destroying insulin producing beta cells of the pancreas. Rodents are commonly used as a model to study aspects of diabetes relating to human health. Appropriate use of streptozotocin is important for ensuring optimal animal health and for preventing human health risks.

#### 3. BENEFITS & RISKS

##### 3.1 Benefits

- 3.1.1 Streptozotocin can avoid some of the limitations associated with the alternative method of genotype related spontaneous diabetes such as unpredictable disease onset.
- 3.1.2 Streptozotocin has a rapid induction

##### 3.2 Risks

- 3.2.1 There are known human safety risks associated with the use of streptozotocin.
- 3.2.2 Diabetes and multi-organ streptozotocin toxicity can adversely affect the wellbeing of rodents and potentially cause death.

#### 4. PROCEDURE/PROTOCOL

- 4.1 Streptozotocin is usually prepared with tri-sodium citrate and should be kept at an appropriate temperature prior to preparation. Streptozotocin should be used within 5 minutes of preparation and excess discarded due to the potential to degrade.
- 4.2 Streptozotocin should be administered at least 7 days prior to the study, including administration of insulin, to allow blood glucose stabilisation.

- 4.3 Streptozotocin may cause toxicity to the liver, kidney, lung, intestines, testes and brain so caution is advised where pharmaceuticals or genotypes may compound impacts on these organs.
- 4.4 Streptozotocin may be given to mice as a single high dose (100-200mg/kg) or multiple low doses (20-40mg/kg daily for 5 days). Fasting of mice prior to streptozotocin injection must not exceed 6 hours duration and must not be done overnight. A single dose of approximately 50mg/kg has been sufficient for several studies involving rats. Justification for the streptozotocin doses proposed for the species and strain must be provided in the AEC application.
- 4.5 Younger and smaller animals may experience more severe diabetes and therefore streptozotocin should not be administered to animals less than 6 weeks of age, 20g weight for mice, and 100g weight for rats.
- 4.6 Rats may experience hypoglycaemia within the 48 hours after streptozotocin administration, which has the potential to cause death or severe illness. Sucrose (15g/L) must be provided during the first 48 hours for rats and blood glucose performed daily.
- 4.7 Hyperglycaemia often occurs within the first 2-5 days of administration of high doses of streptozotocin.
- 4.8 Intraperitoneal or intravenous administration routes are usually employed. Intraperitoneal administration can be inconsistent due to accidental delivery into organs. Intravenous administration may be more difficult for multiple low doses due to thrombus formation or inflammation at the injection site. The pH of streptozotocin should be tested and if it is not close to 7.0, intravenous administration may be preferred over intraperitoneal.
- 4.9 Weight loss, diarrhoea and death can occur in the first five days after administration. Polyuria and polydipsia may also occur after day 5 with further deaths possible. Investigators should indicate in their animal ethics application the likely animal numbers that will be excluded from the study due to humane end points. More frequent enclosure and water bottle changing is required to prevent dehydration and maintain hygiene. A uniquely coloured cage card must be placed on the enclosures to alert personnel of increased monitoring requirements. Water and bedding must be checked at least daily. Reduction in stocking density can assist in maintaining cleaner enclosures and sufficient levels of water.
- 4.10 Where insulin is used, the AEC application must include the dose, frequency, route of administration, and storage.
- 4.11 Consultation should be made with the relevant Occupational Health & Safety contact with regard to the use of streptozotocin and compliance with any regulations.
- 4.12 Alloxan is an alternative to streptozotocin, however, due to the fact that toxicity occurs slightly above effective doses of this drug, it is not the drug of choice for this procedure. The minimum effective dose should be used and determined based on existing evidence relating to the sex, strain, age, diet, and health status of the animal. A pilot trial is recommended for use in novel conditions.

## 5. MONITORING & INTERVENTION

- 5.1 Body weight must be measured prior to administration of streptozotocin.
- 5.2 Blood glucose and weight must be measured at least three times a week after streptozotocin administration. Where animals are of an age where they are still growing, body condition scoring must be used in addition to weight.
- 5.3 In addition to the above, monitoring criteria should include: diarrhoea, skin tenting, sunken eyes, water consumption, urine soaked bedding, ketoacidosis and the criteria listed in the OREI Sample Monitoring Sheet.
- 5.4 Blood glucose of rats must be measured daily for the first two days after streptozotocin administration.
- 5.5 Blood glucose should be measured using a drop of blood on a glucometer.
- 5.6 Mice and rats that have a blood glucose of lower than 70mg/dl (4mmol/L) are susceptible to neurological symptoms such as coma. These animals must either be humanely killed or administered a glucose solution. Where glucose is administered these animals must be monitored every 15 minutes and blood glucose taken every hour. The Animal Facility Manager/Animal Welfare Officer should also be notified immediately if treatment is commencing for animals with a low blood glucose. Animals must be humanely killed immediately if deterioration is noticed, if the animal is minimally responsive to provocation, or if no improvement is noticed within 60 minutes.
- 5.7 Mice and rats that have a blood glucose of greater than 400mg/dl (22mmol/L) are at risk of further morbidity and death. These animals must either be humanely killed or treated (eg. administered insulin), monitored twice daily and weighed daily.
- 5.8 Where insulin is used, blood glucose and monitoring must be done daily for at least two days after commencement

## 6. ADDITIONAL INFORMATION

- OREI Sample Monitoring Sheet: <http://www.orei.unimelb.edu.au/content/forms-templates-guidance-documents>

## 7. ENFORCEABLE REQUIREMENTS

- 7.1 Adherence to doses above.
- 7.2 Identification in the animal ethics application of the likely number of animals that will be excluded from the study due to (a) humane endpoint criteria and (b) failure to develop or maintain a diabetic state.
- 7.3 Adherence to monitoring described above.

## 8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

## 9. UNEXPECTED ADVERSE INCIDENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

## 10. GLOSSARY

Scientific Term	Lay Description
Diabetes mellitus	High blood sugar due to insufficient insulin or failure of bodies cells to respond to insulin.
Hyperglycaemia	High blood glucose levels
Hypoglycaemia	Low blood glucose levels
Polyuria	Increased urination
Polydipsia	Increased drinking
Morbidity	Disease
Mortality	Death

## 11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Deeds, MC., Anderson, JM., Armstrong, AS., Gasineau, DA., Hiddinga, HJ., Jahangir, A., Eberhardt, NL., Kudva, YC. 2011. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. *Laboratory Animals* 45: 131-140
- Tesch, GH., Allen, TJ. 2007. Rodent models of streptozotocin-induced diabetic nephropathy. *Methods in Renal Research* 12: 261-266