

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Streptozotocin (STZ) in Animals

1. Health hazards	<p><i>Streptozotocin</i> is an antibiotic that is produced by <i>Streptomyces achromogenes</i>, it is a toxic and carcinogenic material.</p> <p>Streptozotocin (STZ) is commonly used to induce experimental diabetes in animals. It specifically targets beta cells, entering via the glucose transporter GLUT2 and causing alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, depletion of cellular NAD⁺ and ATP, and formation of superoxide radicals, leading to the destruction of beta cells.</p> <p>Streptozotocin is non-volatile and thus primarily represents a risk in its crystalline or aerosolized forms.</p>
2. Designated Area	ABSL-2 facility.
3. Training	Hazardous chemical training and training on this SOP is required before working with STZ. This should include but is not limited to reviewing the MSDS, training on the physical hazards of the chemicals, symptoms of exposure, appropriate work practices, and proper use of PPE.
4. Personal Protective Equipment (PPE)	<p>Chemically-resistant gloves or double nitrile gloves, Chemical safety goggles, Lab coat and mask. Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat. Personnel should not work with STZ if skin is cut or scratched.</p> <p>Hands must be washed upon changing gloves and upon exiting animal room.</p> <p>**Women who are pregnant, breast feeding, or planning to get pregnant should not be exposed to, or handle this chemical in any form.</p>
5. General . Precautions for Animal Use	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p> <p>Care should be taken to avoid generating aerosol during the preparation or injection procedure.</p>

	<p>STZ may be excreted by the animals within the first 48 hours post injection, therefore the lab must change the bedding 48 hours after administration.</p> <p><i>Animals injected with streptozotocin will become diabetic and produce increased amounts of urine. They will need more frequent cage changing to maintain sanitary cage conditions.</i></p>
6. Environmental / Ventilation Controls	<p>The preparation of STZ including reconstitution, weighing, and diluting should be performed in a fume hood or biological safety cabinet (class II Type B). Work should be done over absorbent pads.</p> <p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p>
6. Special Handling Procedures & Storage Requirements	<p>Handling:</p> <p>STZ should be handled in containment and done over absorbent pads. Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity, Leurlock syringes are recommended). The fume hood or other approved containment must be cleaned upon completion of tasks.</p> <p>When transporting STZ, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers.</p> <p>All equipment must be decontaminated prior to removal from the room housing the infected animals.</p>
7. Precautions for Animal Use	<p>No recapping needles. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection. Once STZ is injected, animals, animal waste and cages are considered hazardous for a minimum of 48 hours.</p> <p>Hands must be washed upon exiting animal room.</p>
8. Animal handling practices	<ol style="list-style-type: none"> 1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done only by the researchers. 2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages. 3. Injecting animals with STZ: Animals will be injected IP with STZ within Class II Biosafety cabinet or designated chemical fume hood. <p>All needles will be disposed of in sharps container – do not recap or bend needles.</p> <ol style="list-style-type: none"> 4. Infected animals considered hazardous for a minimum of 24 hours after each administration of STZ; take precautions to avoid the creation of aerosols when changing

or washing cages, or cleaning the room.

A respirator is recommended for personnel that are immunocompromised or pregnant and for healthy personnel if work is done outside the ventilated cabinet.

5. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time.

6. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration.

7. All surfaces and racks that may be contaminated will be decontaminated with detergent solution followed by water ASAP.

8. The first cage change after each drug administration is to be done no sooner than 24 hours after the administration. The bedding is considered contaminated and requires special handling.

**All bedding from the first 24 hours post-STZ injection should be handled using procedures that minimize the creation of dust and aerosols, and bedding should be changed in a fume hood or a specially designed animal bedding disposal cabinet.

When changing cages, use the following technique:

- Transfer the animals to clean cages .
- Insert the used cages in a plastic bag .
- Twist the ends of full bags, and seal with tape. Label with wide tape or other Type, of label marked “**toxin- Streptozotocin**”.
- Transport the bags of cages to a HEPA filtered dumping station that draws air away from the use, (**it is recommended to use a mask**) or fume hood.
- If local ventilation controls are not available for opening cages or dumping Bedding, an N-99 respirator and safety goggles must be worn.
- All contaminated bedding will be labeled as hazardous materials and handled accordingly :
incinerated or placed in chemical waste bags for disposal.
- After this first cage change there is no need for further special precautions to be taken regarding the animals or the cages as long as the animals have not received any more STZ.
- The cages should then be put in plastic bags (marked “toxin- Streptozotocin”) and sealed for transport to the washroom.

	<ul style="list-style-type: none"> In the washroom ,cages should be unloaded from the bags with the appropriate PPE as mentioned above and run through the cage wash in the conventional manner. Note- cage wash personnel should take extra precautions (additional PPE) when handling cages that may have STZ contamination.
<p>9. Spill and Accident Procedures</p>	<p><u>Incompatible materials :</u></p> <p>Strong oxidizing agents, Strong acids, Strong bases.</p> <ol style="list-style-type: none"> Spills must be cleaned immediately by properly protected trained personnel. Minor Liquid Spills: should be cleaned immediately by personnel wearing a gown, goggles,two pairs of gloves (nitrile). Use absorbent pads to wipe liquid. The spill area should then be cleaned thoroughly with a detergent solution followed by clean water. Place waste in plastic bag and then in the chemical waste container. Powder/Major Spills: should be cleaned immediately by personnel wearing a gown, goggles, chemically-resistant gloves and respirator, with a damp paper towel. For powder or major liquid spills outside of a fume hood or approved containment, personnel should be instructed to leave the laboratory and entrance should be restricted for at least 30 min. Contain or absorb spill with absorbent pad or vermiculite. Collect and place waste in plastic bag and then in the chemical waste container. The spill area should then be cleaned thoroughly with a detergent solution followed by clean water- prevent runoff into drains. Place waste in a plastic bag and then in the chemical waste container. Prevent, by all means available, spillage from entering drains. <p><u>Exposure:</u></p> <ol style="list-style-type: none"> In case of skin contact or injection with STZ, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center, Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.
<p>10. Waste Disposal</p>	<p>Dispose all waste material in the appropriate chemical waste container.</p> <p>Unused solutions of STZ , solid waste ,any empty containers that held STZ will be</p>

	<p>disposed of as hazardous chemical material.</p> <p>Glassware and other non-porous materials can be decontaminated by soaking in bleach solution for 24 hours.</p> <p>Needles/syringes should be disposed in a chemical waste sharps container. Label the container as "toxin- Streptozotocin" .</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with STZ in Animals, and agree to follow these procedures.</p>	
Name:	Title:
Signature:	Date:

Dr. Esther Michael – Biosafety Manager

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