

August 18

SOP # 005 for *Streptozotocin* (STZ)

Standard Operating Procedure for STZ in Animals	
1. Health Hazards	<p>Streptozotocin (STZ) is a highly hazardous substance, suspected carcinogen, mutagen and teratogen that is harmful to the following organs: blood, kidneys, nervous system, liver, digestive system, skin, eyes, bone marrow, muscle tissue and pancreas.</p> <p>STZ is used to produce an animal model of Type I Diabetes. 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>Pregnant or breast-feeding women should not work with STZ</p>
2. Physical & Chemical Properties/Definition of Chemical Group	STZ is non-volatile and thus only represents a risk in its crystalline and solubilized forms. STZ and its metabolites are primarily excreted in the urine and to a much lesser extent in feces
3. Designated Area	ABSL2 Facility (Animal Biosafety Level 2)
4. Training Requirements	Hazardous chemical training and training on this SOP is required before working with STZ. This should include but is not limited to reviewing the SDS, training on the physical hazards of the chemicals, symptoms of exposure, appropriate work practices, and proper use of PPE.
5. Personal Protective Equipment (PPE)	Double nitrile gloves or compatible chemical-resistant 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
6. General Precautions for Animal Use	Tools should be adapted for BSL-2 (use safety syringe, blades and needles where possible). Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection. STZ may be excreted by the animals within the first 24 hours post injection therefore <u>only the lab staff</u> must change the bedding at least 24 hours after the last administration.
7. Environmental /Ventilation Controls	A. The preparation of STZ including reconstitution, weighing, and diluting should be performed in a fume hood or Class II Type B Biological Safety Cabinet (BSC) (total exhaust cabinet) . Work should be done over absorbent pads. B. Work with animals should be conducted in ABSL-2 facility , over absorbent pads in a Class II type A2 BSC.
8. Special Handling Procedures & Storage Requirements	A. 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). B. The fume hood or other approved containment must be cleaned and decontaminated upon completion of tasks. C. When transporting STZ, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers. D. All equipment must be decontaminated prior to removal from the room housing the infected animals.
9. Precautions for Animal Use	A. Animals should be restrained or anesthetized during injection. B. NO recapping needles. C. Have a sharps container in close vicinity. D. Once STZ is injected, animals, animal waste and cages are considered hazardous for a minimum 24 hours. E. Hands must be washed upon exiting animal room

<p>E. Animal Handling Practices</p>	<p>A. Animals must be housed in filter top cages marked as biohazards (including the name of the chemical hazard, STZ), in negative pressurized IVC.</p> <p>B. Handling the cages (including bedding) will be done only by the researchers at least for 1 day after the last administration and first cage change.</p> <p>C. Use a class II A2 BSC 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.</p> <p>D. <u>Injection animals with STZ:</u></p> <ol style="list-style-type: none">1. Injection of STZ will be conducted within BSC or certified Chemical fume hood2. All needles will be disposed of in sharps container - <i>do not recap or bend needles.</i>3. Injected animals considered hazardous for a minimum of 24 hours after each administration of STZ.4. Take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room5. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time. <i>A respirator is recommended for personnel that are immunocompromised or pregnant and for healthy personnel if work is done outside the ventilated cabinet.</i>6. Dead animals must be placed in primary plastic bags, and then will be transferred to 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 STZ administration is to be done no sooner than 24 hours after the administration. The bedding is considered contaminated and requires special handling <p><u>When changing cages use the following technique:</u></p> <ul style="list-style-type: none">• Transfer the animals to clean cages• Insert the used cages in a plastic bag.
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	<ul style="list-style-type: none"> • Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked <u>"Hazardous Chemical - Streptozotocin "</u>. • Transport the bags of cages to a HEPA filtered dumping station that draws air away from the user (it is recommended to use a mask or fume hood). • If local ventilation controls are not available for opening cages or dumping bedding, a 3M8835 respirator and safety goggles must be worn. • All contaminated bedding will be labeled as hazardous materials and handled accordingly: • <u>incinerated or placed in chemical waste bags for disposal.</u> • 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 "<u>Toxin - Streptozotocin</u>") and sealed for transport to the washroom. • 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 that meet the criteria for extra precautions above (pregnant exc.) should take extra precautions (additional PPE) when handling cages that may have STZ contamination.
<p>F. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> 1. Incompatible materials : Strong oxidizing agents, Strong acids, Strong bases. 2. Spills must be cleaned immediately by properly protected trained personnel. 3. <u>Minor Liquid Spills:</u> should be cleaned by personnel wearing a gown, goggles and 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

	<p>clean water. Place waste in plastic bag and then in the chemical waste container.</p> <p>4. <u>Powder/Major Spills:</u> should be cleaned by personnel wearing a gown, goggles, and chemically resistant gloves. 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. In addition to the above specified PPE, a respirator and safety goggles, should also be worn. Contain or absorb spill with 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. <u>Prevent, by all means available, spillage from entering drains.</u></p> <p>5. <u>Exposure:</u></p> <ul style="list-style-type: none"> • <i>In case of skin contact</i> or injection with STZ, wash the affected area with soap and water for at least 15 minutes. Consult with a Medical doctor in an Emergency Room (ER) • <i>For eye exposure</i>, flush with water for at least 15 minutes. <p>In any case</p> <ul style="list-style-type: none"> • Consult with Medical doctor in ER • Report incident to supervisor • Report the accident/injury to the Safety Unit Tel: 2146/7.
<p>G. Waste Disposal</p>	<p>Dispose all waste material in the appropriate chemical waste container. Unused solutions of STZ and contaminated solid waste will be disposed of as hazardous chemical material.</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>	
<p>Name: _____</p>	<p>Title: _____</p>