Virginia Commonwealth University – Office of Environmental Health & Safety

STREPTOZOTOCIN:
SAFE WORKING PRACTICES INFORMATION PAGE

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BACKGROUND

Streptozotocin (Streptozocin, STZ, Zanosar®, CAS No. 18883-66-44) is a naturally occurring compound, produced by the bacterium *Streptomyces achromogenes*, that exhibits broad spectrum antibacterial properties (Vavra et al., 1959). Antibacterial therapy, however, is not currently a widely used application for STZ (NTP, 2005). Primary contemporary uses for STZ include treatment of metastasizing pancreatic islet cell tumors, malignant carcinoid tumors, and as an investigational drug for diabetes research due to its specific toxicity associated with pancreatic β-cells (NTP, 2005). Streptozotocin is a mixture of α- and β-stereoisomers that appear as a pale yellow or off-white crystalline powder. STZ is very soluble in water, ketones, and lower alcohols and only slightly soluble in polar organic solvents (NTP, 2005). Streptozotocin functions as a DNA synthesis inhibitor in both bacterial and mammalian cells (Bolzan and Bianchi, 2002). In bacterial cells, a specific interaction with cytosine moieties leads to the degradation of the bacterial DNA (Reusser, 1971). In mammalian cells, the mechanism of action that results in cell death has not been fully identified, but is thought to be a result of DNA and chromosomal damage brought forth by mechanisms involving free radical generation during STZ metabolism (Bolzan and Bianchi, 2002). In many animal species, STZ induces diabetes that resembles human hyperglycemic nonketotic diabetes mellitus (Weir et al., 1981). This effect has been extensively studied and appears to be mediated through a lowering of beta cell nicotinamide adenine dinucleotide (NAD^+) and results in histopathologic alteration of pancreatic islet beta cells (Karunanayake et al., 1974). When administered intravenously, plasma levels of STZ rapidly decrease within 15 minutes and concentrate in the liver and kidneys (Sicor Pharmaceuticals, 2003). As much as twenty percent of the drug (or metabolites containing an N-nitrosourea group) is metabolized and/or excreted by the kidneys (Sicor pharmaceuticals, 2003). The interaction with DNA and STZ's ability to produce cytotoxic effects in animals makes exposure to STZ a significant health and safety threat to laboratory staff, animal handlers, and other personnel who may be subject to accidental exposure. Due to this health and safety threat, the Institutional Biosafety Committee (IBC) has classified STZ as a reportable hazardous chemical that must be reported on Institutional Animal Care and Use Committee (IACUC) protocols.

PURPOSE

The purpose of this page is to provide the research community sufficient information regarding specific health effects, exposure routes, implementation of proper work methods, and provision of suitable personal protective equipment for development of research protocols that include measures for effectively reducing risk of occupational exposure to STZ.

OCCUPATIONAL EXPOSURE HAZARDS

Primary routes of occupational exposure to STZ include: inhalation, ingestion, accidental injection, and dermal absorption (Calbiochem, 2005). Industrial hygiene and epidemiological case studies involving STZ are limited; however, the available scientific literature indicates that chronic long-term exposure to STZ could lead to a number of potentially serious health effects:

1. **Carcinogenicity:** Streptozotocin is "*reasonably anticipated to be a human carcinogen*" based on sufficient evidence of carcinogenicity in experimental animals (IARC, 1978, 1982). When administered intravenously, STZ has been shown to induce tumors in rat kidney (Arison et al., 1967), liver and pancreas (Rakieten et al., 1968; Rakieten et al., 1971; Rakieten et al., 1976; Iwase et al., 1989), and liver tumors in hamsters (Sibay and Hayes, 1969). Streptozotocin administered by intraperitoneal injection induces lung, kidney, pancreatic, liver, and uterine tumors in laboratory animals (Weisburger et al., 1975; Berman et al., 1973). Streptozotocin caused neoplastic transformation in primary human kidney cells at doses of 1mM (Robbiano et al., 1996). Although data regarding the carcinogenic effect of STZ in humans is currently not available, the International Agency for Research on Cancer (IARC) emphasizes that: "*STZ should be regarded for practical purposes as if it were carcinogenic to humans"* (IARC, 1978). Streptozotocin is classified by IARC in group 2B, possibly carcinogenic to humans (IARC, 1978).

2. **Cytotoxicity/Genotoxicity:** Streptozotocin is cytotoxic to pancreatic β-cells (Beppu et al, 1987) and oxyntic mucosal cells of the stomach (Brenna et al., 2003) and effects can be seen within one hour after administration (Junod et al., 1967). Cytotoxic effects of STZ are dependent upon DNA alkylation by site specific action with DNA bases (Tjalve, 1983; Bennett and Pegg, 1981; Randerath et al., 1981) and by free-radical generation during STZ metabolism (Bolzan and Bianchi, 2002). Types of DNA lesions formed by STZ include monoadducts, single and double stranded breaks, and alkali-labile sites. Severe DNA damage by STZ results in cell death by apoptosis or necrosis (Bolzan and Bianchi, 2002).

3. **Teratogenicity:** Reproductive studies reveal that STZ is teratogenic in laboratory animals. Embryonic and neonatal developmental delays have been associated with STZ use that are irreversible by insulin administration (Diamond et al., 1989). Streptozotocin is rapidly absorbed into fetal circulation after intravenous administration (Sicor Pharmaceuticals, 2003), thus causing a decrease in insulin stores and mild hyperglycemia in male progeny of laboratory rodents (Serradas et al., 1989).

4. **Reproductive Toxicity:** Streptozotocin adversely affects fertility when administered to male and female rats. In males, the toxic effects on fertility were manifested through disruption of testicular function (Schein and Winokur, 1975) caused by a decrease in testosterone levels resulting in denervation-like supersensitivity (Longhurst et al., 1989). In females, ovarian disruption (Schein and Winokur, 1975) is attributed to a decrease in viable oocyte and delayed oocyte maturation (Diamond et al., 1989).

SAFE WORK METHODS

The list of potential STZ-related health hazards identified above necessitates that principal investigators conduct thorough risk assessments and prepare protocols which include measures for minimizing staff exposure potential. To date, governmental regulatory agencies have not established exposure limits for STZ. In lieu of the availability of regulatory guidance, the prudent course for principal investigators to follow is to either eliminate or reduce exposure potential as much as feasible through implementation of the safe work methods listed below.

1. Administrative Controls.

a. Management considerations for STZ and other potentially hazardous chemicals must be included in the laboratory Chemical Hygiene Plan.

b. Protocols involving the *in vivo* use of STZ must include completion of IACUC Hazardous Chemical Information Page and approval through the Institutional Animal Care and Use Committee.

c. Principal investigators will develop and implement standard operating procedures (SOPs) by which laboratory staff will prepare/administer STZ with minimal potential for exposure.

d. All tasks having potential for occupational STZ exposure (mixing of doses, dose preparation, administering of injections, etc.) will only be conducted by competent staff who have received appropriate training (OSHA: "Worker Right to Know") regarding the specific STZ-related health and safety risks, SOPs, and procedures to be followed in event of an exposure incident.

e. Laboratory personnel using STZ in any of the procedures noted above are also required to complete applicable modules of the VCU Laboratory Safety Training Modules.

f. Laboratory personnel must be instructed to use extreme caution when performing injections involving STZ since accidental needle stick presents an exposure threat.

g. Exposures involving STZ or any other acutely hazardous material should be reported to Employee Health as soon as possible.

2. Personal Protective Equipment. Streptozotocin exposure may often be attributable to the wearing of inadequate PPE. Staff involved in any tasks where potential for STZ exposure exists must don the following PPE:

a. Examination gloves: Use powder-free latex, nitrile, or rubber examination gloves which cover hands and wrists completely through overlapping sleeve of lab coat when working with STZ. Wearing of two sets of gloves ("double gloving") is advised whenever performing tasks involving STZ and other hazardous/antineoplastic drugs. Laboratory personnel should thoroughly wash hands with soap and water before and immediately upon removal of examination gloves.

b. Safety glasses or safety goggles (ANSI Z-87 approved) are considered the minimum appropriate level of eye protection. The IBC recommends donning of full-face shield when conducting tasks posing potential for any generation of aerosol or droplets.

c. Lab coats or disposable coveralls that provide complete coverage of skin not otherwise protected by PPE and/or attire. Laboratory personnel whose clothing has been contaminated by Streptotozocin should change into clean clothing promptly. Do not take contaminated work clothes home – contaminated clothing should be disposed of as regulated medical waste (RMW).
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d. Appropriate laboratory attire: laboratory personnel handling STZ should don attire which when worn in combination with lab coat and other PPE provides entire coverage of the body. Short pants/dresses and open-toed shoes are not appropriate laboratory attire.

e. If an aerosol exposure threat exists, all procedures should be conducted in an approved chemical fume hood whenever possible (see Engineering Controls below). If an approved chemical fume hood cannot be utilized, an appropriate air-purifying respirator must be utilized for all procedures where exposure potential is present. A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use. Prior to instituting respiratory protection to personnel, the laboratory must participate in the university Respiratory Protection Program.

3. Work Practices:

a. Procedures with the potential for producing STZ aerosols should be conducted within an approved chemical fume hood whenever possible.

b. Needles used for STZ injection will be disposed of in approved sharps containers immediately following use.

c. Needles used for STZ injection should never be bent, sheared, or recapped. If recapping is absolutely necessary, a "Needle Recapping Waiver" must be submitted for IBC review/approval prior to proceeding.

d. Bench paper utilized during preparation of STZ stock should be lined with an impervious backing to limit potential for contamination of work surfaces in the event of the occurrence of minor spills.

e. Areas where STZ is prepared and/or administered should be cleaned and decontaminated immediately following each task. Bench tops, BSC interiors, equipment, and laboratory surfaces with potential for STZ contamination should be routinely cleaned with bleach water (20% bleach), sulfamic acid solution, or other suitable deactivating agent: prepare fresh stock only as needed.

f. Do not eat, smoke, or drink where STZ is handled, processed, or stored, since exposure may occur via ingestion. Wash hands carefully before eating, drinking, applying cosmetics, smoking, or using the toilet.

4. Engineering Controls:

a. Use of chemical fume hood is recommended for all tasks with potential of aerosolizing STZ. In all cases where engineering controls alone do not sufficiently reduce exposure potential, provision of appropriate PPE for suitably minimizing hazard will be required.

b. Syringes used for STZ injection must be safety engineered (self-sheathing syringes, luer-lock syringes, etc.). Exceptions will be considered by the IBC on a case-by-case basis.

c. Animals should be appropriately restrained and/or sedated prior to administering injections and other dosing methods.

d. Laboratories and other spaces where handling of STZ occurs must be equipped with an eyewash station that meets American National Standards Institute (ANSI) and OSHA requirements.

5. Waste Disposal:

a. Streptozotocin is a RCRA-listed hazardous material, surplus stocks and other waste materials containing greater than trace contamination (> 3%) must be disposed of through the university hazardous waste disposal program.

b. The available scientific literature indicates that STZ and its metabolites are primarily excreted in urine and to a much lesser extent in feces. The drug undergoes rapid renal clearance within 48 hours after an acute administration. (Karunanyake et al., 1974). The metabolism and potential risks associated with STZ use require that all potentially contaminated carcasses, bedding, and other materials be disposed of as Regulated Medical Waste (RMW) through incineration.

c. All contaminated sharps waste materials must be placed in proper sharps container and disposed of as RMW.

6. Spills: Laboratory personnel must don appropriate PPE prior to attempting to manage any spill involving hazardous drugs/antineoplastic agents. University policy for addressing spills involving STZ is provided below:

a. Small spills (typically involving less than 5 mg of material) of STZ powder should be wet-wiped with cloth/gauze that is dampened with soapy water. Effected surfaces should be thoroughly wet-wiped three times over – with clean damp cloth used for each wipe down. Following completion all cloth and other materials utilized during spill clean-up with potential for STZ contamination must be disposed of as RMW.

b. Small spills (typically involving less 5 ml of material) of liquid STZ should be covered/absorbed with absorbent material. Areas affected by liquid spills should be triple cleaned with soap and water following removal of absorbent paper.

c. For larger spills of STZ, contact the OEHS emergency line (828-9834) for assistance.

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