McGill

STANDARD OPERATING PROCEDURE #711

USE OF TNBS IN RODENTS

1. PURPOSE

This Standard Operating Procedure (SOP) describes the guidelines for the use of 2,4,6-trinitro benzene sulfonic acid (TNBS) in rodents.

2. CONSIDERATIONS

TNBS is an extremely irritating and toxic chemical used to induce a rodent model of intestinal inflammation. TNBS is extremely destructive to the tissue of the mucous membranes and upper respiratory tract. It is toxic if swallowed and is harmful if absorbed through skin. TNBS causes skin and eye burns. It is extremely flammable.

This SOP aims to ensure that the potential of exposure is reduced as much as possible and that these agents pose no risk to research staff, animal care personnel, and other personnel working in the animal facility.

To minimize the risk of exposure, the Principal Investigator and/or delegate(s) must identify all points of hazard and put in place safe work practices for all steps involving contact with the hazardous chemicals and drugs, as per procedures presented in this SOP and in consultation with the McGill Environmental Health and Safety (EHS) officer.

3. RESPONSIBILITY

Principal investigator (PI) and their research staff, animal care staff, veterinary care staff.

4. MATERIALS

4.1. Personal protective equipment (PPE):
- 4.1.1. Safety glasses or goggles
- 4.1.2. Nitrile gloves
- 4.1.3. Disposable gown and sleeves

4.2. Chemical fume hood or Type II B2 Biological Safety Cabinet

4.3. Absorbent pads

4.4. Disinfectant solution (e.g., accelerated hydrogen peroxide, bleach)

4.5. Compressed cotton fiber bedding pads (iso-PADS® Enrichment Bedding)

4.6. Disposal bags or containers

5. PROCEDURES

5.1. Prior Requirements:
- 5.1.1. Use of TNBS must be described in the Facility Animal Care Committee (FACC) approved Animal Use Protocol (AUP). The MSDS must be attached to the AUP.
- 5.1.2. The following information must be provided in the AUP (Hazardous Material Information section):
  - 5.1.2.1. Name of the hazardous agent
  - 5.1.2.2. Room(s) where the chemicals/drugs will be prepared
  - 5.1.2.3. Room(s) where the chemicals/drugs will be administered to animals
  - 5.1.2.4. Route(s) of administration
  - 5.1.2.5. Once metabolized, specify if substance is still considered hazardous
  - 5.1.2.6. Specify for how long the animals and cages will be considered hazardous
  - 5.1.2.7. Specify how the substance is excreted (urine, feces, exhaled, dermal)
  - 5.1.2.8. Describe how bedding and syringes will be disposed of
  - 5.1.2.9. Describe special caging requirements

5.2. General precautions:

5.2.1. Pregnant or breast-feeding women should not work with TNBS.

5.2.2. The following personal protective equipment must be worn at all times when handling TNBS (in addition to the personal protective requirements of the animal room):

5.2.2.1. 2 pairs of nitrile gloves

5.2.2.2. Disposable gown

5.2.2.3. Safety glasses or goggles

5.2.3. PPE should be discarded as hazardous materials.

5.2.4. Any handling, including weighing of powder, preparation of dilutions, injection in rodents and any procedure with the potential of producing aerosols, must be conducted in a certified chemical fume hood or in a Type II B2 Biological Safety Cabinet (BSC).

5.2.5. All containers of TNBS must be clearly labeled and adequately stored when not in use.

5.2.6. TNBS must be transported in unbreakable containers.

5.2.7. Work areas should be protected from spills by placing an absorbent pad with an impervious backing (absorbent material facing up). The absorbent pad is disposed of as a hazardous material.

5.2.8. Areas where TNBS is prepared and/or administered must be cleaned and decontaminated immediately following each procedure.

5.2.9. Needles and sharps used with TNBS must be disposed of immediately in a sharps container. Do not bend or recap needles. Safety needles should be used whenever possible.

5.2.10. Thoroughly wash hands after handling or administering TNBS.

5.2.11. In the event of accidental exposure, promptly complete a McGill University Accident, Incident & Occupational Disease Report form: https://www.mcgill.ca/ehs/forms/forms/accident-and-incident-report

5.3. Storage precautions:

5.3.1. TNBS is light sensitive and labile; it should be kept in the dark at 4◦C .

5.3.2. Keep containers tightly closed, preferably in a locked cabinet or explosion proof refrigerator/freezer.

5.3.3. Keep container in a cool, well-ventilated area away from sources of ignition. Keep container closed to prevent drying out as TNBS may be explosive if dry. Do not use material which has been stored beyond recommended shelf-life.

5.3.4. Store away from oxidizers.

5.3.5. Dispose of empty containers through the Waste Management department.

5.4. Animal Handling and Husbandry:

5.4.1. Research staff must inform the animal facility supervisor at least 48 hours before administering TNBS to animals. This will ensure adequate preparation and availability of necessary equipment provided by the animal facility (e.g., disposal container, PPE, bedding pads).

5.4.2. Clearly indicate the hazard on the room door.

5.4.3. All animal handling must be conducted in a certified chemical fume hood or in a Type II B2 Biological Safety Cabinet (BSC).

5.4.4. Consider using compressed cotton fiber bedding pads (iso-PADS®) instead of standard bedding. The pads are very absorbent, will minimize the creation of aerosols and are easier to dispose of.

5.4.5. All cages housing animals that have been treated with TNBS must be clearly labeled with the following information:

5.4.5.1. “TNBS”

5.4.5.2. Date of administration

5.4.6. Animal cages should not be changed for a minimum of three days after the date of TNBS administration.

5.4.7. Cage bedding is considered contaminated until at least 7 days after TNBS is no longer administered and must be disposed of in the following manner:

5.4.7.1. Handle all cages in a certified chemical fume hood or Type II B2 BSC.

5.4.7.2. Place the dirty bedding or bedding pad in a biohazard bag inside the chemical fume hood or BSC. Close the biohazard bag and place it in a second biohazard bag.

5.4.7.3. Rinse the cage with disinfectant solution and paper towels inside the chemical fume hood or BSC.

5.4.7.4. Place used paper towels in a biohazard bag inside the chemical fume hood or BSC.

5.4.7.5. Place the biohazard bags in a biohazard box, tape closed and send boxes to incineration (not autoclaving).

5.4.7.6. Place clean autoclaved bedding or bedding pads in the cages (without sending them to the cage wash).

5.4.7.7. Repeat steps 5.3.7.1 to 5.3.7.6 until 7 days after the hazardous agent is no longer administered.

5.4.7.8. Then wash the dirty cages by hand as in step 5.3.7.3, stack them in a biohazard bag and bring to cage wash area.

5.4.7.9. Wearing mask, gown and nitrile gloves, open the bags and place the cages on the tunnel washer conveyor to be washed (no need to autoclave first).

5.5. Waste disposal:

5.5.1. All items contaminated or potentially contaminated with TNBS (e.g., needles, gloves, bedding, paper towels) must be discarded in a biohazard bag and/or box and incinerated.

5.5.2. Dead animals must be double-bagged before disposal.

5.5.3. Containers are incinerated by McGill's Waste Management department.

5.6. Small spills and leakage:

5.6.1. Use absorbent paper to pick up all liquid spill material. Seal the absorbent paper, as well as any of your clothing which may be contaminated, in an air-tight plastic bag for eventual disposal.

5.6.2. Wash any surfaces you may have contaminated with disinfectant solution.

5.7. In case of accidental exposure:

5.7.1. Potential routes of exposures include: inhalation, eye contact, skin absorption, ingestion and unintentional injection.

5.7.2. Report the incident immediately to your supervisor. A McGill University Accident, Incident & Occupational Disease Report form must be completed: https://www.mcgill.ca/ehs/forms/forms/accident-and-incident-report

5.7.3. Splash in eyes:

5.7.3.1. Flush eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center.

5.7.3.2. If required, transport the victim after flushing eyes to a hospital.

5.7.4. Skin exposure:

5.7.4.1. Immediately flush affected skin with water while removing and isolating all contaminated clothing.

5.7.4.2. Gently wash all affected skin areas thoroughly with soap and water.

5.7.4.3. If symptoms such as redness or irritation develop, seek medical attention.

5.7.5. Inhalation:

5.7.5.1. Immediately leave the contaminated area; take deep breaths of fresh air.

5.7.6. Immediately call a physician or poison control center Ingestion:

5.7.6.1. Do not induce vomiting.

5.7.6.2. If the victim is conscious and not convulsing, rinse mouth with water and seek medical attention