
1. PURPOSE

This Standard Operating Procedure (SOP) describes the guidelines for the use of N-Butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in rodents.

2. CONSIDERATIONS

N-Butyl-N-(4-hydroxybutyl)nitrosamine (BBN) is a carcinogen used to induce urinary bladder cancer in animal models.

This SOP aims to ensure that the potential of exposure to BBN is reduced as much as possible and that use of this agent poses 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 BBN, as per procedures presented in this SOP and in consultation with the McGill Environmental Health and Safety (EHS) officer.

All hazardous agents must be listed in an approved Animal Use Protocol (AUP).

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. NIOSH-approved, fit-tested, full face respirator or a self-contained breathing apparatus (SCBA)
- 4.1.2. Tyvek® coverall with hood
- 4.1.3. Compatible chemical-resistant gloves (Nitrile)
- 4.1.4. Shoe covers

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

4.3. Absorbent pads

4.4. Accelerated hydrogen peroxide solution (Accel™)

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 BBN 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. House animals in the Goodman Cancer Centre animal facility carcinogen room (3245).
 - 5.2.2. Pregnant or breast-feeding women should not work with hazardous agents.
 - 5.2.3. The following personal protective equipment must be worn at all times when handling hazardous agents (in addition to the personal protective requirements of the animal room):
 - 5.2.3.1. NIOSH-approved, fit-tested, full face respirator or a self-contained breathing apparatus (SCBA)
 - 5.2.3.2. Tyvek® coverall with hood
 - 5.2.3.3. 2 pairs of nitrile gloves
 - 5.2.3.4. Shoe covers
 - 5.2.4. PPE should be discarded as hazardous materials.
 - 5.2.5. 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).
 - 5.2.6. All containers of BBN must be clearly labeled and adequately stored when not in use.
 - 5.2.7. BBN must be transported in unbreakable containers.
 - 5.2.8. 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.
 - 5.2.9. Areas where BBN is prepared and/or administered must be cleaned and decontaminated immediately following each procedure.
 - 5.2.10. Needles and sharps used with BBN must be disposed of immediately in a sharp container. Do not bend or recap needles. Safety needles should be used whenever possible.
 - 5.2.11. Thoroughly wash hands after handling or administering BBN.
 - 5.2.12. 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. Keep containers tightly closed, preferably in a locked cabinet. Keep container in a cool, well-ventilated area away from sources of ignition.
 - 5.3.2. 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 BBN 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 a hazardous agent must be clearly labeled with the following information:
 - 5.4.5.1. "BBN"
 - 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 BBN administration.
- 5.4.7. Cage bedding is considered contaminated until at least 7 days after BBN 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 Accel™ 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 BBN 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 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 BBN (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 Accel™ 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.
 - 5.7.5.2. Immediately call a physician or poison control center.

5.7.6. Ingestion:

- 5.7.6.1. Do not induce vomiting. Volatile chemicals have a high risk of being aspirated into the lungs during vomiting which increases the medical problems.
- 5.7.6.2. If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and immediately call a hospital or poison control center.
- 5.7.6.3. If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. Immediately seek emergency medical care.

SOP REVISION HISTORY

DATE OF MODIFICATION	DETAILS
March 2016	Updated links to EHS forms