May 21

SOP # 011 for N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN)

Standard Operating Procedure for BBN in Animals	
1. Health Hazards	N-Butyl-N-(4-hydroxybutyl) nitrosamine (**BBN**) is a carcinogen used to induce urinary bladder cancer in animal. **BBN** can be toxic if swallowed and can cause skin irritation **BBN** is commonly administered *via* the oral route, in drinking water or by gavage, at a dose that ranges from 0.01-0.05%. However, it can also be administered subcutaneously. Routes of entry are through inhalation and ingestion. Acute toxicity: LD50 Oral - Rat - 1.800 mg/kg **BBN** can be toxic if swallowed, can cause skin irritation. **Pregnant or breast-feeding women should not work with BBN**
2. Physical & Chemical Properties/Definition of Chemical Group	CAS#: 3817-11-6; Molecular Formula: $C_8H_{18}N_2O_2$ Class: Highly toxic by ingestion, target organ effect-carcinogen Form (physical state): Liquid
3. Designated Area	**ABSL2 Facility**
4. Training Requirements	Hazardous chemical training and training on this SOP is required before working with **BBN**. 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 (3M8835). 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 **BBN** 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.

	BBN may be excreted by the animals within the first 7 days post injection therefore **only the lab staff** must change the bedding at least 7 days after administration.
7. Environmental /Ventilation Controls	A. The preparation of **BBN** including reconstitution, weighing, and diluting should be performed in a **certified fume hood** or **class II Type B (preferably)** biological safety cabinet (BSC) or Class IIA2 BSC. B. Work should be done over absorbent pads. C. Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet
8. Special Handling Procedures & Storage Requirements	D. **BBN** 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). A. The fume hood or other approved containment must be cleaned and decontaminated upon completion of tasks. E. When transporting **BBN**, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers. B. 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. F. Once **BBN** is administered, animals, animal waste and cages are considered hazardous for a minimum of 7 days. D. Hands must be washed upon exiting animal room
D. Animal Handling Practices	A. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard), in negative pressurized IVC. B. Handling the cages (including bedding) will be done only by the researchers. C. Use a class II 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. 1. All needles will be disposed of in sharps container - ***do not recap or bend needles***.

	G. Infected animals considered hazardous for a minimum of 7 days after each administration of **BBN**. 2. 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.* 3. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time. 4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious **waste incineration**. 5. All surfaces and racks that may be contaminated will be decontaminated with detergent solution followed by water ASAP. 6. The first cage change after each drug administration is to be done no sooner than 3 days after the administration. The bedding is considered contaminated and requires special handling for 7 days/ ***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- BBN".*** • 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 googles 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 or after 7 days, 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 **BBN**. • The cages should then be put in plastic bags (marked "Toxin-**BBN**") 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 **BBN** contamination.
E. Spill and Accident Procedures	1. Spills must be cleaned immediately by properly protected trained personnel. 2. ***Minor Liquid Spills:*** should be cleaned immediately 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 clean water. Place waste in plastic bag and then in the chemical waste container. 3. ***Powder/Major Spills:*** should be cleaned immediately by personnel wearing a gown, goggles, and two pairs of gloves (nitrile). 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 googles, 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. Prevent, by all means available, spillage from entering drains. 4. ***Exposure***:

	• ***In case of skin contacts*** or injection with **BBN**, wash the affected area with soap and water for at least 15 minutes. Consult with a Medical doctor in an Emergency Room (ER). • ***For eye exposure***, flush with water for at least 15 minutes. **In any case** • **Consult with Medical doctor in ER** • **Report incident to supervisor** • **Report the accident/injury to the Safety Unit Tel: 2146/7.**
F. Waste Disposal	Dispose all waste material in the appropriate chemical waste container. Unused solutions of **BBN** and contaminated solid waste will be disposed of as hazardous chemical material.
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with **BBN** in Animals and agree to follow these procedures.	
Name: _____	Title: _____
Signature: _____	Date: