

August, 2018

**SOP # 003: Standard Operating Procedure for N-Nitroso-N-methylurea
or N-Methyl-N-nitrosourea (NMU)**

Standard Operating Procedure for NMU in Animals	
1. Health Hazards	<p>NMU is a highly toxic and potent carcinogen, mutagen, and teratogen. Routes of potential exposure to N-Nitrosamines are ingestion, inhalation, and dermal contact. Data in rodents suggests that N-Nitrosamines are excreted in the urine of animals after administration, consequently, these instructions MUST be followed when handling animals and bedding for three (3) days after the final administration</p> <p>Pregnant or breastfeeding women, or either gender trying to conceive should consult an occupational doctor prior to handling N-Nitrosamines or animals that have been administered with N-Nitrosamines.</p>
2. Physical & Chemical Properties/Definition of Chemical Group	<p>CAS# : 684-93-5; Molecular Formula: C₂H₅N₃O₂ Class: Highly toxic by ingestion, target organ effect-carcinogen</p>
3. Designated Area	ABSL2 Facility
4. Training Requirements	<p>Hazardous chemical training and training on this SOP is required before working with NMU.</p> <p>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.</p>
5. Personal Protective Equipment (PPE)	<p>Any handling of N-Nitrosamines, including weighing, solution preparation, and drawing doses MUST be done in certified Chemical Fume Hood or class II Type B biological safety cabinet (total exhaust cabinet).</p> <p>Minimum Personal Protective Equipment (PPE) that MUST be worn when handling N-Nitrosamines:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Double nitrile gloves MUST be long enough so that there is no skin exposed between the glove and sleeve (or sleeve covers). <input type="checkbox"/> Chemical safety Goggles <input type="checkbox"/> Lab Coat, <p><i>Personnel should not work with NMU if skin is cut</i></p>
6. Environmental /Ventilation Controls	<p>❖ The preparation of NMU including reconstitution, weighing, and diluting should be performed in a</p>

<p>And General Precautions for Animal Use</p>	<p>certified fume hood or class II Type B biological safety cabinet (total exhaust cabinet).</p> <ul style="list-style-type: none"> ❖ Work should be done over absorbent pads. ❖ Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A2 biological cabinet. Animals should be restrained or anesthetized during injections if needed. ❖ Utilize safe sharps procedures (Have a sharps container in close vicinity) <p>Minimum PPE that MUST be worn <i>when handling animals</i> and bedding of animals that have been administered NMU:</p> <ol style="list-style-type: none"> 1. Double Nitrile Gloves (gloves MUST be long enough so that there is no skin exposed between the glove and sleeve or sleeve covers) 2. Chemical safety Goggles 3. Lab Coat. 4. 3M8835 Respirator <p>NMU may be excreted by the animals within the first 3 days post treatment therefore <i>only the lab staff</i> must change the bedding at least 3 days after final administration.</p>
<p>7. Special Handling Procedures & Storage Requirements</p>	<ol style="list-style-type: none"> A. NMU should be handled in containment and done over absorbent pads. Utilize safe sharps procedures B. The fume hood or other approved containment must be cleaned and decontaminated upon completion of tasks. C. When transporting NMU, 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.

<p>8. Precautions for Animal Use and handling practice</p>	<p>A. Animals must be housed in filter top cages marked as Biohazards (including the name of the Hazard), in negative pressurized IVC.</p> <p>B. Handling the cages (including bedding) will be done only by the researchers during the treatment</p> <p>C. Have a sharps container in close vicinity.</p> <p>D. Once NMU is administered, animals, animal waste and cages are considered hazardous for a minimum of 3 days.</p> <p>E. Use a class II Biological Safety Cabinet at all times, when performing work on these animals and/or when moving animals from dirty to clean cages.</p> <ol style="list-style-type: none">1. All needles will be disposed of in sharps container - <i>do not recap or bend needles.</i>2. Infected animals considered hazardous for a minimum of 3 days after the last administration of NMU3. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time4. Take precautions to avoid the creation of aerosols when changing or washing cages. <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>5. Dead animals must be placed in primary plastic bags, which are then placed in plastic bags for incineration.6. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach solution followed by water ASAP7. The bedding is considered contaminated and requires special handling, and disposal <i>as hazardous chemical waste</i>8. Hands must be washed upon exiting animal room
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When changing cages use the following technique:

- Transport the cages to a HEPA filtered dumping station that draws air away from the user (it is recommended to use a mask or fume hood).
- 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 "NMU".
- 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: incinerated or placed in chemical waste bags for disposal.
- After 3 days from the last treatment, change to a clean cage and 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 " NMU"
- The cages should then be put in plastic bags (marked " N-Nitroso-N-methylurea ") 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 "N-Nitroso-N-methylurea contamination.

<p>9. Spill and Accident Procedures</p>	<ol style="list-style-type: none">1. Spills must be cleaned immediately by properly protected trained personnel2. <u>Minor Liquid Spills:</u> 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 0.5% sodium hypochlorite solution followed by clean water. Place waste in plastic bag and then in the chemical waste container.3. <u>Powder/Major Spills:</u> 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 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 sodium hypochlorite 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>4. <u>Exposure:</u><ul style="list-style-type: none">• <i>In case of skin contact</i>, wash the affected area with soap and water for at least 15 minutes.• <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.
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10. Waste Disposal	Dispose all waste material in the appropriate chemical waste container. Unused solutions of " N-Nitroso-N-methylurea 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 " N-Nitroso-N-methylurea in Animals, and agree to follow these procedures.	
Name: _____	Title: _____
Signature: _____	Date: _____