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| PI: |  | Image result for GHS pictogram https://tse1.mm.bing.net/th?&id=OIP.Mae346b32bfd518c515449ea7c0ba85c3o0&w=300&h=300&c=0&pid=1.9&rs=0&p=0&r=0 |
| Agent(s): | ENU, N-NITROSO-N-ETHYLUREA |
| Date SOP Created: |  |

*Instructions: Insert specific details pertaining to your research and delete irrelevant procedures; contact EH&S at 642-3073 or OLAC at 642-9232 as needed for assistance.*

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| Hazard Information | N-Nitroso-N-Ethylurea (ENU) is toxic, carcinogenic, mutagenic, and teratogenic. ENU may cause cancer and heritable genetic damage. ENU may cause harm to the unborn child. ENU is harmful if swallowed, inhaled, or absorbed through the skin.  Engineering controls such as fume hoods and biological safety cabinets must be used as primary containment in order to limit personnel exposure to ENU. Engineering controls must be inspected in order to ensure efficient removal of hazard, and must have a visual indication of airflow, and alarms to indicate that airflow has fallen below acceptable standards. It may be appropriate to wear N95 particle respirators when handling stock powder – contact EH&S for advice.  Avoid direct contact with skin and mucous membranes of the eyes, nose and mouth. No food or drink is allowed in the lab. Remove gloves and wash hands carefully before leaving. Please note, the highest risk of human exposure is via accidental parenteral injection; careful handling and disposal of sharps is required. Deposit used sharps directly into a rigid sharps container. **NEVER** recap needles.  Hazards specific to your agent and route of administration: |
| Personal Protective Equipment (PPE) | when working with powder form  Non-permeable static free gloves  Long-sleeved lab coat  Safety glasses or goggles  Respiratory protection may be required if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.  when working with ENU in solution  Non-permeable gloves  Long-sleeved lab coat  Safety glasses or goggles  when working with animals:  Disposable gown  Gloves  Safety glasses or goggles or face shield  Respiratory protection may be required if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.  *Additional PPE specific to your research:* |
| Preparation | *List procedures used. Be specific about the physical form (solid, liquid, etc.) and locations for work (bench top, fume hood, biosafety cabinet), and personal protective equipment (PPE) to be worn when handling the material.*  ENU will be purchased/obtained from *(List provider)* in 1 gram isopacs. Order ENU isopacs only as needed and use as soon as possible. Package will be kept intact with shipping documentation and/or maintained in double containment with proper labeling, including “hazardous, toxic, potent mutagen”, PI name and contact information. Inspect the containers for damage during shipment. If damaged, contact the vendor immediately, and decontaminate and dispose of the shipment immediately.  All handling of powder must be performed within a fume hood in *(List location)*. The fume hood will be posted as “Restricted Area -ENU in Use - Toxic/Mutagen”. Place an absorbent pad inside of the fume hood during handling. Never handle ENU powder. ENU should be dissolved within the isopac bottle by injecting solvent into the isopac. All dilutions will be made by individuals wearing required PPE listed above, in the presence of another trained individual. If aliquoting samples, resuspension of the agent will be performed with extremely careful and slow titration, rinsing down the walls of the tube in the process avoiding foaming and aerosolization. Aliquots of 5 mLs of inactivation solution (see disinfectant section below) into a 50 ml conical tube will be made and open tubes will be placed in a tube rack. This tube will serve as a waste receptacle for contaminated pipet tips. ENU vials will be placed in an unbreakable, easily decontaminated, clean secondary storage container and transferred to storage at the appropriate temperature. ENU stocks will be stored in a clearly marked secondary container labeled with the appropriate hazard symbol. Decontaminate all work surfaces and the exterior of all materials leaving the fume hood with inactivation solution and let stand for 24 hours before removing “Restricted Area” posting.  *Other specific preparation steps (with location):* |
| Transportation | ENU will be carried in an easily decontaminated, leak-proof, secondary container labeled with a health hazard and acute toxicity pictograms and PI name and contact information to *(list approved location)*. Avoid the use of glass containers. Handling will only be done by trained personnel. |
| Use | To ensure the safety of research staff, solutions of ENU should be handled and prepared inside a chemical fume hood. Any visible contamination or spills should be cleaned with the inactivation solution (for 24 hours) and then washed with soap and water. Any wipes contaminated with ENU must be disposed as hazardous waste.  It is recommended that animal bedding should be made of material to minimize dust generation, such as corn cob bedding. Cage changes should take place in a  Chemical fume hood whenever possible. If one is not available, use an approved biosafety cabinet. A plastic liner which can simply be rolled up and disposed of should be placed under the bedding when cage changes cannot take place in a chemical fume hood.  Smaller animals being housed during the course ENU administration must be kept in filter-top microisolator cages to minimize the aerosolization of potentially contaminated bedding and excreta. Animals being administered ENU will be housed in disposable caging for at least 72 hours post- final administration.  *Description of treatment procedures:*  *Example: “Dissolve ENU in acetone to prepare 0.04M concentration. Use 25μL to paint the shaved interscapular (region) skin of rats daily for 5 weeks.”* |
| Disposal and Disinfection If unsure, contact EH&S at 642-3073 to determine disposal procedures. | * Decontaminate all work surfaces and equipment with inactivation solution of sodium thiosulfate and high pH (10% sodium thiosulfate, 1% sodium hydroxide (~pH 10). * Formula for inactivation solution: 50 gm Na2S203 (Sodium Thiosulfate),   1 gm NaOH, up to 250 mls with dH20   * All used sharps must be immediately placed into a rigid sharps container. DO NOT recap needles. When 2/3 full, these containers should be placed in a red barrel for disposal as biohazardous waste. * All potentially contaminated lab debris (gloves, pipets, containers) should be inactivated in 10% sodium thiosulfate, 1% sodium hydroxide for 24 hours in the fume hood where the chemical mutagenesis procedure is performed. Waste should then be collected in a yellow bag in a rigid container within NAF 120F, LSA 640, or Minor 599E *(choose location or list approved location)* for disposal as chemotherapy waste. * Any waste liquid, if not absorbed, should be decanted from solid waste and disposed as chemical waste, see [*http://ehs.berkeley.edu/hazardous-materials*](http://ehs.berkeley.edu/hazardous-materials) for guidance. * After treatment, disposable cages may be returned directly to standard ABSL1 housing but the cage card (obtain from OLAC) must be labeled to indicate the hazard type, agent, date of administration, and that OLAC should not change the cage for 72 hours post-final injection. Complete the “OLAC Do Not Change” card with PI responsible cage change dates as applicable. After 72 hours post final treatment, animals can be transferred to clean standard cages by the researcher within a functioning chemical fume hood or biosafety cabinet. Within the chemical fume hood or biosafety cabinet, used cages and bedding should be bagged within yellow bags and disposed of as trace chemotherapy waste. Water not contaminated with ENU can be disposed of by normal OLAC procedures. * Dispose of animals that die during the first 24 hours by placing them into the sodium thiosulfate inactivation bath, then bag and dispose in the biohazardous waste barrel in cold room. 24 hours after treatment, carcasses can be disposed of in a normal manner. |
| Spill Response and Emergency Procedures | Recommended disinfectants: 10% sodium thiosulfate, 1% sodium hydroxide (for >24 hour contact time)  *Disinfectant to be used:*  Injury: If eye or skin contact occurs, wash affected areas with copious amounts of water for 15 minutes and IMMEDIATELY seek medical advice. If inhaled, move individual to fresh air and IMMEDIATELY seek medical advice, call 911. [Rescue breathing, CPR may be needed.] If swallowed, seek IMMEDIATE medical advice.  Report the incident to your supervisor and the Occupational Health Clinic at 2-6891 for follow up.  Medical attention during normal business hours: Tang Center Urgent Care (2-3188 or 3-7197); after hours go to urgent or emergency care: Alta Bates Hospital at 2450 Ashby (204-4444).  If a spill should occur: Avoid generating dust. With gloved hands, remove any contaminated clothing and put in yellow waste bag. Notify other workers in the area of the spill and control traffic through area. Wear shoe covers if the spill is on the floor. Put on fresh gloves and cover spill area with paper towels. Pour  10% bleach over towels from edges of spill to center; be careful not to splatter. Decontaminate all objects in spill area. Allow 30 minutes of contact time. Pick up any sharps, including broken glass, with forceps and place in sharps container. Wipe area with bleach and clean towels again, mop if the spill is on the floor. Remove gloves and foot covers before leaving area of the spill, put in yellow waste bag, and wash hands. Monitor for signs of exposure.  For exposures or emergency clean up response, notify EH&S at 642-3073 immediately. All spills must be notified to EH&S within 8 working hours. |
| Hazard Communication (signs, cage cards, etc.) | All researchers handling this material must read and sign this document.  When ENY is in use, the doors will remain closed and the room should be posted to indicate “ENU in Use-Authorized Personnel Only”. Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while ENU is in use. Postings will not be removed until all surfaces have been decontaminated with inactivation solution for 24 hours.  After 72 hours of final treatment, animals should be transferred to clean cages by the researcher within a functioning chemical fume hood or biosafety cabinet and the “OLAC Do Not Change” card should be removed to indicate OLAC may resume care. Within the chemical fume hood or biosafety cabinet, used cages should be bagged within yellow waste bags and disposed as trace chemotherapy waste.  EH&S and an OLAC veterinarian must review and date this SOP prior to starting this work within an animal facility. Obtain cage cards and hazard labels from OLAC. During injections this SOP must be posted in a plastic sleeve on the door of NAF 120F, LSA 640, Minor 599E *(choose location or list approved location)* to notify OLAC staff and other personnel. |
| Unique Instructions | Before you start work: Review hazards of agents you are handling, complete required reading indicated below, sign and date below, rehearse all handling tasks with a placebo for practice, demonstrate worker proficiency to a supervisor and post signage. All high-risk tasks, such as work with stock material (i.e. making dilutions) should be conducted with two trained individuals present.  Coordinate use of NAF 120F, LSA 640, Minor 599E *(choose location or list approved location)* with the OLAC Facility Manager.  *Other unique procedures:* |
| Additional Information or References | Refer to applicable protocols and authorizations, e.g. the lab’s Biohazard Use Authorization, MAUP/eProtocol, SDS available at <http://ehs.berkeley.edu/hazardous-materials/safety-data-sheets-formerly-msds>, your lab’s chemical hygiene plan, or contact your supervisor or EH&S at 642-3073 for further guidance.  Useful additional information:  *Other required protocols or references:* |

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| **Print Name (last, first)** | **Signature** | **Date Plan Reviewed** |
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PERSONNEL SIGNATURES

EH&S Review (Name/Date):­ 642-3073

OLAC Representative Review (Name/Date): 642-9232