1. PURPOSE

This document describes the guidelines for the use of N-Nitrosamines in animal research. Globally Harmonized System of Classification and Labeling of Chemicals (GHS) classifies 15 N-Nitrosamine compounds as Category 1B Carcinogens, substances presumed to have carcinogenic potential for human, based on animal evidence (for full list see Appendix).

2. SCOPE

This guideline applies to all research personnel or others at Western University and affiliated institutions, who are working with, or could potentially be exposed to N-Nitrosamines.

3. INTRODUCTION

N-Nitrosamines have been classified as reasonably anticipated to be carcinogenic based on sufficient evidence of carcinogenicity from studies in experimental animals. 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 (See 7.4).

4. EXPOSURE CONTROLS

4.1. Use of specific N-Nitrosamine MUST be described in the Animal Use Subcommittee (AUS) approved Animal Use Protocol (AUP).

4.2. Laboratory workers MUST receive specific training regarding the proper handling of N-Nitrosamines, documented in their laboratory safety manual.

4.3. Pregnant or breastfeeding women, or either gender trying to conceive should consult institutional specific Occupational Health (See 5.6.2) prior to handling N-Nitrosamines or animals that have been administered N-Nitrosamines.

4.4. Any handling of N-Nitrosamines, including weighing, solution preparation, and drawing doses MUST be done in certified Chemical Fume Hood. Minimum Personal Protective Equipment (PPE) that MUST be worn when handling N-Nitrosamines:

   4.4.1. Double Nitrile Rubber (0.11mm) Gloves
   4.4.1.1. Gloves MUST be long enough so that there is no skin exposed between the glove and sleeve
   4.4.2. Canadian Standards Association (CSA) Approved Safety Glasses
   4.4.3. Lab Coat, Tyvek™ or Back-Closure Gown

4.5. Minimum PPE that MUST be worn when handling animals and bedding of animals that have been administered N-Nitrosamines:

   4.5.1. Double Nitrile Rubber (0.11mm) Gloves
   4.5.1.1. Gloves MUST be long enough so that there is no skin exposed between the glove and sleeve
   4.5.2. CSA Approved Safety Glasses
   4.5.3. Lab Coat, Tyvek™ or Back-Closure Gown
   4.5.4. Individually Fit-tested NIOSH-Approved N-95 Respirator

4.6. All administrations, cage manipulations, and handling of animals that have been administered N-Nitrosamines MUST be performed in a certified Biological Safety Cabinet (BSC) for three (3) days after the final administration.
5. PROCEDURES

5.1. All procedures **MUST** be completed while wearing the appropriate PPE stated above.

5.2. Conducting animal work in a BSC:

5.2.1. A layer of towels moistened with appropriate disinfectant (See 5.3) **MUST** be placed on the work surface of the BSC prior to opening cages and handling animals that have been exposed to N-Nitrosamines.

5.2.2. The BSC **MUST** be wiped down with paper towel moistened with disinfectant (See 5.3) at the end of each procedure. After wiping with disinfectant, BSC **MUST** be wiped with alcohol to prevent corrosion of BSC.

5.3. Areas where N-Nitrosamines are prepared and/or administered **MUST** be cleaned and decontaminated immediately following each procedure. Spills or surfaces potentially contaminated with N-Nitrosamines should be routinely cleaned with the appropriate solution:

5.3.1. Clidox - 1:5:1 Solution (base : water : activator) - Fume Hood or BSC

5.3.2. Bleach Solution (1:10 Dilution) - Floor & Cage Dunking

5.4. Animal husbandry:

5.4.1. Cages of animals treated with N-Nitrosamines **MUST** be clearly labeled with Hazardous Chemical Cage Card including:

5.4.1.1. Specific N-Nitrosamine Chemical Name

5.4.1.2. Date of N-Nitrosamine Administration

5.4.1.3. Contact Name and Numbers (Both Laboratory & After Hours)

5.4.2. Animal cages should not be changed for a minimum of three (3) days after the final N-Nitrosamines administration.

5.4.3. On the first cage change following N-Nitrosamine administration, the cage bedding is considered contaminated and **MUST** be changed in the following manner:

5.4.3.1. Within the BSC, empty & scrape out the dirty bedding from one cage at a time from up to 14 cages into a garbage bag or labeled hazardous waste bag and placed in container for disposal according to institutional specific hazardous waste program (See 5.5)

5.4.3.2. When finished dumping & scraping up to 14 cages seal the bag and wipe the outside of bag with the appropriate disinfectant (see 5.3).

5.4.3.3. Dirty Water – dispose of directly down the sink drain. It does NOT need to be treated.

5.4.3.4. Once removed from the BSC the cages, bottles, sipper tubes and other housing supplies are to be dunked in an appropriate disinfectant (see 5.3) and placed on the cart for transport to cage wash area.

5.4.3.5. After this first cage change, new cages can be handled using universal laboratory precautions & PPE.

5.5. Laboratory Waste & Carcass Disposal:

5.5.1. Items contaminated or potentially contaminated with N-Nitrosamines and infected carcasses **MUST** be double bagged, labeled as Hazardous Waste and placed in specified containers for removal by institutional specific hazardous waste management program. Western's Hazardous Material Management Handbook, LHSC Waste Management Program, St. Joseph's Waste Management Program

5.6. Emergency Procedures:

5.6.1. In the case of an exposure to eyes or skin, flush the area for 15 to 20 minutes with running water.

5.6.2. During Business Hours bring the MSDS (See Appendix) to Institutional specific Occupational Health:

5.6.2.1. UWO Workplace Health UCC25 Ext. 82047

5.6.2.2. Hospital Occupational Health & Safety Services; VH-Ext. 52286, UH-Ext 33201, or SJHC Ext. 64332

5.6.3. After Business Hours bring the MSDS (See Appendix) to the nearest Hospital Emergency Department

5.6.4. Inform supervisor, who shall complete an Accident/Incident Reporting & Investigation Form Western's form, LHSC's AEMs reports (Intranet only), St. Joseph's Form (See OHSS Office for the form)
6. APPENDIX

International Agency for Research on Cancer (IARC) List of Carcinogenic N-Nitrosamines

For current MSDS, search Product # on Sigma-Aldrich Website: [http://www.sigmaaldrich.com/safety-center.html](http://www.sigmaaldrich.com/safety-center.html)

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<th>Chemical Name</th>
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<td>N-Methyl-N'-Nitro-N'-Nitrosoguanidine</td>
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7. RESOURCES & RELATED DOCUMENTS

7.1. ACVS Chemical SOP SAF-003 [Hyperlink](#)
7.2. U.S. National Toxicology Program *13th Report on Carcinogens - N-Nitrosamines* (2014) [Hyperlink](#)