

Chemical Hygiene Plan for the Purchase and Use of In Vivo Agents

March 2011

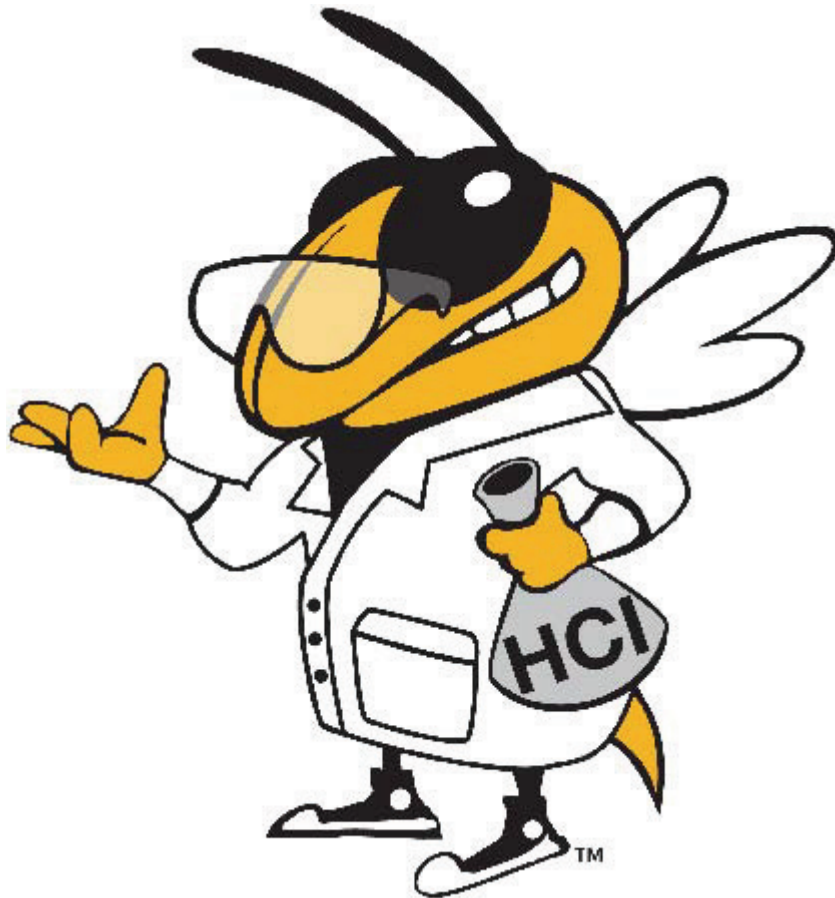


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1. Purpose

The purpose of this program is to ensure that all persons who could potentially be exposed to antineoplastic agents and other chemicals used in in vivo procedures, to be referred to as in vivo agents (IVAs), in Georgia Tech labs have been informed of the presence of these chemicals in their work place and given adequate training in procedures for avoiding exposure.

For the purpose of this document, IVAs are anything injected into live vertebrate animals. This includes antineoplastic agents, other drugs or chemicals, including but not limited to Doxorubicin, Bleomycin, N-Nitroso-N-ethylurea, and Streptozotocin.

2. Scope

This policy applies to all persons conducting research in Georgia Tech Facilities, including but not limited to faculty, staff, students, and visiting scientists.

3. Pre-Approval and Pre-Communication

- 3.1.** Any use of in IVAs involving vertebrate animals must be pre-approved by the Georgia Tech Institute Animal Care and Use Committee (IACUC).
- 3.2.** It is the Principal Investigator's responsibility to communicate any intended use of any in vivo agents to Georgia Tech EHS (404-894-4635) prior to the purchase of the material so that EHS can conduct a risk assessment for the proposed activity.
- 3.3.** It is the responsibility of the Laboratory and Chemical Safety Manager to review the available information, including but not limited to Material Safety Data Sheets (MSDS), drug package inserts, and published studies to determine if the IVA or its metabolic by-products pose any hazard to PRL staff and also to determine how those hazards can best be avoided.
- 3.4.** Any intended use of IVAs involving vertebrate animals must also be communicated to the Director of the Georgia Tech Physiology Research Laboratory (PRL), Dr. Laura O'Farrell (laura.ofarrell@gtrc.gatech.edu), prior to the acquisition of the animals.
- 3.5.** Researchers who administer IVAs to animals are responsible for creating or obtaining and placing individual cage stickers indicating the agent named and dates and times when special handling must occur after administration of these agents. In addition, researchers

must verbally inform PRL staff that these stickers have been placed. No one is to post signs or stickers about hazards within the PRL without verbally informing PRL staff.

4. General Safe Handling and Storage Procedures

- 4.1. Review the Material Safety Data Sheet (MSDS) *prior to* handling the material at (<http://www.ehs.gatech.edu/chemical/chematix.php>) or the manufacturer's website
- 4.2. Nitrile gloves shall be worn. The thicker, longer style (surgeon's gloves) that cover the lab coat cuffs are recommended.
- 4.3. Whenever possible, IVAs should be purchased in sealed rubber capped vials so that they can be solubilized by injecting water into the vial cap without having to open the vial.
- 4.4. If the material cannot be purchased as described above, weigh out on a draft-protected balance (clean around balance after use, *whether or not you think you've spilled anything*).
- 4.5. When working with high concentrations (i.e. stocks or powder) or for a prolonged period of time, *double gloving* can further reduce the risk of exposure, especially if the outer glove is replaced whenever significantly contaminated.
- 4.6. All use of IVAs, particularly aerosol-producing procedures, should be conducted in a fume hood or biological safety cabinet. If this is not possible, contact GT EHS at **404-894-4635** for procedures on how to obtain and be cleared for a respiratory protection device. Dust/surgical masks do not provide adequate protection against chemical agents.
- 4.7. Areas where IVAs is prepared and/or administered must be cleaned and decontaminated with *soap solution and water immediately following each task*. Potentially contaminated areas include bench tops, biological safety cabinet interiors, centrifuges equipment, personal protective equipment (PPE), intravenous bags and tubing.
 - 4.7.1. Countertops should be well wetted with soap solution (Sporicidin or equivalent), wiped dry, and then wiped down twice with wet towels or sponges. Wipes/sponges should be discarded as hazardous material.
 - 4.7.2. Centrifuges and other equipment may be cleaned by wiping with soap solution followed by wiping with a wet towel or sponge. Wipes/sponges should be discarded as hazardous material.
- 4.8. Always wash hands thoroughly after handling IVAs, even when gloves are used.
- 4.9. Store IVAs away from incompatible materials. This will vary, but is most likely to include strong acids and bases, and in some cases (Doxorubicin) sun light.
- 4.10. It is recommended that personnel with preexisting dermatitis, cardiovascular impairment, or women who are pregnant consult a physician prior to working with IVAs.

5. Safe Handling Procedures for those Administering IVAs

- 5.1.** Refer to the Handling Procedures for the IVA you are using (Appendix A)
- 5.2.** It is recommended that animals be anesthetized prior to the administration of IVAs.
- 5.3.** Persons administering IVAs must be careful to avoid needle sticks from syringes and other sharps used in administration. Needles should be discarded in specifically designated sharps containers without recapping.
- 5.4.** The entire procedure area must be cleaned using soap and water as described in 4.7.1 after the animals are returned to their cages.
- 5.5.** Wash hands thoroughly after administering IVAs.

6. Protective Measures for Those Handling Animals/Bedding of Animals Injected with IVAs

- 6.1.** Refer to the Handling Procedures for the specific IVA you are using (Appendix A)
- 6.2.** Right-To-Know Training which includes specific information about the IVA being used, is required prior to working with IVA- dosed rats. Contact EHS Chemical Safety at 404-385-2964.
- 6.3.** The following PPE required for PRL staff and others working with IVA exposed animals, carcasses, animal bedding, or spill clean-up.
 - 6.3.1.** Safety glasses
 - 6.3.2.** Long-sleeve lab coat or gown (change immediately after handling (IVA dosed animals).
 - 6.3.3.** Double nitrile gloves (no latex or vinyl)

7. Handling of IVA Dosed Animals

- 7.1.** The procedures for how animals are handled after dosing will vary from drug to drug and be based on the metabolic fate of the drug after it is introduced into the animal and how and when the material is excreted. Researchers should consult with EHS, the Institute

Veterinarian, and others, as appropriate, to develop specific procedures based on the following questions/issues:

- 7.1.1. Is the material broken down in the body? What are the metabolites? Are they hazardous?
- 7.1.2. Is the material excreted intact? If so, how (urine, feces, exhaled), and for how long?
- 7.1.3. How and over what period of time are the metabolites excreted?
- 7.2. The above information will determine:
 - 7.2.1. How will the animals be housed after dosing? Will they go back on regular bedding or wire floor cages?
 - 7.2.2. How will the bedding be disposed of? If the bedding is hazardous waste, how long after dosing must it be treated as such?
 - 7.2.3. Can the animals be returned to the ventilated cage racks right away or will they need to be kept in a fume hood? How long must they stay in the fume hood?
 - 7.2.4. Can the dosed animals be handled in the housing room or only in the Animal Transfer Station?
 - 7.2.5. IVA-contaminated sharps may not be discarded in general use sharps containers. Researchers must supply and remove their own IVA sharp containers each time they work in the PRL.

8. PRL Sharps Use Procedure

- 8.1. A “sharp” is any discarded material that may cause punctures or cuts and may have been exposed to infectious or potentially infectious agents. This includes, but is not limited to, needles, IV tubing, and syringes with needles attached, and scalpel blades.
- 8.2. Contaminated sharps must be placed in puncture-proof and leak-proof containers which are picked up by EHS for incineration.
- 8.3. Sharps should never be resheathed prior to disposal unless the sharp comes equipped with a safety device designed to be engaged after usage.
- 8.4. Use sharps with safety devices whenever possible or use needleless systems to conduct research.
- 8.5. Sharps should be disposed of as biohazardous waste. The outer box must be labeled to indicate that the box contains sharps to allow for proper disposal.
- 8.6. For each procedure, a small sharps container shall be used that is labeled with the name of the IVA. This container shall be returned to the lab of origin after the procedure.

- 8.7.** The entire procedure area must be cleaned with soap and water (as described in 4.7.1) after the animals are returned to their cages. Wash hands thoroughly after handling IVAs.

9. Spill Procedures

- 9.1.** A *minor* spill is any spill where the individual responsible for the spill feels they are capable of handling the spill safely without the use of respiratory protection or the assistance of specially trained emergency response personnel. (Please note that the typical threshold that GT EHS uses to define a minor spill is 1-4 L of material) For IVAs the threshold may include spills less than 10 mL and which generate little aerosol. Clean up spills using *soap and water only!* (See 4.7.1)
- 9.2.** A *major* spill is any spill that requires outside emergency response personnel and/or requires a respirator to avoid inhalation of the IVA. This includes spills of more than 10 mL or with considerable generation of aerosol. Clean up spills using *soap and water only!* (See 4.7.1)
- 9.3.** Individuals should become familiar with proper clean up procedures before a spill occurs (Spill kits with instructions, absorbents, and protective equipment are located inside PRL Procedure Room in case of emergency). Emergency Procedures should be posted in the lab. For a copy of the GT Laboratory Emergency Procedures contact GT Chemical Safety at 404-385-2964.
- 9.4.** For *Minor Spills*:
- 9.4.1.** Control the area of the spill by restricting access.
- 9.4.2.** For liquid spills: Clean the contaminated surface from *outer edge to center* of spill to avoid spreading the spill.
- 9.4.3.** For powders: Place wet paper towels over the spilled material to avoid aerosolizing material. Clean spill from *outer edge to center* to prevent spread of contaminated area.
- 9.4.4.** Removal of spilled materials should be followed by a soap and water clean up using the same outer edge-to-center technique:
- 9.4.4.1. Wipe the area down with a soapy rag
- 9.4.4.2. Dry with a clean rag
- 9.4.4.3. Wipe the area again with a wet rag (no soap)
- 9.4.4.4. Dry with a clean dry rag
- 9.4.4.5. Repeat with wet rag
- 9.4.4.6. Dry with a clean dry rag.

9.4.5. Bag cleanup materials in a Biohazard bag and handle as hazardous waste (See Disposal Procedures).

9.4.6. Call GT EHS at **404-894-6224** for hazardous waste pick up

9.4.7. Replenish spill clean up supplies.

9.5. For *Major Spills*:

9.5.1. Call GT Police (**404-894-2500**) for help (provide your name, phone number, location, name of the material spilled, and approximate amount of the material spilled)

9.5.2. Control the area of the spill by restricting access

9.5.3. For fume hood spills, close the hood sash

9.5.4. Evacuate the lab

9.5.5. Post warning signs

9.5.6. Pull fire alarm if necessary

9.5.7. Remain on site to speak to first responders

9.5.8. Clean up spills using *soap and water only as described in 9.4.4.1-9.4.4.6!*

9.6. Any residue, contaminated soil, water, and other debris resulting from the clean up of an IVA spill are considered hazardous waste and must be bagged and/or boxed and disposed of in labeled biohazard containers. (see section 10).

10. Disposal Procedures

10.1. All IVA exposed carcasses or tissues, animal bedding, spill clean-up materials, sharps containers, syringes, needles, and/or activated or inactivated solutions are considered biohazardous and must be disposed of as biohazardous waste.

10.2. All materials contaminated with IVAs are to be disposed of in red bag/biohazard boxes, including gloves, disposable gowns, gauze, etc. (this includes but not limited to bedding of IVA dosed animals, body fluids/blood of IVA dosed animals).

10.3. Tissue samples (cultures) can be autoclaved prior to being prepared for disposal via hazardous waste stream

10.4. No waste materials containing IVAs shall be disposed of in sinks or with general trash.

10.5. Absorbent materials used for spill clean up shall be disposed of as biohazardous waste.

- 10.6. All IVA waste containers must be suitable for transportation and must not be leaking (Doxorubicin, for example, is strongly absorbed from solution by glass or Teflon but not by polypropylene, PVC, or siliconized glass).
- 10.7. Double bag waste in red biohazard bags and box.
- 10.8. Write Principal Investigator's name on front of box.
- 10.9. Write PRL on front of box.
- 10.10. Check the boxes for "Chemotherapeutic" (if appropriate) and "Incinerate Only" (for all IVAs).
- 10.11. Contact **GT EHS at 4-6224** for hazardous waste pickup or use Chematix to submit a waste pickup request.
- 10.12. All bedding of animals dosed with IVAs will be disposed of as bio-hazardous waste.
- 10.13. When animal waste is scheduled to be disposed, it will be collected in a biohazard bag and stored as biological waste.
- 10.14. All personnel handling IVAs waste must wear double gloves, lab coats, and safety glasses.

11. Exposure/ Personnel Decontamination Procedures

- 11.1. Exposure Control: IVAs are frequently received as lyophilized powders which can become airborne and may result in personal exposure and area contamination. Avoid creating dust. Solubilize the powder by adding the solvent through the rubber stopper cap. Do not inhale or ingest. Avoid contact with eyes, skin, and clothing.
- 11.2. Shower Procedures: If you are splashed with a chemical on any area of your body you must rinse that area with copious amounts of *tepid* water for no less than 15 minutes. If you are splashed in an area of your body which cannot be put under a sink faucet and flooded immediately – you must use an emergency shower.
 - 11.2.1. If your clothing is splashed or saturated by a spill, remove it on the way to the shower:
 - 11.2.2. Shout for help
 - 11.2.3. Remain in the shower for 15 minutes
 - 11.2.4. Do not re-don contaminated clothing
- 11.3. If medical attention is warranted or desired have someone call the **GT Police (404-894-2500)**. Tell them you need an ambulance.

11.3.1. Report incident to Laboratory and Chemical Safety Manager (404-894-4635)

11.3.2. You must have the street address of where you are located

11.3.3. Have your helper print 3 copies of the MSDS

11.3.3.1. Take one copy of the MSDS with you to the hospital

11.2.7.2. Give one copy to the ambulance crew

11.2.7.3. Give one copy to the GT Police or EHS

11.4. Eye Wash Procedures: If you are splashed in the eyes:

11.4.1. Shout for help

11.4.2. Hold your eyelids open with your fingers as you rinse your eyes for a *full 15 minutes* (move eyes up and down and side to side to fully remove chemical).

11.4.3. Have your helper watch a clock for you to make sure you continue to rinse your eyes for the *full 15 minutes*.

11.4.4. ALL EXPOSURES TO THE EYES REQUIRE MEDICAL FOLLOW UP this means

11.4.4.1. Victim must be transported to medical facility

11.4.4.2. Incident report and first report of injury must be filed

11.4.4.3. EHS recommends you go to Grady Memorial Hospital to ensure proper chemical-exposure care.

11.4.5. If the victim is in respiratory distress call the **GT Police at 404-894-2500 or dial 911**

11.5. MSDSs

11.5.1. Take one copy of the MSDS with you to the hospital

11.5.2. Give one copy to the ambulance crew

11.5.3. Give one copy to the GT Police or EHS

11.6. Inhalation Exposure Procedures

11.6.1. If inhaled or swallowed, seek medical attention immediately: call the **GT Police at 404-894-2500 or dial 911**

11.6.2. Leave the lab / evacuate area if appropriate.

12. Animal Bites and Needle Stick Injuries

Procedures following a bite from an IVA-dosed animal or otherwise invasive incident (i.e. needle puncture) when using an IVA:

- 12.1. Put the animal back in its cage.
- 12.2. Wash the wound for 15 minutes with soap and vigorously running water directed at the wound.
- 12.3. Inform the PRL Manager and the head of your lab.
- 12.4. Make sure that a Georgia Tech Injury Report form is filed. See: www.ehs.gatech.edu/general/injury_illness.php
- 12.5. If medical attention is warranted or desired, the employee may seek medical attention at Concentra Medical Center (Their hours are Monday-Friday, 7:30am- 8:00pm and Saturday-Sunday, 10:00am-4:00pm.) If the injury occurs outside of these hours, the employee may seek medical attention at any of the near by hospital emergency rooms.

13. Additional Information

Non-emergency questions may be directed to Department of Environmental Health and Safety, Chemical Safety Unit: 404-385-2964

14. References

Georgia Institute of Technology Institutional Animal Care and Use Committee. Policies and Procedures. October 2008.

http://www.compliance.gatech.edu/forms/IACUC/Policies_Procedures.doc

NIOSH: Preventing Occupational Exposure to Antineoplastic and Other Hazardous Drugs in Health Care Settings. September 2004.

<http://www.cdc.gov/niosh/docs/2004-165/> .

15. Handling Procedures for Specific In vivo agents

GT EHS will continue to add specific handling procedures to this document as the need arises

15.1. Doxorubicin

Please review the general safe handling and storage procedures sections of this document, *prior to* reading this section.

Any use of doxorubicin involving vertebrate animals must be pre-approved by the Georgia Tech Institute Animal Care and Use Committee (IACUC).

It is the Principal Investigator's responsibility to communicate any intended use of doxorubicin or any other in vivo agents to Georgia Tech EHS (404-894-4635) prior to the purchase of the material.

Any intended use of doxorubicin involving vertebrate animals must also be communicated to the Director of the Georgia Tech Physiology Research Center, Dr. Laura O'Farrell (laura.ofarrell@gtrc.gatech.edu), prior to the acquisition of the animals.

Right-To-Know Training which includes specific information about doxorubicin is required prior to working with doxorubicin-dosed rats.

Doxorubicin

Doxorubicin (trade name Adriamycin) is an antineoplastic chemotherapy drug, a powder or liquid, clear, orange-red in color, and administered only intravenously. Doxorubicin is a mutagen, carcinogen, and teratogen, and is highly irritating to the eyes, skin, mucous membranes and upper respiratory tract. Statistically significant genotoxic effects and genetic damage (for example, increased micronuclei formation and increases in sister chromatid exchange and chromosomal aberrations) have been reported in hospital pharmacists and nurses exposed to in vivo agents. The toxic effects of doxorubicin may be experienced if swallowed, inhaled, ingested or exposed to the skin. It is important that those administering the drug as well as those handling animals/bedding of animals injected with the drug practice appropriate precautions.

15.1.1. General Safe Handling and Storage Procedures

- 15.1.1.1. Store doxorubicin at room temperature in clearly labeled, tightly closed containers *within a designated (labeled/posted) area*. Keep away from direct sunlight or strong incandescent light. Keep away from heat/flame and moisture.
- 15.1.1.2. Store away from incompatibles which include oxidizing agents, strong acids,

and strong bases.

- 15.1.1.3. Doxorubicin, in its powder form, can become airborne and may result in personal exposure and area contamination. Avoid creating dust. Avoid exposure to lyophilized powder by dissolving the material by injecting water through the rubber stopper (do not open the vial until the material is dissolved) If this is not possible, handle the powder in a glove box, biosafety cabinet or fume hood.

15.1.2. Safe Handling Procedures for those Administering Doxorubicin

- 15.1.2.1. Doxorubicin should not be mixed with heparin or fluorouracil since it has been reported that these drugs are incompatible to the extent that they will react and form a precipitate.
- 15.1.2.2. Do not administer doxorubicin solutions that are discolored or contain particulate matter.
- 15.1.2.3. Reconstituted solution and diluted solutions are stable in intravenous bags for 6 hours at room temperature or if refrigerated.
- 15.1.2.4. Doxorubicin is strongly absorbed from solution by glass or Teflon but not by polypropylene, PVC, or siliconized glass. Therefore, all doxorubicin waste containers must be suitable for transportation and must not be leaking.

15.1.3. Safe Handling Procedures for Those Handling Animals/Bedding of Animals Injected with Doxorubicin

- 15.1.3.1. Personal Protective Equipment for PRL staff and others working with Doxorubicin (dox) dosed animals, carcasses, animal bedding, or spill clean-up shall be as described in section 6.1: Safety glasses, long-sleeve lab coat or gown (change immediately after handling dox dosed animals), and double nitrile gloves (no latex or vinyl)
- 15.1.3.2. PPE and handling requirements as outlined in 15.2.2.1 shall be observed for the first 10 days after dosing animals with Doxorubicin after which normal PRL procedures may be observed.
- 15.1.3.3. All dox-dosed animals will be housed in standard cage bedding.
- 15.1.3.4. Dox-dosed animals should only be handled in Animal Transfer Stations (ATS) or Surgery or Procedure Room only, NOT in Housing Room.
- 15.1.3.5. Cages should be changed in ATS only.
- 15.1.3.6. Cages should be dumped only at dump station.
- 15.1.3.7. Scraped out cages should be washed immediately (do not wait to fill up the

cage holder).

- 15.1.3.8. All bedding of animals injected with doxorubicin will be disposed of as biohazardous waste per Section 10 of the GT Program for the Purchase and Use of In Vivo Agents.

15.1.4. Disposal Procedures

- 15.1.4.1. All doxorubicin contaminated materials must be treated as biohazardous waste and disposed of as per Section 10 of the GT Program for the Purchase and Use of in Vivo Agents.

15.1.5. References

NIOSH: Preventing Occupational Exposure to Antineoplastic and Other Hazardous Drugs in Health Care Settings. September 2004.
<http://www.cdc.gov/niosh/docs/2004-165/> .

Adriamycin – Full Prescribing Information.
<http://www.meds.com.leukemia/idamycin/adriamycin.html>

Chematix. Sigma-Aldrich 2007.1 Material Safety Data Sheet.
<http://www.ehs.gatech.edu/chemical/chematix.php>

Georgia Institute of Technology Institutional Animal Care and Use Committee.
October 2008.
http://www.compliance.gatech.edu/forms/IACUC/Policies_Procedures.doc

15.2. Bleomycin

Bleomycin is a mixture of cytotoxic glycopeptide antibiotics isolated from a strain of *Streptomyces verticillus* and is freely soluble in water. It is available as a lyophilized powder for intramuscular, intravenous or subcutaneous injection. Each vial contains sterile Bleomycin sulfate equivalent to 15 units or 30 units of Bleomycin. Sulfuric acid or sodium hydroxide may be used, if necessary to adjust the pH.

Bleomycins are a group of related basic glycopeptides which differ in the terminal amine substituent of the common structural unit, Bleomycin acid. The main components of Bleomycin for Injection are Bleomycins A2 and B2. Chemically, Bleomycin A2 is N1-[3(dimethylsulfonio)propyl]-Bleomycinamide and Bleomycin B2 is N1-[4(aminoiminomethyl)amino]butyl]-Bleomycinamide.

Bleomycin is cytotoxic and possibly carcinogenic. Bleomycin is toxic to the lungs by any means of exposure and causes fibrosis in 10% of the patients who are treated with it. It accumulates in lung, kidney, and skin tissue. It is excreted as bleomycin (intact) by the kidneys and has a half life in blood of 1-5 hour, depending on the route of exposure and the level of kidney function of the patient. Patients with impaired kidney function excrete bleomycin more slowly. Kidney impairment from bleomycin therapy is rare and has been described as “unpredictable”.

Bleomycin has been shown cause harm to the fetuses of pregnant test animals. Exposure should be avoided during breastfeeding.

Exposure to Bleomycin may cause allergic reactions of the eyes or skin. IV Anaphylaxis has occurred in 1% of lymphoma patients treated with bleomycin.

Signs and Symptoms of bleomycin exposure include irritation, redness, stinging, or watering of the eyes; Redness, stinging, burning, and itching of the skin. Inhalation can lead to pneumonitis and pulmonary fibrosis.

15.2.1. General Safe Handling and Storage Procedures

- 15.2.1.1. Store away from incompatibles which include oxidizing agents, strong acids, and strong bases. Protect from heat.
- 15.2.1.2. Bleomycin, in its powder form, can become airborne and may result in personal exposure and area contamination. Avoid creating dust. Avoid exposure to lyophilized powder by dissolving the material by injecting water through the rubber stopper (do not open the vial until the material is dissolved) If this is not possible, handle the powder in a glove box, biosafety cabinet or fume hood.
- 15.2.1.3. Do not inhale or ingest. Avoid contact with eyes, skin, and clothing

15.2.2. Safe Handling Procedures for those Administering / Handling Animals/Bedding of Animals Injected with Bleomycin

- 15.2.2.1. Personal Protective Equipment for PRL staff and others working with bleomycin (bleo) dosed animals, carcasses, animal bedding, or spill clean-up shall be as described in section 6.1: Safety glasses, long-sleeve lab coat or gown (change immediately after handling dox dosed animals), and double nitrile gloves (no latex or vinyl)
- 15.2.2.2. Opening cages and/or handling of bleomycin dosed animals within the first 24 hours of dosing should be avoided whenever possible.
- 15.2.2.3. When opening cages or handling of animals is necessary within the first 24 hours after dosing, PPE and handling requirements as outlined in 15.2.2.1 shall be observed. Normal PRL procedures may be resumed 24 hours post dosing.
- 15.2.2.4. Do not administer bleomycin solutions that are discolored or contain particulate matter.
- 15.2.2.5. Injected animals should be held at least 5 hours (one blood half life) in the fume hood or in exhaust-vented cage racks.
- 15.2.2.6. All bleo-dosed animals will be housed in standard cage bedding.
- 15.2.2.7. Bleo-dosed animals should only be handled in Animal Transfer Stations (ATS) or Surgery or Procedure Room only, NOT in Housing Room.
- 15.2.2.8. Cages should be changed in ATS only.
- 15.2.2.9. Cages should be dumped only at dump station.
- 15.2.2.10. Scraped out cages should be washed immediately (do not wait to fill up the cage holder).
- 15.2.2.11. All bedding of animals injected with bleomycin will be disposed of as bio-hazardous waste as per Section 10 of the GT Program for the Purchase and Use of In Vivo Agents

15.2.3. Disposal Procedures

- 15.2.3.1. All bleomycin contaminated materials must be disposed of as biohazardous waste as per Section 10 of the GT Program for the Purchase and Use of In Vivo Agents

15.2.4. References

[Bleomycin Official FDA information, side effects and uses.](http://www.drugs.com/pro/bleomycin.html)
<http://www.drugs.com/pro/bleomycin.html>

Faulding Labs, Bleomycin Package Insert

Georgia Institute of Technology Institutional Animal Care and Use Committee.

Policies and Procedures. October 2008.

http://www.compliance.gatech.edu/forms/IACUC/Policies_Procedures.doc

NIOSH: Preventing Occupational Exposure to Antineoplastic and Other Hazardous Drugs in Health Care Settings. September 2004.

<http://www.cdc.gov/niosh/docs/2004-165/> .

Rx Med Bleomycin Sulfate

[http://www.rxmed.com/b.main/b2.pharmaceutical/b2.1.monographs/CPS-%20Monographs/CPS%20\(General%20Monographs-%20B\)/BLEOMYCIN%20SULFATE%20USP.html](http://www.rxmed.com/b.main/b2.pharmaceutical/b2.1.monographs/CPS-%20Monographs/CPS%20(General%20Monographs-%20B)/BLEOMYCIN%20SULFATE%20USP.html)

Tiva Sicor Bleomycin Sulfate Material Safety Data Sheet

15.3. N-Nitroso-N-ethylurea

Please review the general safe handling and storage procedures sections of this document, *prior to* reading this section.

Any use of In vivo agents involving vertebrate animals must be pre-approved by the Georgia Tech Institute Animal Care and Use Committee (IACUC).

It is the Principal Investigator's responsibility to communicate any intended use of any in vivo agents to EHS (404-894-4635) prior to the purchase of the material.

Any intended use of in vivo agents involving vertebrate animals must also be communicated to the Director of the Georgia Tech Physiology Research Center, Dr. Laura O'Farrell (laura.ofarrell@grtc.gatech.edu), prior to the acquisition of the animals.

Right-To-Know Training which includes specific information about N-Nitroso-N-ethylurea is required prior to working with N-Nitroso-N-ethylurea-dosed rats.

N-Nitroso-N-ethylurea

N-Nitroso-N-ethylurea (NEU) is unstable at metabolic pHs: Its half life at pH 7 is 1.5 hrs; at pH 8 this drops to 0.1 hrs.

NEU breaks down in the blood to formaldehyde and acetaldehyde

Formaldehyde has a half life of 1 minute in rat blood. It is broken down to formate and carbon dioxide. In 2006 IARC published a monograph that showed that when rats were dosed orally with ¹⁴C labeled formaldehyde, 40% of it was broken down to CO₂ and eliminated through the lungs; 10% of the ¹⁴C was found in the urine and 1% in feces within 12 hours. The remaining formaldehyde incorporates itself into biological macromolecules such as DNA and proteins and remains in the animal. The high level of conversion of formaldehyde to formate is due to the fact that this conversion can take place in all tissues of the body. The IARC study did not distinguish between ¹⁴C labeled formate and formaldehyde in the urine and feces, and due to high dosing it would be safe to assume that at least some of the labeled formaldehyde was excreted intact- within the first few minutes or hours. With a 1 minute half life, however, it is safe to assume that by the time the animals get to 12 hours post exposure; anything being excreted is formate, which is harmless.

Acetaldehyde has an estimated half life of 15 minutes in the blood. It is metabolized in the lungs and liver (of mammals) by acetaldehyde dehydrogenase to acetate and acetic acid. Acetate is excreted in the urine and completely oxidized CO₂ and O₂. Un-metabolized acetaldehyde incorporates itself into DNA and proteins.

Chemical information:

N-Nitroso-N-ethylurea

- CAS – 759-73-9
- ACGIH Threshold Limit Value (TLV): None
- Exposure may occur by oral, respiratory or dermal routes Target organs are liver and kidney
- Mutagen and teratogen
- Reasonably Anticipated to be a Human Carcinogen (IARC)
- Limited human exposure data, large body of animal data

Formaldehyde

- CAS – 50-00-00
- Symptoms include irritation to the eyes, nose, throat, skin
- Exposure may occur by oral, respiratory or dermal routes
- Mutagen and teratogen
- Reasonably Anticipated to be a Human Carcinogen (IARC)
- Limited human exposure data, large body of animal data
- Occurs naturally in most living systems and in the environment
- Indoor air pollutant, typical indoor level is 0.025 – 0.075 ppm
- Outdoor Air Pollutant, typical urban level is 0.025 ppm
- ACGIH Threshold Limit Value (TLV): None.
- ACGIH Short Term Exposure Limit (STEL) (15 minute) 0.3 ppm

Acetaldehyde

- CAS - 75-07-0
- Symptoms include irritation to the eyes, nose, throat, skin
- Exposure may occur by oral or respiratory routes
- Possibly carcinogenic to Humans (IARC 2B)
- ACGIH TLV: None
- ACHIH STEL and Ceiling 25 ppm

15.3.1. General Safe Handling and Storage Procedures

- 15.3.1.1. Store away from incompatibles which include oxidizing agents, strong acids, and strong bases. Protect from heat.
- 15.3.1.2. In its powder form, this material can become airborne and may result in personal exposure and area contamination. Avoid creating dust. Avoid exposure to lyophilized powder by dissolving the material by injecting water through the rubber stopper (do not open the vial until the material is dissolved) If this is not possible, handle the powder in a glove box, biosafety cabinet or fume hood.
- 15.3.1.3. Do not inhale or ingest. Avoid contact with eyes, skin, and clothing

15.3.2. Safe Handling Procedures for those Administering N-Nitroso-N-ethylurea and/or Handling NRU Dosed Animals

GT Environmental Health and Safety conducted air sampling for formaldehyde that included breathing zone air sampling on employees doing the initial cage change and area air sampling in the procedure room in July 2009. All results were negative for elevated formaldehyde. These sampling results are considered a “negative exposure assessment” proving that the procedures outlined in this document are sufficient to avoid formaldehyde over exposure, but are valid for only one year. Please contact EHS (404-894-4635) if you intend to work with NEU after July 1, 2010 so that a new negative exposure assessment can be accomplished. Thank you.

- 15.3.2.1. Personal Protective Equipment for PRL staff and others working with NEU dosed animals, carcasses, animal bedding, or spill clean-up shall be as described in section 6.1: Safety glasses, long-sleeve lab coat or gown (change immediately after handling dox dosed animals), and double nitrile gloves (no latex or vinyl)
- 15.3.2.2. Opening cages and/or handling of NEU dosed animals within the first 24 hours of dosing should be avoided whenever possible.
- 15.3.2.3. When opening cages or handling of animals is necessary within the first 24 hours after dosing, PPE and handling requirements as outlined in 15.3.2.1 shall be observed. Normal PRL procedures may be resumed 24 hours post dosing.
- 15.3.2.4. Do not administer NEU solutions that are discolored or contain particulate matter
- 15.3.2.5. Injected animals should be held at least 3 hours (one blood half life) in the fume hood or in exhaust-vented cage racks.
- 15.3.2.6. All NEU -dosed animals will be housed in standard cage bedding.
- 15.3.2.7. NEU-dosed animals should only be handled in Animal Transfer Stations (ATS) or Surgery or Procedure Room only, NOT in Housing Room.
- 15.3.2.8. Cages should be changed in ATS only.
- 15.3.2.9. Cages should be dumped only at dump station.
- 15.3.2.10. Scraped out cages should be washed immediately (do not wait to fill up the cage holder).
- 15.3.2.11. All bedding of animals injected with NEU will be disposed of as bio-hazardous waste as per Section 10 of the GT Program for the Purchase and Use of In

Vivo Agents

15.3.3. Disposal Procedures

Treat the bedding of NEU dosed animals as chemically contaminated and dispose of it in red biohazard bags for the first 24 hours as per the procedures of Section 10 of the GT Program for the Purchase and Use of In Vivo Agents

15.3.4. References

Bingham, E. Cochrane, B., Powell, C. Patty's Toxicology, Fifth Edition, Wiley-Interscience, New York, 2001.

Georgia Department of Natural Resources, Title 45 Chapter 22. Public Employees Hazardous Chemical Protection and Right to Know Act of 1988.

Klaassen, C., Casarett and Doull's Toxicology, the Basic Science of Poisons, Fifth Edition, McGraw-Hill, New York, 1996

U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Official Citation: Report on Carcinogens, Eleventh Edition; <http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html>

World Health Organization, International Agency for Research on Cancer, IARC Monograph on Formaldehyde, 2-Butoxyethanol, and 1-tert- Butoxy propan-2-ol, Vol. 88, WHO, 2006.

15.4. Streptozotocin

Please review the general safe handling and storage procedures sections of this document, *prior to* reading this section.

Any use of in vivo agents involving vertebrate animals must be pre-approved by the Georgia Tech Institute Animal Care and Use Committee (IACUC).

It is the Principal Investigator's responsibility to communicate any intended use of any in vivo agents to Georgia Tech EHS (404-894-4635) prior to the purchase of the material. Any intended use of in vivo agents involving vertebrate animals must also be communicated to the Director of the Georgia Tech Physiology Research Center, Dr. Laura O'Farrell (laura.ofarrell@grc.gatech.edu), prior to the acquisition of the animals.

Right-To-Know Training which includes specific information about streptozotocin is required prior to working with streptozotocin-dosed rats.

Streptozotocin

Streptozotocin is a glucosamine-nitrosurea compound which was originally developed as an antibiotic. It is structurally similar enough to glucose to be recognized as glucose and transported across cell membranes. It is selectively toxic in vivo to mammalian pancreatic islet beta cells and has limited use in treating cancers of these cells. It has a reported LD50 of 240 mg/kg in mice.

Streptozotocin has been used in research for many years to induce diabetes mellitus in rats and mice. Studies indicate that as much as 77% of injected streptozotocin is either broken down and eliminated or eliminated intact within 6 hours of injection. Of that amount 30% is eliminated within the first hour. The majority of the material/metabolites are eliminated (74%) in urine; the remainder (3%) is eliminated in feces. Most of the remaining material is found in the liver and kidney.

Streptozotocin is cleaved into segments in the liver and degrades in plasma. Its cleavage/degradation products are not capable of causing diabetes but some remain biologically active and capable of crossing cell membranes.

Chemical Information:

Streptozotocin

- CAS – 18883-66-4
- ACGIH Threshold Limit Value (TLV): None
- Exposure may occur by oral, respiratory, or dermal routes. Target organs are liver and kidney
- Mutagen and teratogen
- Possibly carcinogenic to humans (IARC 2B). Limited human exposure data, large body of animal data

15.4.1. General Safe Handling and Storage Procedures

- 15.4.1.1. Store away from incompatibles which include oxidizing agents, strong acids, and strong bases. Protect from heat.

- 15.4.1.2. In its powder form, streptozotocin can become airborne and may result in personal exposure and area contamination. Avoid creating dust. Avoid exposure to lyophilized powder by dissolving the material by injecting water through the rubber stopper (do not open the vial until the material is dissolved) If this is not possible, handle the powder in a glove box, biosafety cabinet or fume hood. (Lyophilized streptozotocin can be purchased in rubber stopper sealed vials form Teva)
- 15.4.1.3. Do not inhale or ingest. Avoid contact with eyes, skin, and clothing

15.4.2. Safe Handling Procedures for those Administering/Working with Streptozotocin Dosed Animals

- 15.4.2.1. Personal Protective Equipment for PRL staff and others working with Streptozotocin dosed animals, carcasses, animal bedding, or spill clean-up shall be as described in section 6.1: Safety glasses, long-sleeve lab coat or gown (change immediately after handling dox dosed animals), and double nitrile gloves (no latex or vinyl).
- 15.4.2.2. Opening cages and/or handling of streptozotocin dosed animals within the first 24 hours of dosing should be avoided whenever possible.
- 15.4.2.3. When opening cages or handling of animals is necessary within the first 24 hours after dosing, PPE and handling requirements as outlined in 15.4.2.1 shall be observed. Normal PRL procedures may be resumed 24 hours post dosing.
- 15.4.2.4. Do not administer s Streptozotocin solutions that are discolored or contain particulate matter.
- 15.4.2.5. Injected animals should be held at least 3 hours in the fume hood or in exhaust-vented cage racks.
- 15.4.2.6. All - Streptozotocin dosed animals will be housed in standard cage bedding.
- 15.4.2.7. Streptozotocin dosed animals should only be handled in Animal Transfer Stations (ATS) or Surgery or Procedure Room only, NOT in Housing Room.
- 15.4.2.8. Cages should be changed in ATS only.
- 15.4.2.9. Cages should be dumped only at dump station.
- 15.4.2.10. Scraped out cages should be washed immediately (do not wait to fill up the cage holder).
- 15.4.2.11. All bedding of animals injected with Streptozotocin will be disposed of as bio-hazardous waste as per Section 10 of the GT Program for the Purchase and Use of In Vivo Agents

15.4.3. Disposal Procedures

Treat bedding as chemically contaminated and dispose of it in red biohazard bags for the first 24 hours as per Section 10 of the GT Program for the Purchase and Use of In Vivo Agents

15.4.4. References

Adolphe, A. Glasofer, E. Troetel, W. Ziegenfull, J. The Fate of Streptozotocin in Patients with Advanced Cancer. Cancer Chemotherapy Reports. May/June 1975

Georgia Department of Natural Resources, Title 45 Chapter 22. Public Employees Hazardous Chemical Protection and Right to Know Act of 1988.

Georgia Institute of Technology Institutional Animal Care and Use Committee. Policies and Procedures. October 2008.

http://www.compliance.gatech.edu/forms/IACUC/Policies_Procedures.doc

Karunanayake, E., Hearse, D. and Mellows, G. The Metabolic Fate and Elimination of Streptozotocin. Biological Society Transactions. 1975 Volume 3

Lee, J., Kim, M., Moon, C., Chang, J. Degradation Products of Streptozotocin Do Not Induce Hyperglycemia in Rats. Biochemical Pharmacology. Volume 46. No 11. 1993.

NIOSH: Preventing Occupational Exposure to Antineoplastic and Other Hazardous Drugs in Health Care Settings. September 2004.

<http://www.cdc.gov/niosh/docs/2004-165/>