



October 20

SOP # 008 for Doxorubicin

Standard Operating Procedure for Doxorubicin in Animals	
Health Hazards	<p>Doxorubicin, also known by the trade name Adriamycin or hydroxydaunorubicin belongs to the anthracycline family (polyketides containing a tetracene quinone ring structure with a sugar attached by glycosidic linkage), is a chemotherapeutic drug. Doxorubicin is an antibiotic anthracycline compound isolated from cultures of <i>Streptomyces peucetius</i> var. <i>caesius</i>. Doxorubicin works by inserting itself into (intercalating) DNA and preventing replication. Doxorubicin may also inhibit polymerase activity, affect regulation of gene expression, and produce free radical damage to DNA. The drug is used to treat a broad range of cancers. The drug is usually administered intravenously as the Doxorubicin hydrochloride salt or encapsulated as a polyethylene glycol coated liposomal particle.</p> <p>Statement of Hazard:</p> <p>In the clinical setting life-threatening heart damage is the most serious side effect of the drug (urine can turn bright red after treatment). Since the amounts used in animal research are far smaller than clinical doses, it is unlikely that anyone working with Doxorubicin or Doxorubicin treated animals would be exposed at the level that produces these effects. However, those with preexisting cardiovascular illnesses may be at increased risk from exposure. The heart problems may occur, months to years after exposure. Doxorubicin may cause a severe decrease in the number of blood cells in the bone marrow. 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 antineoplastic agents. Effects such as severe tissue death (necrosis) have been seen where Doxorubicin has been injected improperly and has leaked out of veins or been injected into surrounding tissue (extravasation).</p> <p><i>Doxorubicin is a mutagen, carcinogen, and teratogen</i>, and is highly irritating to the eyes, skin, mucous membranes, and upper respiratory tract. The toxic effects of doxorubicin may be experienced if swallowed, inhaled, ingested, or exposed to the skin</p> <p>As a precautionary measure, keep away from strong oxidizers (such as bleach).</p> <p>*Pregnant women should not be exposed to or handle this cytotoxic drug in any form.</p>
Designated Area	ABSL2 Facility (Animal Biosafety Level 2)
Training Requirements	Hazardous chemical training and training on this SOP is required before working with Doxorubicin. 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
Personal Protective Equipment (PPE)	<p>Double nitrile gloves or compatible chemical-resistant gloves, Chemical safety goggles, Lab coat and mask.</p> <p>Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p> <p><i>There are no established safe levels of exposure to cytotoxic drugs. Medical opinion is that even small quantities of cytotoxic drugs and their metabolites should be avoided as much as possible. The safest approach therefore is to reduce occupational exposure to levels as low as reasonably achievable (ALARA)</i></p>
General Precautions for Animal Use	<p>Tools should be adapted for BSL-2 (use safety syringe, blades, and needles where possible). Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p> <p>Exposure may occur during preparation and administration of the drugs, handling of body fluids from animals receiving cytotoxic drugs, handling and disposal of cytotoxic wastes and related trace contaminated material, and transportation of cytotoxic drugs. Some cytotoxic drugs have a direct irritant effect on the mucous membranes, eyes and skin. Spills onto skin surfaces that have cuts or abrasions and punctures of the skin with a contaminated needle or broken glass can lead to severe soft tissue injury. They should be treated immediately and observed for potential problems.</p> <p>Doxorubicin may be excreted by the animal post injection; therefore the bedding is considered contaminated.</p>
Environmental / Ventilation Controls	<p>A. The preparation of Doxorubicin including reconstitution, weighing, and diluting should be performed in a chemical fume hood. Work should be done over absorbent pads.</p> <p>B. Whenever possible, doxorubicin should be purchased in sealed rubber capped vials so that it can be solubilized by injecting water into the vial cap without having to open the vial</p> <p>C. Work with animals should be conducted in ABSL-2 facility, over absorbent pads in a Class II type A2 BSC.</p>
Special Handling Procedures & Storage Requirements	<p>A. Handling: Doxorubicin should be handled in containment and done over absorbent pads. Following preparation of Doxorubicin, the work area should be thoroughly cleaned with soap and water or with distel. Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity, Leurlock syringes are recommended). The fume hood or other approved containment must be cleaned upon completion of tasks. Following preparation of Doxorubicin, the work area should be thoroughly cleaned with soap and water or with distel. Any visible contamination or spills should be cleaned with distel and then washed with water. Any wipes contaminated with Doxorubicin must be disposed as Cytotoxic hazardous waste.</p>



	<p>* DO NOT use bleach for disinfection of work surfaces where Doxorubicin has been used.</p> <p>B. All equipment must be decontaminated prior to removal from the room housing the infected animals.</p> <p>C. Wash hands thoroughly after handling doxorubicin</p>
<p>Precautions for Animal Use</p>	<p>D. Animals should be restrained or anesthetized during injection.</p> <p>A. NO recapping needles.</p> <p>B. Have a sharps container in close vicinity.</p> <p>C. Once Doxorubicin is injected, animals, animal waste and cages are considered hazardous.</p> <p>D. Hands must be washed upon exiting animal room</p>
<p>Animal Handling Practices</p>	<p>A. Animals must be housed in filter top cages marked as biohazards (including the name of the chemical hazard, Doxorubicin), in negative pressurized IVC.</p> <p>B. Handling the cages (including bedding) will be done only by the researchers at least for 7 days after the last administration and first cage change.</p> <p>C. Use a class II A2 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.</p> <p>D. Injection animals with: Doxorubicin:</p> <ol style="list-style-type: none"> 1. Injection of: Doxorubicin will be conducted within Class II A2 BSC. 2. All needles will be disposed of in sharps container - do not recap or bend needles. 3. Injected animals considered hazardous for 7 days after each administration of Doxorubicin. 4. Take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room 5. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time. 6. Dead animals must be placed in primary plastic bags, and then will be transferred to incineration. 7. All surfaces and racks that may be contaminated will be decontaminated with detergent solution followed by water ASAP. 8. The first cage change after each drug administration is to be done no sooner than 7 days after the administration. The bedding is considered contaminated and requires special handling. <p>E. When changing cages use the following technique:</p> <ol style="list-style-type: none"> 1. Transfer the animals to clean cages 2. Insert the used cages in a plastic bag. 3. Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked "Hazardous Chemical - Doxorubicin".



	<ol style="list-style-type: none"> 4. 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). 5. If local ventilation controls are not available for opening cages or dumping bedding, a 3M8835 respirator (or other P3 respirator) and safety goggles must be worn. 6. All contaminated bedding will be labeled as hazardous materials and handled accordingly: incinerated or placed in chemical waste bags for disposal. 7. After this first cage change 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 Doxorubicin. 8. The cages should then be put in plastic bags (marked "Toxin - : Doxorubicin") and sealed for transport to the washroom. 9. 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 Doxorubicin contamination.
<p>Spill and Accident Procedures</p>	<ol style="list-style-type: none"> A. Spills must be cleaned immediately by properly protected trained personnel. B. Minor Liquid Spills: should be cleaned 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. C. Powder/Major Spills: should be cleaned by personnel wearing a gown, goggles, and chemically resistant gloves. 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 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 <u>from entering drains</u>. D. Exposure: <ul style="list-style-type: none"> • <i>In case of skin contact</i> or injection with Doxorubicin, wash the affected area with soap and water for at least 15 minutes. Consult with a Medical doctor in an Emergency Room (ER)



	<ul style="list-style-type: none"> • For eye exposure, 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.
Waste Disposal	<p>Dispose all waste material in the appropriate chemical waste container. Unused solutions of Doxorubicin and contaminated solid waste will be disposed of as hazardous chemical material.</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Doxorubicin in animals and agree to follow these procedures.</p>	
<p>Name: _____ Title: _____</p>	

*** Note**

In humans Doxorubicin is excreted primarily in the feces, but also minute amount is eliminated in urine. Due to enterohepatic recirculation the drug excretion continues for several days. At these times there is insufficient information to determine the point at which excretion becomes negligible in rodents. Therefore the 7 days guideline is based on human clinical practice. As a general rule, substances are cleared from the bodies of rats and mice rapidly than from human patients.