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|  | Standard Operating Procedure for Cyclophosphamide (CPX) in Animals |
| **Health Risk**  |  [Cyclophosphamide SDS](https://www.sigmaaldrich.com/US/en/sds/SIGMA/C7397) * CPX is used to treat cancers, autoimmune disorders, and AL amyloidosis. Like other alkylating agents, CPX is teratogenic and contraindicated in pregnant women.
* CPX is converted by the liver into two cytotoxics, acrolein and phosphoramide both are active compounds, that slow the growth of cancer cells by interfering with the actions of DNA within the cancerous cells.

**Statement of Hazard*** **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.**
* **Pregnant women should not be exposed to or handle this cytotoxic chemical in any form as it can cause damage to unborn child.**
* **Infertility due to exposure can affect both females and males.**
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| **Designated Area-North Skaggs Building,** **Room # 028** | * LAR Facility North Skaggs 028 (SB028), accessible by key only (see LAR or IACUC Manager for authorization).
* Designated chemical hazard room.
* A copy of this protocol will be posted outside the SB028 entrance.
* Only research staff and PI will be allowed in SB028 upon start of any study using CPX and until the room has been decontaminated (as instructed by UM EHS) and cleared via wipe-test.
* PI or research staff designated by PI must be available 24 hours a day 7 days a week in the event of an emergency in LAR, such as HVAC/power failure or other disaster. LAR staff will not be responsible for animals on study in SB028. Contact information must be given to LAR manager and posted outisde SB028.
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| **Training** | **Undergraduate students are not permitted to work with CPX or have access to SB028.** * PI and Research staff working with CPX must confirm healthcare insurance coverage and eligibility for the MUS Worker's Compensation Program.
* Research staff and PI are required to review and sign this SOP annually (a copy will be submitted to Occupational Health).
* Prior to being added to the animal use protocol, new research staff who will be handling CPX must review the SOP with the PI both parties must sign the document. A copy of the signed SOP with be sent to Occupational Health. Curry Health physicians and UM occupational health officers retains the right to interview new staff members in person prior to allowing work with CPX in LAR.
* New research staff will be required to submit an Occupation Health Risk Assessment form to Occupational Health.
* Hazardous cytotoxic training and training on this SOP is required before working with CPX. This should include, but is not limited to, reviewing the SDS, training on the physical hazards of the cytotoxic drugs, symptoms of exposure, appropriate work practices, and proper use of PPE. The PI is responsible for training personnel on the hazards of working with CPX.
* The UM Biosafety Officer will perform training and walk-though of SB028 prior to first use of CPX.
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| **Personal Protective Equipment (PPE)** | **Required PPE provided by the PI:*** **Disposable, spill-proof gowns**
* **Protective sleeves**
* **Vented safety goggles**
* **Double ASTM D6978 chemotherapy certified gloves (NO exceptions will be made for the types of gloved used)**
* **Fit-tested N95 (research staff working with CPX must have annual N95 fit test performed by UM EHS)**

Hands must be washed upon exiting SB028.A respirator fitted by UM EHS must be worn for all procedures utilizing CPX that cannot be performed within a BSL-2 cabinet. |
| **General****Precautions and Routes of Exposure** | * **There is no known medical treatment for exposure to cytotoxic drugs only supportive care and limited monitoring.**
* **The main routes of exposure to cytotoxic drugs are through the inhalation of drug particles or aerosols, skin absorption, inadvertent ingestion through contact with contaminated food or cigarettes, and needle stick injuries.**
* **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.**
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| **Environmental / Ventilation** | The preparation of CPX including reconstitution, weighing, and diluting should be performed in a fume hood or biological safety cabinet (class II Type B). Work should be done over absorbent pads. **Following preparation of CPX, the work area should be thoroughly cleaned. A 2% bleach solution can be used to deactivate cyclophosphamide followed by rinsing the surface with water, more than one round of cleaning may be required to decrease the detection level of CPX.** |
| **Special Handling Procedures & Storage Requirements** | Handling: * **Work must be conducted in Chemical Hazard Animal facility (SB028), over absorbent pads in a class II type B biological safety cabinet (BSC).**
* Any visible contamination or spills should be cleaned with 2% bleach solution and then washed with water. Paper towels or absorbent pads used to clean up spills must be disposed as Cytotoxic hazardous waste.
* Utilize safe sharps procedures (i.e., sharps container in the immediate vicinity, Leurlock syringes are recommended). BSC must be cleaned upon completion of tasks with 2% bleach solution.
* Any laboratory equipment or surfaces that have come in contact with CPX must be disposed of (as cytotoxic waste) or decontaminated with 2% bleach solution.
* When transporting CPX, the vials should be placed in sealed and labeled plastic containers.
* All equipment must be decontaminated prior to removal from the room housing the infected animals or disposed of as cytotoxic hazardous waste.

All needles will be disposed of in sharps container kept in BSC– do not recap or bend needles. Label sharp container as cytotoxic waste. Contact EHS for disposal of sharps container at the end of use.  |

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| **Animal handling practices** | 1. Animals MUST be housed in DISPOSABLE cages, supplied by the Investigator and labeled with the following (along with standard animal cage card required information):

 **CHEMICAL HAZARD** **Name of Chemical Hazard Agent**  **Approximate dose** **Date of administration** **Date 72hr bedding change will occur**1. Handling of cages, daily checks and husbandry will be done by researchers and not LAR staff for the duration of housing in SB028.
2. Class II Biological Safety Cabinet will be used at all times when performing work on animals (ie during injections or procedures) or when moving animals from disposable cages to clean cages.
3. **Animals, cages and bedding are considered hazardous for a minimum of 72 hours after each administration of CPX; take precautions to avoid the creation of aerosols. A respirator must be worn for personnel that if work is done outside the ventilated cabinet.**
4. Animals that die within the 72hr after CPX dosing are to be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration. Place contaminated animal carcass in freezer located in SB028 and contact EHS for disposal. Bags must be labeled with "Cytotoxic-CPX" and separated from other non-contaminated carcasses.
5. Euthanasia of animal within 72hr of dosing with CPX will be done via an isoflurane drop jar, provided by PI, followed by a secondary method and stored as stated in #5 above.
6. During CPX administration, mice will be house in disposable cages. Animal cages and bedding are considered hazardous for a minimum of 72hrs after drug administration. No sooner than 72h post CPX injection, mice can be transferred from disposable cages to “standard” LAR cages, transfer of mice will be done in BSC.

When transferring from disposable cages to standard cages, use the following technique:* + Done in biosafety cabinet, one box at a time
	+ Transfer the animals from disposable cage to clean standard LAR cages
	+ Insert the used disposable cages in a clear plastic biohazard bag, double bagged
	+ Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked “Cytotoxic- CPX".
	+ For disposable of cytotoxic waste please contact EHS

*Disposable cages avoid CPX aerosolization and exposure as standard cage changes and cage washing is not necessary.*1. Disposable cages are double bagged in clear Biohazard bags, provided by LAR, store in large biohazard bin that is labeled as cytotoxic waste. Contact EHS for disposal.

**Once mice have been moved to a standard cage, if needed mice can be moved to another LAR room or the BSL-2 room in HS for further experimentation. The following must be done:**1. **Approval must be given by LAR Manager before mice are moved.**
2. **Spray outside of cases with Peroxigard.**
3. **Cover cages with cage cover located at LAR entrance before moving cages through hallways or outside.**
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| **Spill and Accident Procedures** | * Spills must be cleaned immediately by properly protected trained personnel wearing a gown, goggles, two pairs of ASTM D6978 chemotherapy certified gloves and a N95 respirator if in BSC, or ½ mask APR if outside approved containment.
* **Minor Liquid Spills**: should be cleaned immediately by personnel wearing a PPE. Use absorbent pads to wipe liquid. The spill area should then be cleaned thoroughly with 2% bleach solution *(allow at least 15 minutes*) and then wash the area with soap and water. Place waste in plastic bag and then in the cytotoxic waste container.
* **Powder/Major Spills**: should be cleaned immediately by personnel wearing a PPE. 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 googles, should also be worn. Contain or absorb spill with absorbent material, it may be helpful to lightly wet the absorbent material. Wipe the area with 2% bleach solution 1-2 times *(allow at least 15 minutes*) and then wash the area with soap and water.
* Collect and place waste in plastic bag and then in the cytotoxic waste container.

Exposure: * **In the event of an emergency call 911 or campus police at x4000.**
* **In the event of CPX exposure, wash the affected area as described below and then proceed immediately to an emergency room to receive medical care.**
* **In case of skin contact or injection with CPX, wash the affected area with soap and water for at least 15 minutes.**
* **For eye exposure, flush with water for at least 15 minutes. An eye wash station is located in SB028.**
* **If exposed, prepare and file a report with the MUS Worker's Compensation Program and UM's Biosafety Officer.**
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| **Cleaning and decontamination of S028 at the completion of use** | * Clean all surfaces with 2% bleach solution followed by rinsing with water (i.e., animal rack, biosafety cabinet (both inside and out), sink, door handle).
* Clean biosafety cabinet (both inside and out) with Surface Safe two applicator kit (following directions on package). Surface Safe will be supplied by LAR.
* Perform CPX wipe testing on the following surfaces; cage rack, door handles, BSC (inside and out), sink also include a negative control. Testing to be done at Bureau Veritas (or other designated service provider) after completing the chain-of custody form (contact Biosafety Officer for sampling protocol instructions and questions), results will be sent to LAR Manager and IACUC Manager/Biosafety Compliance Officer.
* Sweep the room.
* Mop the floor with Peroxigard diluted to 1:64 in water (2 oz concentrate per gallon of water).
* Dispose of any generated waste: place waste in clear Biohazard bags and double bagged, stored in large biohazard bin that is labeled with "Cytotoxic-CPX" and contact EHS for disposal.
* Dispose of any animal carcasses: place animal carcass in freezer located in SB028 and contact EHS for disposal.
* Inform LAR Manager that room is clean and decontaminated. Room will remain the responsibility of the PI until swipe-test resulted have deemed room clear of CPX.
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|  **Waste Disposal** | Contact EHS for disposal of CPX waste and for any questions (406-243-4504). |
|  **Investigator Responsibility** | * Research Staff must inform LAR Manager prior to use of SB028.
* Hazardous Sign must be posted on door of SB028.
* Staff performing work is properly trained and have read and understand both the SOP and animal use protocol.
* CPX work, including cage changes and daily monitoring, emergency response, will be performed by researchers, not LAR staff.
* Assume cost of disposable animal cages.
* Wipe testing for CPX surface contamination will occur at the completion of the study. Investigator will assume cost.
* Cleaning and decontaminating SB028 after the completion of study.
* Inform LAR Manager study is complete and room is cleaned. Remove any hazardous signage.
* Research staff may NOT include undergraduate students or personnel that does not have health insurance coverage.
* Personnel associated with UM Occupational Health, EHS, and Biosafety programs have plenary access to SB028 to confirm compliance with this SOP.
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**I hereby acknowledge that I have read this Standard Operating Protocol and understand the risks associated with the use of cyclophosphamide (CPX). I further acknowledge that performing research with cyclophosphamide (CPX) is not a condition of employment at the University of Montana and that I may decline to participate in any such work.**

**I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with**

**CPX in Animals, and agree to follow these procedures.**

Personnel:

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| Name: | Title: |  |
| Signature: |  | Date: |

Principal Investigator:

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| Name: | Title: |  |
| Signature: |  | Date: |