Animal protocols involving hazardous chemicals must be planned and conducted appropriately in order to minimize the potential exposure to research personnel, facility animal care staff, and veterinarians. Hazardous chemicals used in laboratory animals, include known or suspect carcinogens, reproductive toxins or highly toxic substances (e.g. anti-neoplastic agents) and nanomaterials. The very nature of antineoplastic agents make them harmful to healthy cells and tissues as well as cancerous cells, even if they are FDA approved for use in human and/or animals. Animals that have been dosed with a toxic chemical may excrete that chemical or their potential toxic metabolites, particularly for seventy-two (72) hours after the last chemical administration. The following procedures are designed to ensure that individuals are:

1. Informed of the potential hazards;
2. How to minimize exposure when performing duties associates with protocols using toxic drugs.

As with any laboratory operation, the Principal Investigator (PI) and researchers must identify and understand the hazards associated with the chemical(s) being used (e.g. toxicity, reactivity, flammability, corrosivity, etc.) before they begin their work so that appropriate controls can be established. This information may be available from Safety Data Sheets (SDS) and other sources of safety information. It is important to understand all the hazards of the chemical and any other special considerations that may be required prior to beginning work. Research staff may be exposed to hazardous chemicals during preparation, handling, and animal dosing. These chemicals may be excreted from the animal and, therefore, be present in the animal’s bedding in low concentrations. Researchers, animal care staff, and veterinarians may be exposed to these hazardous substances or their metabolites during cage handling or handling of medicated water and/or feed. The Institutional Animal Care and Use (IACUC) protocols must be carefully reviewed by EHSO (Environmental Health and Safety Office) for activities occurring in facilities where animals are housed or used to determine if the proposed chemicals are to be considered hazardous. Additional measures to prevent human exposure and contamination will be implemented for all hazardous chemicals. It is incumbent upon the PI to provide accurate hazard information about the research conducted and chemicals used and to comply with these standard procedures and any procedures specifically developed for their protocols. Failure to do so could results in the non-compliance being reported to the respective Federal agency as well as other negative consequences to the University.

This policy describes required procedures that the PI and the individuals involved with the care of animals must follow for:

A) Completion of the IACUC Protocol
B) Coordination with IACUC and EHSO
C) Hazardous chemical preparation and handling and animal dosing
D) Cage management and disposal

Principal Investigator’s Responsibilities:

1. The PI must provide a list of chemicals and information related to their hazards, e.g. Safety Data Sheets (SDS) and/or literature citation on the prior use of the particular chemicals in animals. This information must be included in the UH IACUC protocol. The information should include but not limited to the following:
   a. Specific health risks to humans and animals from possible exposure.
   b. Proposed precautions to be taken to protect people and animals.
   c. Any information on recommended medical surveillance and/or use of antidotes.
   d. Information on how the chemical is metabolized in a specific animal species. Animals dosed with hazardous chemicals may excrete that chemical or metabolites, particularly during the first 72 hours after dosing. A review of peer-reviewed literature may provide this information if documented. Otherwise, in the absence of data, conservative measures will be required.
2. The PI shall provide protocol specific training to his/or staff, and the animal care staff prior to start of the project.
3. Must coordinate the use of the chemical with facility animal care Supervisor or designee prior to start of project (i.e. two (2) weeks before beginning dosing project). Coordination includes confirmation of facility availability; room/facility assignment and provisions for appropriate cage labeling and waste management.

4. Must comply with health and safety requirements set forth by this policy, including the development of standard operating procedures (SOPs) when working with specific chemicals. At a minimum, the SOP should describe:
   a. Use of a chemical fume hood or other appropriate engineering controls.
   b. Appropriate personal protective equipment (PPE).
   c. Methods to restrain or sedate animals per IACUC protocol to reduce the possibility of accidental self-inoculation.
   d. Administration of chemical and methods to minimize risk of accidental exposures (e.g. use of safety syringe).
   e. Methods to be used to clean-up spills and decontaminate lab surfaces and equipment using wet wiping methods and an appropriate cleaning agent.

Training of staff on SOP (ensure research personnel are trained on the SOPs and specific hazards associated with the chemicals. Maintain training documentation). Include a subject matter expert from EHSO to attend the meeting.

**Environmental Health and Safety Office (EHSO) responsibilities:**

1. Will review the information provided by the PI to determine if it is a hazardous chemical. If it is hazardous, advise on:
   a) Specialized personal protective equipment (PPE) in addition to the standard, double nitrile gloves and front closing gown used during chemical administration, animal handling (opening cages), cage changes, waste collection, etc. as described in Work Practices.
   b) Waste disposal. If the method of disposal includes segregating and collecting the bedding for disposal as hazardous waste, facility animal care Supervisor will work directly with EHSO to determine how the waste will be stored while awaiting disposal by a licensed hazardous waste contractor. If the waste is a mixed waste (e.g. biological and chemical), procedures will be determined in collaboration with the Biosafety Office and EHSO.
2. Will review proposed IACUC protocol and provide their comments/recommendations regarding the protocol to the UH IACUC.
3. Will provide appropriate general training such as Laboratory Safety, hazard communication, etc.

**IACUC and Animal Facility Responsibilities:**

1. IACUC incorporates EHSO’s comments/recommendations into their review and approval process.
2. IACUC ensures that the protocol approval letter informs the PI of special handling and disposal methods of chemically contaminated animal carcasses, cages and bedding, and other associated wastes during the 72-hour period following the last chemical administration.
3. The facility animal care Supervisor or designee will inform PI of any additional costs associated with special husbandry procedures and hazardous disposal of animal carcasses, and/or waste that will be charged back to the PI if the method of disposal exceeds what is covered by the current per diem rate for the species.
4. The facility animal care Supervisor or designee will document and archive all training records for individual protocols involving hazardous chemical dosing in animals.

Work Practices for Operations with Chemically Dosed Animals: (See Manoa & JABSOM EHSO SOPs for Tamoxifen and Azoxymethane (AOM) attached)

1. Personal Protective Equipment (PPE) and Use:
   a. Double, disposable, nitrile glove shall be worn when handling contaminated animals and bedding. Gloves shall be inspected for tears or holes prior to donning, changed frequently, and not reused.
   b. PPE specific for the hazard is doffed before exiting the room where chemicals in animals are used.
   c. Individuals shall wash their hands, or use waterless hand soap immediately after removing gloves.
   d. A closed front, wrap around gown shall be worn when handling contaminated bedding. Gowns are disposed of after each use. Plastic apron, rubber boots or disposable booties may also be needed.
   e. A face shield or safety glasses shall be worn when handling contaminated bedding. Individuals wearing contact lenses MUST wear safety glasses. If there is potential hazard from chemical splash, then chemical goggles must be worn in lieu of safety glasses.
   f. If respirators are recommended and/or required, the appropriate elements of the Respirator Protection Program must be implemented. Contact EHSO for guidance on respirator use.
   g. Disposable gowns, gloves, respirators and paper towels are disposed of as hazardous waste.

2. Engineering Controls:
   a. Cages shall be equipped with filtered micro-isolator tops, preferably high efficiency particulate air (HEPA) filters, 0.2 um pore size.
   b. For all cages opened to manipulate animals and soiled bedding during the treatment period and up to 72 hours after the last administration, is done in a biosafety cabinet (BSC) or fume hood. Animal care staff will collect bedding in clear, polypropylene bags and label with agent name and date.
   c. Decontamination of the BSC or fume hood shall consist of surface cleaning with water and detergent followed by a thorough rinsing with clean water. In some cases, regular vivarium cleaning with clidox 1:18:1, or equivalent, followed by 70% isopropyl alcohol may be sufficient. Cleaning shall proceed from the least to the most contaminated areas.

3. Signage:
   a. When animals are dosed with a toxic chemical, their cages must be labeled with:
      i) Name of Principal Investigator
      ii) Chemical name and chemical hazard warning sign
      iii) Date and time of chemical administration
   b. If hazardous chemicals are administered by water/feed, also label the water bottle/feeder with the above information.
   c. Maintain label on cage, water bottle/feeder for 72 hours after the last dosing AND until contaminated bedding is changed, unless longer time frames are required as identified in the risk assessment.
   d. The animal care staff are responsible for posting animal SOP signs on rooms housing dosed animals. Signage on door must include the following:
      . Name of hazardous chemical
Hazardous Chemicals in Animals Policy
First Issued: 6/15/06, Revised: 5/1/07, 7/18/07, 9/17/09, 5/19/14, 6/4/15, 9/22/17, **10/12/21**
IACUC Approved: 6/15/06, 9/17/09, 6/18/15, **10/19/17**

4. Disposal:
   a. Disposal of residual and unused chemicals and solutions as well as animal bedding will be determined by EHSO.
   b. For disposal of sharps, including syringes, refer to: [www.hawaii.edu/ehso/complaince/waste2htm#sharps](http://www.hawaii.edu/ehso/complaince/waste2htm#sharps) or for questions, contact Biosafety Office or EHSO.
   c. Disposal of carcasses by animal care staff through alkaline hydrolysis (tissue digester) or equivalent.
   d. Disposal of bedding by animal care staff shall follow one of the below methods as determined by EHSO:
      i. For all bedding during the treatment period and up to 72 hours after the last administration, animal care staff will collect bedding in clear, polypropylene bags, label with agent name and date, and turn into EHSO for disposal as regulated waste.
      ii. For all bedding during the treatment period and up to 72 hours after the last administration, animal care staff will collect bedding in clear, polypropylene bags, label with agent name and date, and dispose of in the regular trash.
Tamoxifen SOP

Principal Investigator (PI):

Start Date and Duration of Project: Room #:

Approximate # of Cages: Housing:

Hazardous Chemicals Used: Tamoxifen

Tamoxifen is a white, odorless, crystalline solid with a melting point of 140-144 degrees Celsius. It is an antineoplastic agent used to treat breast cancer and is used in campus laboratories and animal facilities for cancer research studies. This document establishes procedures for the safe handling and use of Tamoxifen (CAS# 10540-29-1). Tamoxifen is a known carcinogen (IARC Group 1), toxic, and is considered a reproductive hazard. Pregnant women should not be exposed to or handle this chemical in any form. The Oral LD50 for Tamoxifen is 4,100 mg/kg. AVS staff shall read the safety data sheets (SDS) for all chemicals listed. SDSs are provided by the principal investigator (PI). Institutional Animal Care and Use Committee (IACUC) protocols that include Tamoxifen should reference this SOP to verify that the standard operating procedures are being followed.

Acute Health Effects
- Eyes: Irritation
- Skin: Irritation
- Ingestion: Harmful if swallowed
- Inhalation: Irritation of the respiratory tract.

Chronic Health Effects
Antineoplastic drugs may be potential carcinogens, mutagens, teratogens (harms the fetus), and reproductive hazards.

Regulatory Limits
There are no current established occupational exposure limits for Tamoxifen. 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.

Notification
PI group will email the Operations Manager (wongmich@hawaii.edu) to notify AVS of drug administration 24 hours in advance to request chemical caging if necessary. PI group will fill out the chemical hazard label and place it on the cage card holder. All information will be present, including cage card number, chemical used, start and end dates.

Method of administration:

Cage Labeling
- Chemical Hazard Label
- Tamoxifen
- Date Started

If the PI needs to terminate a cage during the treatment period, date the granite card and turn it in as normal, leave the chemical treated cage on the rack and write “terminated” on the chemical label so AVS knows how to dispose of the cage bedding. Do not place the cage on the floor with other cages.

Disposal Procedures for Carcasses
Place carcasses in the “hazard bin” in the procedure room freezer. Regular disposal in the tissue digester.

Expected Observations of Animals:

What AVS should do if a sick animal is found:

What AVS should do if a dead animal is found:

PI and Technician Contact Information:
General Information Continued

The main routes of exposure to cytotoxic materials are through the inhalation of the material’s 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 material, handling of body fluids from animals receiving cytotoxic drugs, handling and disposal of cytotoxic wastes and related trace contaminated material, and transportation of cytotoxic materials. Some cytotoxic materials 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.

- Review the Product Safety Data Sheet (SDS) prior to use of Tamoxifen.
- Personnel should not work with Tamoxifen if skin is cut or scratched.
- Pregnant or breast-feeding women should not work with Tamoxifen.

Chemical Preparation:

- Dose preparation work must be conducted in a chemical fume hood.

Animal Handling and Dosing Controls:

- Upon first dosage with Tamoxifen and all following handling, cages MUST be opened in the procedure room Biosafety Cabinet. Dosing of animals in a chemical fume hood or ducted (total exhaust) class II, type B2 biological safety cabinet (BSC) is recommended for maximum worker protection.
- Alternatively, when exhaust ventilation is unavailable, the use of a non-ducted class II, type A2 BSC, OR personnel using a powered air-purifying respirator (PAPR) may be considered. The PAPR must be fitted with organic vapor cartridges and p-100 filtration.
- PI will put mice in clean cage bottoms the first day of drug administration. Change filter top to a chemical, HEPA filtered lid. Treat / Dose mice in the BSC. Note on chemical hazard label the agent name and date. Please notify AVS for each cage treated. Where possible work on absorbent pads.
- Contaminated sharps must be placed in puncture proof and leak proof sharps containers. Label as Tamoxifen Sharps.

PPE:

- When in AVS facilities, PPE use must be consistent with the facility policy.
- Consider double gloving (nitrile or compatible cytotoxic-resistant gloves) especially when cleaning. Double gloving is also recommended during animal dosing if dexterity can be maintained.
- Always wash hands thoroughly after handling Tamoxifen.

Cage Changes / Wash Out Period:

- Cage bedding cleanout and waste processing work must be completed in either a chemical fume hood, ducted (total exhaust) class II, type B2 biological safety cabinet (BSC), OR in a non-ducted class II, type A2 BSC, OR personnel using powered air-purifying respirators (PAPRs). The PAPR must be fitted with organic vapor cartridges and p-100 filtration.
- AVS will change the cages in the procedure room BSC during the treatment period. AVS will remove the chemical label and change back to a normal filter top during the last cage change at least 72 hours after the chemical is last administered.
- Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time.
- Consider using compressed cotton fiber bedding pads (iso-PADS) instead of standard bedding. The pads are very absorbent, will minimize the creation of aerosols and disposal is easier. AVS will collect bedding in clear polypropylene bags, label with “Agent name and date” and turn into EHSO.

Normal Cleaning:

- Decon areas where Tamoxifen is prepared and/or administered. The area must be cleaned and decontaminated with a 10% bleach solution immediately following each task. Leave the bleach solution in contact with surfaces for 5 minutes. After wiping up the bleach solution, clean the surface with soap and water. Potentially contaminated areas include bench tops, biological safety cabinet interiors, equipment, reusable personal protective equipment, intravenous bags, and tubing.
- Decon chemical fume hood or BSC after cleaning cages.
- Glassware and other non-porous materials may be decontaminated by soaking them in bleach.

Small Spills (<10ml):

- Spills inside of a chemical fume hood can be controlled by closing the sash to its lowest level. Clean up with dilute bleach followed by soap and water. Collect spilled material and clean up material into appropriately labeled, nonmetallic waste containers. All spill clean-up material should be disposed of as hazardous waste.
- Spills outside of a chemical fume hood must be covered with a paper towel and sprayed / soaked with a 10% bleach solution immediately.
- Do not attempt to clean-up if you feel unsure of your ability to do so or if you perceive the risk to be greater than normal laboratory operations.

Accidental Release (Large Spills):

- If a large spill occurs, notify others in the area and evacuate the room immediately. Contact EHSO during working hours and 6-6911 if after hours.

Accidental Exposure:

- Needle stick or animal bite - Return rodent to cage and wash area for 15 minutes. Immediately notify your supervisor. Seek medical advice immediately about Tamoxifen exposure. Take a copy of the product SDS with you.
- An emergency eyewash station and safety shower should be accessible nearby where Tamoxifen is handled.

Storage, Transportation, and Waste

- Keep containers tightly closed in a cool, dry, and well-ventilated area. Recommended storage temperature 2 - 8 °C. Light sensitive.
- When transporting Tamoxifen, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers. Contact AVS Staff and PI for waste procedures.
Azoxymethane (AOM) SOP

General Information
Azoxymethane is a clear oily liquid. It is a highly toxic and potent carcinogen used to induce colon cancer in rats and mice. It is a known teratogen that is harmful to the following organs: teeth, pancreas, liver, blood, central nervous system, large intestines, heart, nerves, and kidneys. Training on this SOP is required before working with Azoxymethane. This should include but is not limited to reviewing the SDS, training on the physical hazards of the cytotoxics, symptoms of exposure, appropriate work practices, and proper use of personal protective equipment (PPE). The Oral LD50 for Azoxymethane is 5.1 mg/kg.

Institutional Animal Care and Use Committee (IACUC) protocols that include Azoxymethane should reference this SOP to verify that the standard operating procedures are being followed.

Acute Health Effects
- Eyes: Irritation
- Skin: Irritation
- Ingestion: Fatal if swallowed
- Inhalation: Limited data; consult a physician

Chronic Health Effects
Antineoplastic drugs may be potential carcinogens, mutagens, teratogens (harms the fetus), and reproductive hazards.

Regulatory Limits
There are no current established occupational exposure limits for Azoxymethane. 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.

Notification
PI group will email the Operations Manager (wongmich@hawaii.edu) to notify AVS of drug administration 24 hours in advance to request chemical caging if necessary. PI group will fill out the chemical hazard label and place it on the cage card holder. All information will be present, including cage card number, chemical used, start and end dates.

Method of administration:

Cage Labeling
- Chemical Hazard Label
- Azoxymethane
- Date Started
- Date Ending
- Cage card number

If the PI needs to terminate a cage during the treatment period, date the granite card and turn it in as normal, leave the chemical treated cage on the rack and write “terminated” on the chemical label so AVS knows how to dispose of the cage bedding. Do not place the cage on the floor with other cages.

Disposal Procedures for Carcasses
Place carcasses in the hazard bin in the procedure room freezer. Regular disposal in the tissue digester.

Expected Observations of Animals:

What AVS should do if a sick animal is found:

What AVS should do if a dead animal is found:
Azoxyrnethane (AOM) SOP

Pi and Technician Contact Information:

General Information Continued
The main routes of exposure to cytotoxic materials are through the inhalation of the material’s 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 material, handling of body fluids from animals receiving cytotoxic drugs, handling and disposal of cytotoxic wastes and related trace contaminated material, and transportation of cytotoxic materials. Some cytotoxic materials 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.

- Review the Product Safety Data Sheet (SDS) prior to use of Azoxyrnethane.
- Personnel should not work with Azoxyrnethane if the skin is cut or scratched.
- Pregnant or breastfeeding women should not work with AOM.

Chemical Preparation:
- Dose preparation work must be conducted in a chemical fume hood.

Animal Handling and Dosing Controls:
- Due to the limited exposure data of AOM, dosing of animals in a chemical fume hood or ducted (total exhaust) class II, Type B2 biological safety cabinet (BSC) is recommended for maximum worker protection.
- Alternatively, when exhaust ventilation is unavailable, the use of a non-ducted class II, Type A2 BSC in conjunction with personnel using a powered air-purifying respirator (PAPR) may be considered. The PAPR must be fitted with organic vapor cartridges and p-100 filtration.
- Where possible work on absorbent pads.
- Contaminated sharps must be placed in puncture-proof and leak-proof sharps containers. Transport and store sharps containers in a chemical fume hood after use. Label as Azoxyrnethane Sharps.

PPE:
- Personnel must wear appropriate PPE for animal work.
- Consider double gloving (nitrile or compatible cytotoxic-resistant gloves) especially when cleaning. Double gloving is also recommended during animal dosing if dexterity can be maintained.
- Always wash hands thoroughly after handling Azoxyrnethane.

Cage Changes / Wash Out Period:
- Recommend 72 hours. Contamination is likely to persist indefinitely as the waste is adsorbed and absorbed by the bedding limiting evaporation.
- Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time.
- Consider using compressed cotton fiber bedding pads (iso-PADS) instead of standard bedding. The pads are very absorbent, will minimize the creation of aerosols and disposal is easier.
- Cage bedding cleanout and waste processing work must be completed in either a chemical fume hood, ducted (total exhaust) class II, Type B2 biological safety cabinet (BSC), OR in a non-ducted class II, Type A2 BSC in conjunction with personnel using powered air-purifying respirators (PAPRs). The PAPR must be fitted with organic vapor cartridges and p-100 filtration.

Normal Cleaning:
- Decon areas where Azoxyrnethane is prepared and/or administered. The area must be cleaned and decontaminated with a 10% bleach solution immediately following each task. Leave the bleach solution in contact with surfaces for 5 minutes. After wiping up the bleach solution, clean the surface with soap and water. Potentially contaminated areas include bench tops, biological safety cabinet interiors, equipment, reusable personal protective equipment, intravenous bags, and tubing.
- Decon chemical fume hood or BSC after cleaning cages.

Small Spills (<10mL):
- Spills inside of a chemical fume hood can be controlled by closing the sash to its lowest level. Soak a paper towel with a 10% bleach solution and cover the spill for 10 minutes. Collect the spill clean-up materials as hazardous waste.
- Spills outside of a chemical fume hood must be covered with a paper towel and sprayed/soaked with a 10% bleach solution immediately. Leave the bleach solution in contact with surfaces for 10 minutes. After wiping up the bleach solution, clean the surface with soap and water. Leave the area to allow AOM to dissipate if evaporation is suspected.

Accidental Release (Large Spills):
- Evacuate immediately and call 6-6911.

Accidental Exposure:
- Needlestick or animal bite - Return rodent to cage and wash area for 15 minutes. Notify your supervisor immediately. Seek medical advice immediately about Azoxyrnethane exposure. Take a copy of the product SDS with you.
- An emergency eyewash station and safety shower should be accessible nearby where Azoxyrnethane is handled.

Storage and Transportation:
- Keep containers tightly closed in a cool, dry, and well-ventilated area. Recommended storage temperature is -20 °C. Avoid strong oxidizing agents.
- When transporting Azoxyrnethane, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers.
- Contact AVS Staff and PI for specific waste procedures.