# Safe Operating Procedure of using Azoxymethane for Animal Research

**NOTE: You must read this entire document and both you and the Principal Investigator must sign it before commencing any work.**

Project Number (e.g. Tick@Lab): _____{ University SOP}_____
Principal Investigator/Supervisor: _____{ University SOP}_____
Room and Building where SOP is used: Wet labs, LAF labs_____

**Summary of How the Material/Equipment/Process will be Used**

Azoxymethane (AOM) is a non-steroidal anti-estrogenic drug recognized as a human carcinogen, with a high toxicity profile, including a risk of fatality upon ingestion. In animal research, AOM is administered to initiate colon cancer studies. The processes associated with the use of Azoxymethane include:
1) Preparation of Azoxymethane solution
2) Transport of Azoxymethane from research lab to LAF for administration
3) Administration of Azoxymethane into animals
4) Housing and handling of Azoxymethane administrated animals
5) Cage changing and washing of Azoxymethane contaminated cages
6) Disposal of Azoxymethane contaminated waste

**Potential Hazards**

Human Carcinogen Exposure (Process 1-6)
- May cause cancer (hazard statement H350)
- Flammable liquid and vapor (hazard statement H226)

Sickness or Irritation due to Ingestion, Inhalation or Skin Contact (Process 1-6)
- Fatal if swallowed (hazard statement H300)
- If swallowed, immediately call a poison center or seek medical attention (hazard statement H300)
- Causes skin irritation (hazard statement H315)
- Causes serious eye irritation (hazard statement H319)

Needle Stick Injury due to Injection (Process 3)
- Causes skin irritation (hazard statement H315)

Animal Bite (Process 3-5)

Azoxymethane SDS weblink: https://www.sigmaaldrich.com/HK/en/sds/sigma/a5486

**Safety Installations**

Engineering Control Measures
- Certified fume hood (FH) for Azoxymethane preparation (e.g. open, weight, reconstitute and dilute)
- Certified Class II Biosafety cabinet (BSC) for injection, animal handling and cage changing
- Secondary container for transport
- Luer lock syringes for administration
- Animal restraint devices for administration (if applicable)
- Forceps for animal handling
- Animal cage changing station for cage changing
- Individually ventilated cage (IVC) and air handling unit for housing
- Cage liners for housing
- Cage washing machines for washing
- Azoxymethane waste bin for disposal of contaminated waste
- Azoxymethane sharp box for disposal of contaminated syringes
- Eye wash station for emergency use
- Availability of Azoxymethane Safety Data Sheet (SDS) in handling areas

**Work Practices**

LAF Notification
- Inform LAF prior to using Azoxymethane in LAF for better arrangement

Medical Surveillance Program
- Enroll in medical surveillance program and indicate "toxic chemicals injected "

Training
- Complete Azoxymethane specific safety training provided by the PI
- Complete HSEO MC03 Chemical I, MC07 Chemical II, MC06 Biological Safety Training
- Complete general animal user training and animal handling training provided by LAF

SOP
- Establish and follow in-house SOPs for specific process (e.g. Azoxymethane administration, waste collection, cage washing, etc.)

Labelling and Warning Sign
- Complete LAF blue cage card "Health Hazard Card" with administration information and place in each Azoxymethane administrated animal cage until 10 days post-administration.
- Affix a Azoxymethane warning sign with hazard information on each of the Azoxymethane administrated animal holding room
- Label all Azoxymethane tubes, containers, waste bin, waste bag with appropriate hazard warnings and chemical identification.
- Post a "Azoxymethane Hazard" sign on the FH and BSC which used for Azoxymethane preparation and administration

Proper Storage
- Store Azoxymethane in a lockable secondary container, away from light and avoid strong oxidizing agents
- Use zipper bags and secondary containers during transport to avoid spillage

Good Hygiene Practice
- Minimize exposure by implementing control measures and wear adequate PPE
- Clean work surfaces with detergent and water, followed by sodium hypochlorite and rinse thoroughly
- Wash hands immediately after handling Azoxymethane and Azoxymethane-contaminated waste

**Specific Experimental Procedures**

Preparation, Transportation and Administration of Azoxymethane (process 1-3)

- Purchase only the necessary quantity of Azoxymethane
- Wear adequate PPE when handling Azoxymethane
- Inspect the outer package of Azoxymethane to make sure it is intact
- Open, weigh and prepare Azoxymethane solution inside a certified FH
- A plastic-backed absorbent pad should be placed under the work surface during preparation and administration process to avoid contamination
- Place a waste bag on the work surface for collection of contaminated wastes
- Aliquot the Azoxymethane solution into leak-proof, screw cap tubes and place inside a secondary container labelled with chemical name and hazard warning labels. Store the container in a fridge.
- During transportation, Azoxymethane solution tubes must be stored in a zipper bag inside a secondary container (only bring the required amount to LAF)
- Conduct the administration inside a certified BSC
- Do not recap the syringes. Dispose of syringes used into Azoxymethane sharp box inside BSC
- Dispose of all contaminated wastes (e.g. tubes, tips, absorbent pad, syringes, etc.) into a waste bag inside FH/BSC and then discard into designated Azoxymethane chemical waste bin
- Clean work surfaces with detergent and water, followed by sodium hypochlorite and rinse thoroughly.

Housing and Handling of Azoxymethane-Administrated Animals (process 4)

- Complete LAF blue cage card "Health Hazard Card" with administration information and place in each Azoxymethane administrated animal cage
- Conduct cage changes within a BSC or Cage Changing Station (CCS)
- Each Azoxymethane administrated animal should return to a cage with cage liner and "Health Hazard Card", then house in designated animal holding room

Cage Changing and Washing of Azoxymethane-Contaminated Cages (process 5)

- After 10 days of last administration, the contaminated cage/water/feed can be changed inside a BSC or CCS
- Dispose all contaminated disposable waste into the cage liner inside BSC/CCS, and bag the cage liners into chemical waste bag (refer to waste disposal for more details)
- After disposing wastes, the "Health Hazard Card" can be removed and considered free of Azoxymethane
- Bag the non-disposable cage accessories' (e.g. lid, cage base, wire bar, etc.) in a bag for LAF collection
- LAF staff collect the bagged cage accessories to cage washing area for normal washing process

**Personal Protective Equipment**

Skin Protection, Eye Protection, Face Protection and Respiratory Protection

- Wear double layer gloves. One glove should be placed under the coat / coveralls and one over.
- Lab coat or coveralls
- Sleeve covers if wrist is exposed
- Safety glasses or chemical goggles or face shield
- N95 mask or PAPR if work is not conducted in BSC or fume hood

**Waste Disposal**

Azoxymethane-Contaminated Waste Disposal (Process 6)

- Discard all contaminated wastes during preparation and administration into waste bags.

**Double bags** all wastes.

- Tie up the waste bags and place in designated Azoxymethane chemical waste bin for HSEO collection
- The waste bin should be kept closed at all times
- For contaminated sharps waste box, place the sharp box in designated Azoxymethane chemical waste bin for HSEO collection
- For Azoxymethane-administrated animal carcasses, **double bags** before placing in clinical waste fridge for contractor collection

Bedding and Cage Liner Disposal (Process 6)

- Place the bedding and cage liners in a chemical waste bag, and tie up the bag
- Place the waste bag in designated Azoxymethane chemical waste bin for HSEO collection

**Spills and Incidents**

Minor Spill of Azoxymethane

- Absorb the spill with absorbent pad or paper towel with adequate PPE
- Cover, Clean work surfaces with detergent and water, followed by sodium hypochlorite and rinse thoroughly
- Collect the wastes in a plastic bag and dispose in a Azoxymethane-contaminated waste bin

Major Spill of Azoxymethane

- Avoid inhalation and generating dust
- Remove contaminated clothing with gloved hands, remove gloves and place near the spill
- Remove all ignitable sources. If a fire is visible, DO NOT turn on the emergency ventilation.
- Evacuate other workers within the laboratory.
- Call Security Control Center Ext 8999 for assistance and do not allow anyone to enter the affected area
- HSEO will be notified by Security Control Center to clean up the spill

Bites by Animals Treated with Azoxymethane

- Wash bite area with running water for 15 minutes
- Seek medical attention. Bring SDS to the clinic / doctor.

**Emergency Procedures**

Exposure

• If Azoxymethane comes into contact with the eye, face and skin, wash the area with running water for 15 mins using the nearest emergency facilities and seek medical advice immediately.

• Notify HSEO of any exposures.

Emergency Response

• Notify the Security Control Center by dialing ext. 8999 and provide information on the incident, including the chemical involved, the location, and any injuries.

**References**


- Azoxymethane Safety Data Sheet (SDS). Sigma-Aldrich. Retrieved February 20, 2025 from https://www.sigmaaldrich.com/HK/en/sds/sigma/a5486
- National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 33184, Azoxymethane. Retrieved February 20, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Azoxymethane.
- National Cancer Institute at Frederick (2014). Chemical Safety Practices Recommendations. Retrieved February 20, 2025 from https://ncifrederick.cancer.gov/ehs/Safety/Media/Documents/CSPR_Azoxymethane.pdf
- Panpan Wang, Yifei Jia, Rongrong Wu, Zhiqiang Chen, Ru Yan. Human gut bacterial β-glucuronidase inhibition: An emerging approach to manage medication therapy, Biochemical Pharmacology, Volume 190, 2021. ISSN 0006-2952. https://doi.org/10.1016/j.bcp.2021.114566.


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