

August 18

SOP # 001 for *7,12-Dimethylbenz[a]anthracene* (DMBA)

<b>Standard Operating Procedure for DMBA in Animals</b>	
1. Health Hazards	<p><i>7,12-Dimethylbenz[a]anthracene</i> (DMBA) is highly carcinogenic in experimental animals. Large single and multiple doses produce tumors of the skin, breast, and stomach or leukemia regardless of route of administration. Skin of mice is particularly sensitive to low, topically applied doses. DMBA is a strong mutagen after metabolic activation.</p> <p>OSHA Hazards - It is a carcinogen mutagen and teratogen substance. DMBA is absorbed through the skin and respiratory and intestinal tracts; and by intravenous and intraperitoneal injection, ingestion, and inhalation. It is carcinogenic and may irritate tissues and induce sensitivity</p> <p><b>Pregnant or breast-feeding women should not work with DMBA</b></p>
2. Physical & Chemical Properties/Definition of Chemical Group	<p>CAS#: 57-97-6 ; Molecular Formula: C<sub>20</sub>H<sub>16</sub> OSHA Hazards - Toxic by ingestion, carcinogen mutagen and teratogen substance. Form (physical state): Powder or Liquid (light yellow) Boiling point: 122-123 °C</p>
3. Designated Area	<b>ABSL2 Facility (Animal Biosafety Level 2)</b>
4. Training Requirements	Hazardous chemical training and training on this SOP is required before working with DMBA. This should include but is not limited to reviewing the MSDS, training on the physical hazards of the chemicals, symptoms of exposure, appropriate work practices, and proper use of PPE.
5. Personal Protective Equipment (PPE)	Double nitrile gloves or compatible chemical-resistant gloves, Chemical safety goggles, Lab coat and mask (3M8835). Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat. Personnel should not work with DMBA if skin is cut or scratched
6. General Precautions for Animal Use	Tools should be adapted for BSL-2 (use safety syringe, blades and needles where possible). Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection.

	DMBA may be excreted by the animals within the first 72 hours post injection therefore <u>only the lab staff</u> must change the bedding at least 3 days after administration.
7. Environmental /Ventilation Controls	<p>A. The preparation of DMBA including reconstitution, weighing, and diluting should be performed in a <b>fume hood</b> or <b>class II Type B biological safety cabinet (total exhaust cabinet)</b>. Work should be done over absorbent pads.</p> <p>B. Work with animals should be conducted in <b>ABSL-2 facility</b>, over absorbent pads in a class II type A1 or A2 biological cabinet</p>
8. Special Handling Procedures & Storage Requirements	<p>A. DMBA should be handled in containment and done over absorbent pads. Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity, Leurlock syringes are recommended).</p> <p>B. The fume hood or other approved containment must be cleaned and decontaminated upon completion of tasks.</p> <p>C. When transporting DMBA, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers.</p> <p>D. All equipment must be decontaminated prior to removal from the room housing the infected animals.</p>
9. Precautions for Animal Use	<p>A. Animals should be restrained or anesthetized during injection/topic application.</p> <p>B. NO recapping needles.</p> <p>C. Have a sharps container in close vicinity.</p> <p>D. Once DMBA is injected, animals, animal waste and cages are considered hazardous for a minimum of 3 days.</p> <p>E. Hands must be washed upon exiting animal room</p>
E. Animal Handling Practices	<p>A. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard), in negative pressurized IVC.</p> <p>B. Handling the cages (including bedding) will be done only by the researchers at least for 3 days.</p> <p>C. Use a class II Biological Safety Cabinet 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. <u>Injection/topical application with DMBA:</u></p> <ol style="list-style-type: none"> <li>Topical application of animals or IP injection with DMBA will be conducted within <b>Class II Type B</b> Biosafety cabinet or certified <b>Chemical fume hood</b> or other Local exhaust ventilation (e.g. <b>"snorkel or elephant"</b>(trunks) <b>hood connected</b> to building exhaust system).</li> <li>All needles will be disposed of in sharps container - <b>do not recap or bend needles.</b></li> <li>Infected animals considered hazardous for a minimum of 3 days after each administration of DMBA.</li> <li>Take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. <i>A respirator is recommended for personnel that are immunocompromised or pregnant and for healthy personnel if work is done outside the ventilated cabinet.</i></li> <li>Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time.</li> <li>Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious <b>waste incineration.</b></li> </ol>

	<p>7. All surfaces and racks that may be contaminated will be decontaminated with detergent solution followed by water ASAP.</p> <p>8. The first cage change after each drug administration is to be done no sooner than 72 hours after the administration. The bedding is considered contaminated and requires special handling</p> <p><b><u>When changing cages use the following technique:</u></b></p> <ul style="list-style-type: none"> <li>• Transfer the animals to clean cages</li> <li>• Insert the used cages in a plastic bag.</li> <li>• Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked "<b><u>Toxin- DMBA</u></b>".</li> <li>• 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).</li> <li>• If local ventilation controls are not available for opening cages or dumping bedding, a 3M8835 respirator and safety goggles must be worn.</li> <li>• All contaminated bedding will be labeled as hazardous materials and handled accordingly:</li> <li>• <b><u>incinerated or placed in chemical waste bags for disposal.</u></b></li> <li>• 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 DMBA.</li> <li>• The cages should then be put in plastic bags (marked "Toxin-DMBA ") and sealed for transport to the washroom.</li> <li>• 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 DMBA contamination.</li> </ul>
<p>F. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> <li>1. Spills must be cleaned immediately by properly protected trained personnel.</li> <li>2. <b><u>Minor Liquid Spills:</u></b> should be cleaned immediately 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.</li> <li>3. <b><u>Powder/Major Spills:</u></b> should be cleaned immediately by personnel wearing a gown, goggles, and two pairs of gloves (nitrile). 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-</li> </ol>

	<p>prevent runoff into drains. Place waste in a plastic bag and then in the chemical waste container. <u>Prevent, by all means available, spillage from entering drains.</u></p> <p>4. <b><i>Exposure:</i></b></p> <ul style="list-style-type: none"> <li>• <b><i>In case of skin contact</i></b> or injection with DMBA, wash the affected area with soap and water for at least 15 minutes. Consult with a Medical doctor in an Emergency Room (ER)</li> <li>• <b><i>For eye exposure</i></b>, flush with water for at least 15 minutes.</li> </ul> <p><b>In any case</b></p> <ul style="list-style-type: none"> <li>• <b>Consult with Medical doctor in ER</b></li> <li>• <b>Report incident to supervisor</b></li> <li>• <b>Report the accident/injury to the Safety Unit Tel: 2146/7.</b></li> </ul>
G. Waste Disposal	<p>Dispose all waste material in the appropriate chemical waste container. Unused solutions of DMBA 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 DMBA in Animals, and agree to follow these procedures.</p>	
Name:	Title:
Signature:	Date: