Tel-Aviv University –Safety Unit

Standard Operating Procedure for 7,12-Dimethylbenz[a]anthracene (DMBA) in Animals	
1. Health hazards	DMBA is a highly potent carcinogen, neoplastigen (contributes an abnormal proliferation of cells), tumorigen ,and teratogen (causes developmental malformations),that causes cancer and heritable genetic damage and is used to induce cancer in animal models. DMBA may absorbed through skin, may cause reproductive damage. Contact can irritate and burn the skin, eyes, nose, throat and lungs. DMBA may damage the liver and kidneys.
2. Designated Area	ABSL-2 facility.
3.Training	Hazardous cytotoxic 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 cytotoxics, symptoms of exposure, appropriate work practices, and proper use of PPE.
4. Personal Protective Equipment (PPE)	Double nitrile gloves, Cytotoxic safety goggles, Lab coat and mask . 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.*** 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.
5.General . Precautions for Animal Use	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. Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. 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 the research staff must change the bedding 72 hours after administration.**
6. Environmental / Ventilation Controls	The preparation of DMBA 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. Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.
5. Special Handling Procedures & Storage Requirements	Handling: 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). The fume hood or other approved containment must be cleaned upon completion of tasks. When transporting DMBA, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers. **All equipment must be decontaminated prior to removal from the room housing the infected animals.**
8. **Precautions for Animal Use**	No recapping needles. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection. **Once DMBA is injected, animals , animal waste and cages are considered hazardous for a minimum of 72 hours.** Hands must be washed upon exiting animal room
7. **Animal handling**	1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done

practices	only by the researchers. 2. 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. 3. **Injecting animals with DMBA:** Animals will be injected IP with DMBA within Class II Biosafety cabinet or designated cytotoxic fume hood. All needles will be disposed of in sharps container – do not recap or bend needles. 4. Infected animals considered hazardous for a minimum of 72 hours after each administration of DMBA; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. A respirator is recommended for personnel that are immunocompromised or pregnant and for healthy personnel if work is done outside the ventilated cabinet . 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, which are then placed in biosafety bags for infectious waste 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 3 days after the administration. The bedding is considered contaminated and requires special handling. <u>When changing cages, use the following technique</u>: • Transfer the animals to clean cages . • Insert the used cages in a plastic bag . • Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked “cytotoxic- DMBA". • Transport the bags of cages to a HEPA filtered dumping station that draws air away from the use. .(it is recommended to use a mask) or fume hood. • If local ventilation controls are not available for opening cages or dumping bedding, an N-99 respirator and safety googles must be worn. • All contaminated bedding will be labeled as hazardous materials and handled accordingly :

	incinerated or placed in cytotoxic waste bags for disposal. • 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. • The cages should then be put in plastic bags (marked “cytotoxic- DMBA") and sealed for transport to the washroom. • 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.
9. Spill and Accident Procedures	1. Spills must be cleaned immediately by properly protected trained personnel. 2. **Liquid Spills:** should be cleaned immediately by personnel wearing a gown, goggles, 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 cytotoxic waste container. 3. **Powder Spills**: should be cleaned immediately by personnel wearing a gown, goggles, two pairs of gloves (nitrile). For powder 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. The spill area should then be cleaned thoroughly with a detergent solution followed by clean water. Place waste in a plastic bag and then in the cytotoxic waste container. <u>Exposure</u>: 4. In case of skin contact or injection with DMBA, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center. 5. For eye exposure, flush with water for at least 15 minutes. Consult with

	Employee Health Center, Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.
10. Waste Disposal	Dispose all waste material in the appropriate cytotoxic waste container. Unused solutions of DMBA and containmented solid waste will be disposed of as hazardous cytotoxic material.
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with DMBA in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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