

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Dacarbazine in Animals

1. Health hazards	<p>Dacarbazine, also known as DTIC, DIC, and Imidazole Carboxamide, is an antineoplastic chemotherapy drug used in the treatment of various cancers, among them malignant melanoma, Hodgkin lymphoma, sarcoma, and islet cell carcinoma of the pancreas. Dacarbazine is a member of the class of alkylating agents, which alkylates and cross-links DNA during all phases of the cell cycle, resulting in disruption of DNA function, cell cycle arrest, and apoptosis.</p> <p>Dacarbazine is a prodrug that requires metabolic activation by the hepatic cytochrome P450 system to the resulting: 5-aminoimidazole carboxamide and the active methylating intermediate methyldiazonium ion.</p> <p>Resulting DNA methylation products are: 3-methyl adenine, 7-methyl guanine, and O-6-methyl guanine, which are responsible for the cytotoxic activity.</p> <p>Statement of Hazard:</p> <p>Heating may cause an explosion.</p> <p>May cause CANCER.</p> <p>May cause heritable genetic damage.</p> <p>Harmful by inhalation, in contact with skin and if swallowed.</p> <p>Irritating to eyes, respiratory system and skin.</p> <p>May be harmful to the fetus/embryo.</p> <p>May possibly affect fertility</p> <p>As a precautionary measure, keep away from strong oxidizers (such as bleach).</p> <p>*Pregnant women should not be exposed to or handle this chemical in any form - May damage fertility or the unborn child - May cause genetic defects.*</p>
2. Designated Area	ABSL-2 facility.
3. Training	Hazardous chemical training and training on this SOP is required before working with Dacarbazine. 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.
4. Personal Protective Equipment (PPE)	<p>Double nitrile gloves or compatible chemical-resistant gloves, Chemical 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.</p> <p><i>Pregnant women should not be exposed to or handle this chemical in any form.</i></p>
5. General . Precautions for Animal Use	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p> <p>Dacarbazine may be excreted by the animals within the first 48 hours post injection, therefore the lab must change the bedding 48 hours after administration.</p>
6. Environmental / Ventilation Controls	<p>The preparation of Dacarbazine 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.</p> <p>Following preparation of Dacarbazine, the work area should be thoroughly cleaned with soap and water or with virusolve.</p> <p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p>
5. Special Handling Procedures & Storage Requirements	<p>Handling: Dacarbazine should be handled in containment and done over absorbent pads.</p> <p>Any visible contamination or spills should be cleaned with virusolve and then washed with water. Any wipes contaminated with Dacarbazine must be disposed as Chemical hazardous waste.</p> <p>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.</p> <p>Any laboratory equipment or surfaces that have come in contact with Dacarbazine must be disposed of (cytotoxic chemical waste) or decontaminated (wipe with virusolve follow by water soaked paper towels) Non-porous material (e.g. glassware) can be decontaminated by soaking in virusolve for 24 hours.</p> <p>Upon completion, soak all surgical equipment in 80%(v/v) ethanol for at least one hour before washing with soap and water and autoclaving.</p>

	<p>When transporting Dacarbazine, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers.</p> <p>All equipment must be decontaminated prior to removal from the room housing the infected animals.</p> <p>DO NOT use bleach for disinfection of work surfaces where Dacarbazine has been used.</p> <p>Hands must be washed upon exiting animal room.</p>
<p>8. Precautions for Animal Use</p>	<p>No recapping needles. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection. Once Dacarbazine is injected, animals , animal waste and cages are considered hazardous for a minimum of 48 hours.</p> <p>Hands must be washed upon exiting animal room.</p>
<p>7. Animal handling practices</p>	<ol style="list-style-type: none"> 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 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 Dacarbazine: Animals will be injected IP with Dacarbazine within Class II Biosafety cabinet or designated chemical 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 48 hours after each administration of Dacarbazine; 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 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 virusolve followed by water ASAP. 8. The first cage change after each drug administration is to be done no sooner than 48

	<p>hours after the administration. The bedding is considered contaminated and requires special handling.</p> <p><u>When changing cages, use the following technique:</u></p> <ul style="list-style-type: none"> • Transfer the animals to clean cages . • Decontaminate the used cages with virusolve. • 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 “toxin- Dacarbazine. • Transport the bags of cages to a HEPA filtered dumping station that draws air away from the use (or BSC Type II), it is recommended to use a fume hood. • If local ventilation controls are not available for opening cages or dumping bedding, an N-99 respirator and safety goggles must be worn. • All contaminated bedding will be labeled as hazardous materials and handled accordingly : incinerated or placed in chemical 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 Dacarbazine. • Use virusolve to decontaminate the cages, then put in plastic bags (marked “toxin- Dacarbazine) 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 Dacarbazine contamination.
<p>9. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> 1. Spills must be cleaned immediately by properly protected trained personnel wearing a gown, goggles, two pairs of gloves (nitrile) and respirator mask covering the mouth and nose . 2. 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 virusolve (<i>allow at least 15 minutes</i>) and then wash the area with

	<p>soap and water. Place waste in plastic bag and then in the chemical waste container.</p> <p>3. 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 goggles, 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 virusolve 1-2 times (<i>allow at least 15 minutes</i>) and then wash the area with soap and water.</p> <p>Collect and place waste in plastic bag and then in the chemical waste container.</p> <p>**Prevent, by any means available, spillage from entering drains or water courses.**</p> <p><u>Exposure:</u></p> <p>4. In case of skin contact or injection with Dacarbazine, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center.</p> <p>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.</p>
<p>10. Waste Disposal</p>	<p>Dispose all waste material in the appropriate chemical waste container.</p> <p>Unused solutions of Dacarbazine 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 Dacarbazine in Animals, and agree to follow these procedures.</p>	
<p>Name:</p>	<p>Title:</p>
<p>Signature:</p>	<p>Date:</p>

Dr. Esther Michael - Biological Safety Office, : 640-9966

