## Standard Operating Procedure for 6-OHDA in Animals

### Health Hazards

6-Hydroxy dopamine (6-OHDA) is neurotoxin that destroys catecholamine terminals and can cause Parkinsonism, ataxia, and other motor disturbances in humans if it enters the blood stream in significant amounts. At low concentrations 6-OHDA can cause irritations to the respiratory tract, digestive tract, the eyes, and skin.

**Statement of Hazard:**

- Harmful if swallowed
- Harmful in contact with skin
- Causes skin irritation
- Causes serious eye irritation
- Harmful if inhaled
- May cause respiratory irritation.

There has been concern that this material can cause cancer or mutations, but there is not enough data to make an assessment.

**Chronic exposure** to phenethylamines excites the central nervous system and induce tolerance; in extreme cases they produce amphetamine-like responses including personality changes, compulsive and stereotyped behavior and may induce psychosis with auditory and visual hallucinations and paranoid delusions.

*Pregnant and lactating women should not be exposed to 6-OHDA or handle animals that have been administered 6-OHDA or be extra careful and use additional PPE (use respirators).*

*Immunocompromised individuals should also use extreme caution when handling 6-OHDA.*

### Designated Area

**ABSL2 Facility (Animal Biosafety Level 2)**

### Training Requirements

Hazardous chemical training and training on this SOP is required before working with 6-OHDA. This should include but is not limited to reviewing the SDS, training on the physical hazards of the chemicals, symptoms of exposure, appropriate work practices, and proper use of PPE.

### Personal Protective Equipment (PPE)

- 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.

**Personnel should not work with 6-OHDA if skin is cut or scratched**

### 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.

Exposure may occur during preparation and administration of the drugs and handling of cytotoxic wastes from animals receiving cytotoxic drugs.
| Environmenta l/ Ventilation Controls | A. The preparation of 6-OHDA including reconstitution, weighing, and diluting should be performed in a chemical fume hood or biological safety cabinet (*class II Type B*). Work should be done over absorbent pads.  
B. Whenever possible, 6-OHDA should be purchased in sealed rubber capped vials so that it can be solubilized by injecting water into the vial cap without having to open the vial  
C. Work with animals should be conducted in ABSL-2 facility, over absorbent pads in a Class II type A2 BSC. |
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| Special Handling Procedures & Storage Requirements | A. **Handling:** 6-OHDA should be handled in containment and done over absorbent pads. Following preparation of 6-OHDA, the work area should be thoroughly cleaned with soap and water or with Distel (disinfectant). Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity, Luerlock syringes are recommended). The fume hood or other approved containment must be cleaned upon completion of tasks.  
Following preparation of 6-OHDA, the work area should be thoroughly cleaned with soap and water or with Distel. Any visible contamination or spills should be cleaned with Distel and then washed with water. Any wipes contaminated with 6-OHDA must be disposed as Cytotoxic hazardous waste.  
B. All equipment must be decontaminated prior to removal from the room housing the infected animals.  
C. **Wash hands thoroughly after handling** 6-OHDA.  
D. When transporting 6-OHDA, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers.  
**Storage:** Store in a well-ventilated place. Keep container in a dry place, tightly closed. Do not store next to strong oxidizing agents or strong bases |
| Precautions for Animal Use | E. Animals should be restrained or anesthetized during injection.  
A. NO recapping needles.  
B. Have a sharps container in close vicinity.  
C. Once 6-OHDA is injected, animals, animal waste and cages are considered hazardous.  
D. Hands must be washed upon exiting animal room |
| Animal Handling Practices | A. Animals must be housed in filter top cages marked as biohazards (including the name of the chemical hazard, 6-OHDA), in negative pressurized IVC.  
B. Handling the cages (including bedding) will be done only by the researchers at least for 7 days after the last administration and first cage change.  
C. Use a class II A2 BSC 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.  
D. **Injection animals with 6-OHDA:**  
   1. Injection of 6-OHDA will be conducted within Class II A2 BSC.  
   2. All needles will be disposed of in sharps container - do not recap or bend needles. |
3. Injected animals considered hazardous after each administration of 6-OHDA.
4. Take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room.
5. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time.
6. A respirator is recommended for personnel that are immunocompromised or pregnant and for healthy personnel if work is done outside the ventilated cabinet.
7. Dead animals must be placed in primary plastic bags, and then will be transferred to incineration.
8. All surfaces and racks that may be contaminated will be decontaminated with detergent solution followed by water ASAP.
9. The bedding is considered contaminated and requires special handling on the first cage change after each drug administration.

E. When changing cages use the following technique:
1. Transfer the animals to clean cages.
2. Insert the used cages in a plastic bag.
3. Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked "Hazardous Chemical – 6-OHDA".
4. 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).
5. If local ventilation controls are not available for opening cages or dumping bedding, a 3M8835 respirator (or other P3 respirator) and safety goggles must be worn.
6. All contaminated bedding will be labeled as hazardous materials and handled accordingly: incinerated or placed in chemical waste bags for disposal.
7. 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 6-OHDA.
8. The cages should then be put in plastic bags (marked as "Toxin – 6-OHDA") and sealed for transport to the washroom.
9. 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 6-OHDA contamination.

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<tr>
<th>Spill and Accident Procedures</th>
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<tr>
<td>A. Spills must be cleaned immediately by properly protected trained personnel (do not let product enter drain).</td>
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<td>B. <strong>Minor Liquid Spills:</strong> should be cleaned 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.</td>
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solution followed by clean water. Place waste in plastic bag and then in the chemical waste container.

C. **Powder/Major Spills**: should be cleaned by personnel wearing a gown, goggles, and chemically resistant gloves. 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- prevent runoff into drains. Place waste in a plastic bag and then in the chemical waste container.

Prevent, by all means available, spillage from entering drains.

D. **Exposure**:

- **In case of skin contact** or injection with 6-OHDA, wash the affected area with soap and water for at least 15 minutes. Consult with a Medical doctor in an Emergency Room (ER)
- **For eye exposure**, flush with water for at least 15 minutes.

In any case

- Consult with Medical doctor in ER
- Report incident to supervisor
- Report the accident/injury to the Safety Unit Tel: 2146/7.

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<tr>
<th>Waste Disposal</th>
<th>Dispose all waste material in the appropriate chemical waste container. Unused solutions of 6-OHDA and contaminated solid waste will be disposed of as hazardous chemical material.</th>
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I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with 6-Hydroxy dopamine (6-OHDA) in animals and agree to follow these procedures.

| Name: __________________ | Title: ____________________ |