**Standard Operating Procedure (SOP) for systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in non-human primates**

**Preface**

Because of its relative selectivity for dopamine neurons, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is frequently used to induce parkinsonism in primates. MPTP can be used by systemic (s.c., i.m., or i.v.) injections, or through intracarotid injections. The procedures described here apply to the use of systemic injections of the compound.

MPTP use poses health risks for the personnel administering the agent. Accidental MPTP exposure can produce parkinsonism in humans. MPTP may enter the body via absorption through the skin or eyes, injection, inhalation of vapors or powders, or by ingestion. Many of the points made below are in place to address these safety concerns.

An essential part of the biocontainment effort is focused on quarantine the animals during the MPTP exposure. To this end, they are placed into a dedicated room prior to the injections and remain in this room for a 72 hour period following the injections. During this time all of their excreta are collected and disposed of in dedicated biohazardous waste containers for later incineration. The relevant animal housing and husbandry details are mentioned in sections 3.4-3.6

1. **Personnel education**

* Personnel working with MPTP need to be fully informed of the potential hazards before beginning to work with the chemical. Personnel who administer MPTP may have to undergo specialized training provided by occupational health personnel.
* The use of full-body personal protective equipment (see below) is essential. This includes the use of a well-maintained half-mask or full-face respirator with appropriate filter cartridges (at Emory University, a 3M™ Multi Gas/Vapor Cartridge/Filter 60926, P100 is used for this purpose). Personnel using respirators undergo annual health assessments to assess their fitness for using the respirator.
* Most institutions will have a Chemical Safety Board which needs to approve the use of this agent.
* Personnel has access to doses of selegiline (5 mg tablets) to use in cases in which accidental exposure to MPTP has occurred. Personnel receives training on when and how to use this agent.

**2. Materials needed**

*Personal protective equipment (PPE)*

* Disposable water-repellent coverall (i.e., Tyvek suit)
* Two pairs of chemical-resistant (nitrile) gloves that cover the wrists of the coveralls
* Headcover
* Shoe covers
* Safety goggles or face shield (if using a half-face respirator)
* Half- or full-face mask respirator (requires annual medical clearance and fit-test)
* Cut-resistant gloves and sleeves

*Facility requirements*

* Given the extent of preparation and effort required for prevention of biohazard exposure after the injections, it is best to carry out the MPTP injections in a dedicated room
* The MPTP solutions should be prepared in a chemical fume hood or Type II B2 Biological Safety Cabinet

*Other materials*

* MPTP (e.g., Sigma-Aldrich, catalog number M0896-10MG)
* Sterile saline for injection
* Blunt needles
* Syringes
* Absorbent pads
* 1% bleach solution in water
* Biohazard disposal bags and boxes/containers
* Selegiline, 5mg tablets (optional)
  + The Containment Manager routinely inspects the selegiline lock box in the MPTP anteroom to ensure that the initial unexpired prophylaxis dose is available, in a box labeled “For prophylaxis after MPTP exposure - Only for personnel cleared to take selegiline.”

**3. Procedures**

**3.1 Receipt of MPTP**

* The package delivered to the research lab is placed into a chemical fume hood dedicated to MPTP solution preparation
* Don a lab coat and two pairs of nitrile gloves and inspect the packaging to ensure no damage.
* Open the box and visually inspect the bottle.
  + If all packaging and bottles are intact, the MPTP bottle is removed from the packaging and then stored in a locked cabinet.
  + If the package or its contents is damaged, or the lid is not secure:
    - close the box and chemical fume hood sash and don all PPE listed above
    - Add 1% bleach solution to each vial to inactivate the MPTP. Discard the MPTP in a dedicated biohazard container
    - Notify the manufacturer that the product arrived damaged and was discarded
    - Contact the Environmental Health and Safety Office to dispose of the biohazard container

**3.2. Preparation of MPTP solutions**

* MPTP solutions are considered to be stable for 24 hours at 4 °C, 1-3 months at -20 °C, and 2-6 months at -80 °C. However, given the toxicity of the agent, storage should involve dedicated MPTP storage freezers. The general consensus is that is preferable to use freshly prepared MPTP solutions.
* Only investigators and/or staff members trained in and familiar with the use of MPTP should prepare and administer MPTP.
* MPTP should be prepared under fume hood in the designated MPTP preparation suite.
* Anybody working with MPTP needs to wear the PPE mentioned above.
* A warning sign is posted on the outside of the suite door to alert others that work with MPTP is underway, and that PPE is required to enter.
* All surfaces that may come in contact with MPTP have to be covered with absorbent plastic-backed disposable bench paper. The plastic-backed paper is disposed of into biohazard bins after each procedure or whenever a spill occurs.
* MPTP solution is prepared under the fume hood, by adding the appropriate amount of diluent (saline) into the MPTP bottle. MPTP-HCl dissolves rapidly in aqueous solution. The appropriate amount of MPTP can be retrieved from the MPTP bottle with a needle/syringe.
* Before removal from the hood, the outside of the container and all other items used during preparation are wiped down with 1% bleach solution.
* Contaminated equipment is rinsed with 1% bleach solution before disposal.
* If the MPTP solution needs to be transported out of the MPTP preparation suite, the solution is stored in a closed, unbreakable, secondary container. The containers need to be labeled to indicate their contents ("Danger: MPTP; dangerous chemical; Neurotoxin"). An absorbent material is placed in the secondary container to absorb the MPTP solution in the event of a spill.

**3.3 MPTP administration procedures**

* Prior to MPTP injections, animals are moved into the approved MPTP housing area.
* Needle-safe systems, needle-locking or single-unit syringes are used.
* Syringes are filled for dosing in the chemical fume hood using a blunt needle to draw MPTP from the vial into the syringe.
* Institution Animal Care and Use Committees, or veterinary SOPs may limit the maximal dose of MPTP that can be administered to animals (per day or per week). As an example, an IACUC policy at Emory University limits the total daily dose given by systemic injections to 0.8 mg/kg.
* After withdrawal of MPTP for injection, the remaining MPTP solution in the vial is inactivated with the bleach solution and discarded into a biohazard material (sharps) container.
* The needle and syringe are disposed of post-injection immediately into a sharps container.
* The injection site is inspected for leaking or spilled solution. Leaking or spilled solution is absorbed with an alcohol-dampened pledget or sterile gauze.
* Order of PPE removal (after completion of injections)
  + First remove shoe covers and disposable coveralls (Tyvek suit).
  + Remove outer layer of gloves
  + Remove respirator
  + Clean respirator after each use.
    - Cartridges are removed and wiped down using a 1% bleach solution.
    - Respirator (without cartridges attached) is sprayed with 1% bleach solution or washed with soap and water.
    - Respirator is allowed to air dry in an appropriate area (e.g., locker).
    - Dry respirator and cartridges are placed in a sealed plastic bag for storage.
  + Head cover is removed.
  + The inner layer of the gloves is removed.
  + Disposable garments are placed in a biohazard bag, and the bag is marked as “pathological waste. “
  + Bags are placed in the biohazard container pending incineration
  + Thoroughly wash exposed areas of skin.

**3.4. MPTP Animal Room Arrangements**

* For the time of the injection and a 72-hour post-injection containment period, animals are placed into the dedicated MPTP treatment suite.
* The MPTP treatment room may be shared by several animals receiving MPTP treatment concomitantly. These can either be in a single rack of cages, or separate racks. If animal(s) have to enter an isolation room during the quarantine period of (an)other animal(s), the animals that receive MPTP injections later are housed in a different cage rack.
* All animals housed in the same rack must leave the MPTP room simultaneously.

**3.5. Animal Housing and Husbandry**

* Animals injected with MPTP are housed in areas isolated from the general animal colony.
* Access to the MPTP animal room is limited to trained personnel for a minimum of 72 hours following the injection of MPTP.
* Special arrangements for animal housing are made with the Veterinary and Animal Care Departments (see above).
* Given safety concerns, animals are housed in a single-tier rack whenever possible or in the lower cages of double-tier racks.
* During the 72-hour quarantine period. animal cages are labeled to indicate clearly that the cages contain animals treated with MPTP.
* PPE required for entry into the NHP animal area during periods that MPTP or its metabolites are likely to be in the excreta of treated animals are listed above.
* PPE is removed immediately after exiting the MPTP isolation room, following the procedures mentioned above.
* As aerosols of MPTP and/or its metabolites may be generated from bedding, excreta, or the animal’s hair/coat, special precautions are taken during cage cleaning activities to reduce the creation of aerosols
  + Cage pans are lined with disposable plastic-backed absorbent pads. Dry bedding can be used as an alternative if necessary.
  + Prior to removal of pads or bedding, excreta are sprayed with 1% bleach solution.
  + If dry bedding is used, the bedding is dampened with 1% bleach and allowed 10-15 minutes of contact before disposal to reduce airborne particles.
  + Absorbent pads, bedding, and excreta are carefully removed from the drop pan and placed in a biohazard bag for incineration.
  + The animal is transferred to a clean cage after 72 hours following the MPTP injection.
  + Cages and pans are sprayed with 1% bleach solution before cage washing.
* Once the animal(s) have been transferred to clean cages, and the room has been decontaminated, the use of special MPTP PPE is not required, and the room may be cleaned according to standard husbandry procedures.

**3.6 Transport of MPTP-treated animals**

* Animal transport during the quarantine period should be avoided if at all possible
* If animal transport is unavoidable, the animal is anesthetized. Then, all surfaces the animal touches are covered with absorbent, plastic-backed disposable paper, and the surfaces are later decontaminated with a 1% bleach solution.
* A diaper is placed on the animal.
* The animal is transported wrapped in a plastic bag or similar (avoid wrapping the head of the animal).

**3.7 Procedures in case of a spill**

* Evacuate people from the immediate area.
* Don the appropriate PPE (if not already wearing it).
* If possible to do so without contaminating yourself, spray the spill with 1% bleach solution.
* Cover the spill with an absorbent, plastic-backed pad.
* If available, contact the Environmental Health and Safety Office Spill Team if assistance is needed. Provide information on the agent, the size and location of the spill, and contact information.
* When they arrive, inform the spill response team of any actions and specifics related to the spill.
* Remove all contaminated clothing and place it in a sealed plastic bag, making sure not to contaminate yourself or the environment.
* All contaminated materials used to clean spill are disposed of in the MPTP suite.
* PPE removal after decontamination of the area follows the rules mentioned above.

**3.8 Exposure**

* Prompt medical attention is essential should an MPTP exposures occur
* In the event of a recognized exposure to MPTP, personnel who have completed a evaluation and have been trained in the use of selegiline can take 4 5-mg tablets (total of 20 mg) of selegiline HCl immediately.
* The exposure site is washed immediately with soap and water for 15 minutes. If eyes or mucous membranes are involved, these are flushed for 15 minutes with water.
* Follow all reporting requirements at your institution.

**References**

* National Institutes of Health, Office of Research Services. “Procedures for Working with MPTP or MPTP-treated Animals.” Retrieved from: https://www.ors.od.nih.gov/sr/dohs/Documents/Procedures\_for\_Working\_with\_MPTP\_or\_MPTP\_Treated\_Animals.pdf.
* Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M. The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety. J Neurochem. 2001 Mar;76(5):1265-74.