



Guidelines for the Safe Use of MPTP

INTRODUCTION:

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) SOP

MPTP is a neurotoxin. Its metabolism is capable of producing severe irreversible brain damage by injuring the nigrostriatal dopaminergic pathway. Administration of the drug can result in a Parkinson's Disease-like appearance within the recipient. Signs and symptoms of the on-set of disease include slowness of movement, postural instability, rigidity and tremors.

Extreme caution should be used while working with this chemical. The lipophilic chemical can reach and cross the blood-brain barrier within one minute. In the brain, it is metabolized to MPDP+ and finally the active form of the neurotoxin MPP+.

MPTP has the potential to enter the body through the skin and mucous membranes by absorption or injection, by inhalation of vapors or particles in the air and by ingestion. MPTP is excreted through the urine of experimental animals.

The goal of this protocol is to provide information about the proper handling of MPTP within the laboratory and the animal care facility. It assumes that all minimum laboratory safety requirements have been met.

RESPONSIBILITIES:

Training requirement:

Prior to commencing any work with MPTP and annually thereafter, the Principal Investigator shall conduct formal training of all staff that will be engaged in the use and / or handling of the chemical. The training must include an understanding of all the hazards of MPTP use, storage and handling including using appropriate personal protective equipment (PPE), work area decontamination and emergency procedures. The staff should be given a copy of the MPTP SOP and SDS at this training. This training must be documented for each employee and this documentation submitted to the Office of Research Safety Affairs or entered into the EHS Assistant database accessible through the Research Safety Affairs website.

Occupational Health:

Staff must be enrolled with Occupational Health (OH) for medical evaluation prior to initiating any work and annually thereafter. The OH number is 448-5630. Selegiline hydrochloride (CAS # 14611-52-0) / deprenyl, a MAO-B inhibitor, is a potential antidote for MPTP exposure.

Presumptive therapy can also be discussed with OH.

If the antidote is stored within the lab, it must be checked regularly to ensure it is in the proper location and within the expiration date.

Required respirator use necessitates medical clearance and fit-testing by OH.

Signage requirement:

When MPTP is in use, there must be appropriate signage posted. Signs should be posted outside of the laboratory and on the equipment (i.e. chemical fume hood) until the chemical has been

properly stored and all work areas decontaminated. Animals treated with MPTP require signage on the cages and the animal room door(s).

Personal Protective Equipment:

Please read the SDS for MPTP. Handling of MPTP requires stringent compliance with wearing / using personal protective equipment (PPE). The following list is the minimum that must be worn.

- 1.) Chemical goggles
- 2.) Disposable nitrile gloves; recommend double-gloving. No Latex gloves may be worn. Hands should be washed upon glove removal. Gloves should be changed frequently and immediately if they become contaminated, punctured or torn.
- 3.) A disposable water repellent laboratory coat, jumpsuit or appropriate protective clothing. The lab coat should have elastic wrist cuffs or the cuffs should be tucked into the glove cuffs. It should be discarded after working with MPTP.
- 4.) Booties and head cover
- 5.) Respirator – a NIOSH approved air-purifying respirator. Sigma SDS outlines appropriate full-face particle respirators type N99 or type P100 respirator cartridges as a back-up to engineering controls. A full-face supplied air respirator is required when no engineering controls are available such as cleaning up a spill outside of a chemical fume hood. Disposable N-95 respirator will protect against respirable particles but should not be considered sufficient for chemical protection. Required respirator use necessitates medical clearance and fit-testing by Occupational Health, 448-5630.

PPE should be removed in the anteroom or immediately prior to exiting any room with MPTP in use.

Sequence for PPE removal:

- 1.) garments
- 2.) gloves
- 3.) chemical goggles
- 4.) respirator

Place all disposable PPE in hazardous waste box. Wash the chemical goggles with mild detergent and water; thoroughly wash hands, face and neck.

Preparation and Handling:

Please consult with OHS regarding the need for a MPTP antidote in the laboratory.

The door to the laboratory **MUST** be kept closed and the laboratory must be under negative pressure to the surrounding areas (rooms and hallway).

MPTP solutions must be handled in a chemical fume hood. **DO NOT** use a biological safety cabinet (BSC) unless it is a Class II B2 with 100% exhaust. The hood or Class II B2 BSC must be certified within the last 12 months and used within the operational parameters.

Disposable plastic-backed bench paper (i.e. puppy pee pads, bench paper, etc) must be used to line the chemical fume hood. It should be changed at the completion of work or daily, whichever is shorter, and / or after a spill.

Generate the smallest amount of MPTP solution required for the experiment.

It is preferable to purchase the chemical in pre-weighed bottles that can be reconstituted with sterile solvent. Solutions can be sterilized with a 0.22 μ m disposable filter if sterile solvent is not available. Do Not Autoclave. If chemicals must be weighed on a balance, this activity must be conducted within the chemical fume hood or using closed / sealed vessels that are loaded within the chemical fume hood. The closed vessels may be weighed outside the hood but all reconstituting and dilutions must be prepared within the hood. MPTP solutions will oxidize at room temperature and should be used on the same day as prepared. Excess MPTP solution should be inactivated by the injection of bleach into the glass vial.

Disposal labware is highly recommended. Disposal animal cages are required. All reusable laboratory glassware must be decontaminated prior to reuse.

Transportation of MPTP on Campus:

MPTP may be transported in a labeled, sealed, leak-proof primary container inside a labeled, sealed, leak-proof unbreakable secondary container lined with absorbent material. This secondary container must be opened in the chemical fume hood.

Transportation of MPTP from Campus:

MPTP is a regulated hazardous material and requires DOT training to ship the material from campus. Contact the Office of Research Compliance for more information.

Storage:

Solutions of MPTP must be stored in a secured location for access to authorized users only. The solutions should be sealed and maintained in unbreakable secondary containers. All containers should comply with the OSHA labeling requirement and list the chemical name and hazard warnings. The secondary container should also be labeled.

An inventory of MPTP should be maintained in a logbook noting available quantity, quantities dispersed for experiments and any amounts discarded with a note on the method of inactivation of the neurotoxin.

MPTP powder can be stored in a desiccator at room temperature. It should also be secured in the same manner as MPTP solutions. MPTP solutions can be stored at -80 $^{\circ}$ C for a couple of months but general lab safety practices should prohibit this practice.

Decontamination:

After completing work with MPTP, the work area and any equipment used must be decontaminated. Work surfaces and equipment should be cleaned with a 1-10% bleach solution made on the day of the experiments. The bleach solution should have a minimum contact time

of 10 minutes. The chemical fume hood must be decontaminated before any other chemical work is allowed within the hood.

If glassware that must be decontaminated, the rinsate must be collected as chemical waste.

Chemical Hygiene Plan:

The SOP and SDS for MPTP must be included in the laboratory specific component of the Chemical Hygiene Plan.

Syringe Use:

ABSOLUTELY NO RECAPPING OF NEEDLES (unless by mechanical means.)

Syringes must be filled with MPTP in the chemical fume hood. Animals must be injected in the chemical fume hood. It is recommended that these activities take place in the same chemical fume hood to limit transport of MPTP within syringes. If MPTP syringes must be filled prior to transport, the needles should be capped mechanically and transported in a labeled, sealed, leak-proof unbreakable secondary container lined with absorbent material. This secondary container must be opened in the chemical fume hood.

Air from filled syringes must be expelled into sterile dampened gauze that is held tightly to the needle. Immediately after injection, draw 1% bleach into the needles and then the needles and syringe must be placed into a sharps container within the chemical fume hood. The injection site on the animal must be inspected to ensure that there is no leakage of MPTP from the site. If any MPTP is found, it should be carefully absorbed with dampened gauze. All contaminated non-sharp materials must be disposed of into the proper hazardous waste stream.

Needles-safe products such as needle-locking syringes or disposable syringe-needle units with the needle integral to the syringe must be used. Used needles must not be manipulated (bent, broken, recapped, sheared or removed) before disposal.

ANIMAL WORK:

The PI must inform the Animal Facility veterinarian of the planned use of MPTP. The PI should instruct the veterinarian of the hazards associated with MPTP including MPTP-treated animals. The PI should supply the veterinarian with proper safety procedures for caring for these animals in case emergency care is required. However, the PI and staff are expected to conduct all animal care activities during the first five days after MPTP administration.

MPTP Administration to Animals:

The required PPE for injections, entering MPTP-animal room prior to cage change and during cage changes is to wear a disposable Tyvek coveralls (elastic wrists, attached boots and attached hood), double nitrile gloves, N-95 disposable respirator and chemical goggles. The procedure room must be under negative pressure to the surrounding areas (rooms and hallway).

Animals must be chemically or physically restrained prior to commencing any procedures. All administration must be performed in a chemical fume hood or Biological Safety Cabinet. Animals should be positioned away from researchers in case the animal urinates. Work surfaces

must be covered with absorbent, plastic-packed, disposable bench paper. MPTP-treated animals should not be transported within the first 5 days after inoculation.

MPTP-treated animals must be segregated for the first five days post-injection. Animal cages should be disposable and covered with filter bonnets. The cages should be located within a chemical fume hood or ventilated rack. The animal cages must also be individually labeled. Use of low-dust bedding is recommended. The room door must be posted with a warning sign and the required PPE to enter.

Mice are less sensitive to MPTP than primates and female mice less sensitive than male counterparts so care must be taken with experimental doses. MPTP intoxication results in a transient drop in body temperature that needs to be modulated. Animals should be given time (4-7 days) to acclimate to the procedure room prior to the administration of MPTP to allow for monoamine stabilization.

If animal tissues must be collected within five days post-MPTP administration, animals should be euthanized in the chemical fume hood. All work areas should be lined with plastic-backed bench paper. Take care to prevent blood splatters and releases of urine and feces.

Cage Changes:

Cage changes can be performed five days post-injection. Cage changes must be performed by laboratory personnel trained to handle MPTP and may not be performed by LACU staff. Disposable cages must be bagged carefully in the chemical fume hood to avoid the creation of dusts. It is recommended to wet the bedding with bleach solution since MPTP is excreted in the urine of treated animals. All cages are disposed of in the proper hazardous waste stream. PPE must be worn and the chemical fume hood decontaminated after cage changes. The room used for the MPTP procedures should be decontaminated before returning to general use. If ventilation racks were used, they should be decontaminated while in the procedure room and then moved to the cage wash area for immediate cleaning. Drinking bottles, if not disposable, need to be decontaminated.

The animals are transferred to clean standard caging. Signage and PPE are not required after the cage change.

EMERGENCIES:

Spills of Solution:

Inside chemical fume hood:

- 1.) Close sash and notify people within the lab to evacuate immediately.
- 2.) Have a co-worker post sign on door not to enter the laboratory while you remove potentially contaminated PPE and washed hands with soap and running water.
- 3.) Have co-worker call 8-6114 to notify Research Safety Affairs of the spill for clean-up.
- 4.) Small spills may be decontaminated with a copious 10% bleach solution.

Outside chemical fume hood:

- 1.) Notify people within the lab to evacuate immediately.

- 2.) Have a co-worker post sign on door not to enter the laboratory while you remove potentially contaminated PPE and washed hands with soap and running water.
- 3.) Have co-worker call 8-6114 to notify Research Safety Affairs of the spill for clean-up.
- 4.) Contact Occupational Health at 448-5630 about possible exposure incident to determine if additional treatment at OHS or the local emergency room is necessary.

Spills of Powder:

Inside chemical fume hood:

- 1.) Cover powder with disposable towel and saturate with bleach solution for at least 10 minutes.
- 2.) Collect materials and put into hazardous waste container for proper disposal.
- 3.) Repeat decontamination of area with fresh bleach solution.
- 4.) Contact Research Safety Affairs (8-6114) to inform that a spill occurred and how it was cleaned up.

Outside chemical fume hood:

Follow solution spill instructions

Accidental Exposure:

Recognized percutaneous or mucous membrane exposure:

- 1.) Immediately ingest antidote: four Selegiline HCl 5 mg capsules.
- 2.) Remove contaminated clothing.
- 3.) Wash contaminated skin with soap and running water for 15 minutes. Eyes and mucous membranes should be irrigated with running water or saline for 15 minutes. Access to a safety shower or eyewash must be available. A disposable laboratory coat may be donned after rinsing while waiting for medical attention.
- 4.) Notify your supervisor or co-worker immediately.
- 5.) Contact Occupational Health at 448-5630 about exposure incident to determine additional treatment at OH or the local emergency room.
- 6.) If the exposure occurs after hours, contact the CorVel 24/7 nurse triage at 1-866-245-8588 to seek medical care guidance and to initiate a claim.

Possible exposure:

- 1.) Remove contaminated clothing.
- 2.) Wash contaminated skin with soap and running water for 15 minutes. Eyes and mucous membranes should be irrigated with running water or saline for 15 minutes. Access to a safety shower or eyewash must be available. A disposable laboratory coat may be donned after rinsing while waiting for medical attention.
- 3.) Notify your supervisor or co-worker immediately.
- 4.) Contact Occupational Health at 448-5630 about exposure incident to determine additional treatment at OH or the local emergency room.
- 7.) Contact the CorVel 24/7 nurse triage at 1-866-245-8588 to seek medical care guidance and to initiate a claim.

Sample of appropriate signage for animal cages and equipment.



Designated Area

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

NEUROTOXIN

IN USE

Sample Door Sign



Designated Area

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

NEUROTOXIN

IN USE

Authorized Personnel Only

Contact the Principal Investigator _____ at _____ for more information.

Personal Protective Equipment Required for Entry.

Contact 8-6115 for Chemical Spills

Reference:

- 1) University of Medicine and Dentistry of New Jersey Environmental and Occupational Health and Safety Services Standard Operating Procedure: Safe Work Practices for the use of MPTP in the Laboratory and Vivarium (edition date January 11, 2007).
- 2) Sigma-Aldrich Material Safety Data Sheet 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Product Number M0896 (date updated January 29, 2006)
- 3) Przedborski, S., Jackson-Lewis, V., Naini, A.B., Jakowec, M., Petzinger, G., Miller, R., and A. Muhammad. (2001) The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety. *Journal of Neurochemistry* **76**, 1265-1274.
- 4) Huerkamp, M.J. Division of Animal Resources Agent Summary Sheet 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (edition date July 9, 2001).
- 5) Procedures_for_Working_with_MPTP_or_MPTP_Treated_Animals accessed at dohs.ors.od.nih.gov/pdf/Procedures_for_Working_with_MPTP_or_MPTP_Treated_Animals_july%202006.pdf