



## **Procedure for Working with MPTP or MPTP-Treated Animals**

### **Introduction**

MPTP, or 1-methyl-4 phenyl-1,2,3,6-tetrahydropyridine, is a neurotoxic agent that is commonly used in Parkinson's Disease (PD) research due to its ability to induce Parkinsonism in experimental animals. Its toxic metabolite MPPP causes an irreversible PD-like state by selectively destroying dopaminergic neurons in the substantia nigra, thereby diminishing striatum dopamine levels. MPTP has the same effects on humans and the symptoms of MPTP exposure include slowness of movement, postural instability, rigidity and tremors.

When handling MPTP and animals treated with MPTP, it is imperative to take extreme caution and adhere to the following guidelines to minimize exposure and the effects of exposure during normal and emergency situations.

### **Prior to Working with MPTP or MPTP-Treated Animals**

1. The Principal Investigator (PI) must ensure all employees and students who intend to work with MPTP or MPTP-treated animals:
  - a) Have been trained and are familiar with the contents of this procedure;
  - b) Complete a medical screening form for working with MPTP and using presumptive therapy with Selegiline Hydrochloride. The form should be submitted by employees to the Office of Health & Well-being (HWB), and by students to Student Health Services (SHS) for evaluation and to determine if any follow-up is required. Note that for students the P.I. would need to cover the cost for the student to see Student Health Services, as the cost would not be covered by OHIP.
2. PI must obtain the Selegiline Hydrochloride prescription from HWB and store it in a secure location that is readily accessible to those working with MPTP.
3. PI should ensure Selegiline Hydrochloride is routinely checked at the secure location to ensure supply is sufficient and not expired.
4. All employees and students must be re-evaluated by HWB/SHS annually to confirm suitability to work with MPTP and take Selegiline Hydrochloride. See appendix for screening form.

### **Personal Protective Equipment**

1. Personal protective equipment must be worn whenever working with MPTP or MPTP-treated animals and their waste within 72 hours of administration. See the subsections under Safe Work Procedures for task-specific PPE requirements.
2. Double gloves are required when working with MPTP or MPTP-treated animals. Frequent change of outer gloves is recommended, especially after tasks when



contamination can occur. Change gloves immediately if their integrity has been compromised (e.g., punctured or torn). Always wash hands with soap and water after removing gloves.

3. Protective clothing should be removed immediately prior to exiting any room in which MPTP is in use. Remove outer pair of gloves first, then remove garments, chemical goggles, respirator and remove the inner pair of gloves last.
4. Place disposable respirator, gloves, and garments in hazardous waste bags.
5. Wash chemical goggles with a mild detergent and water; thoroughly wash hands, face, and neck.
6. Prior to respirator use, employees must complete respiratory fit-testing training. Contact EHS Occupational Hygiene and Safety to arrange training sessions.

## **Safe Work Procedures**

### ***MPTP Preparation***

1. Personal protective equipment (PPE): Two pairs of chemical-resistant gloves (e.g., nitrile), chemical goggles, and a lab coat must be worn before commencing this step.
2. MPTP must be purchased in hydrochloride or tartrate salt form rather than as free base.
3. MPTP and MPTP-solutions must be stored in labeled, tightly capped containers.
4. The container must be properly labeled with the identity of the hazardous contents (i.e., MPTP) and the appropriate hazard warning (i.e., neurotoxin). See Appendix.
5. The primary container for MPTP must be placed in a sealed, leakproof, unbreakable secondary container, which must also be labeled as described above.
6. MPTP as received from the manufacturer/vendor in its original undiluted form must be stored in a certified chemical fume hood in a labeled, sealed, leakproof secondary container. The hood must be posted with an appropriate hazard label. See Appendix.
7. MPTP solutions must be prepared in a certified chemical fume hood. Appropriate personal protective equipment (PPE) must be worn. All work surfaces must be covered with absorbent, plastic-backed, disposable bench paper. If it is not possible to weigh MPTP in a certified chemical fume hood, then:
  - Tare an empty container with its cap;
  - In a certified chemical fume hood, transfer an approximate quantity of MPTP into the container;
  - Cap and weigh the container;
  - In a certified chemical fume hood, add an appropriate amount of solvent to achieve the desired concentration.
8. Disposable labware should be used when preparing MPTP solutions. If non- disposable glassware is used, it must be single rinsed in 0.1 N HCl prior to washing. The rinsate must be collected and disposed of as chemical waste.



9. MPTP solutions must be collected and disposed of as chemical waste. Refer to the Laboratory Hazardous Waste Management and Disposal Manual for specific instructions or contact EHS Environmental Protection Services for further information.
10. When all work with MPTP is complete, carefully remove all bench paper and dispose of as chemical waste. Wipe all surfaces with a 1% bleach solution.
11. MPTP solutions must always be transported in a labeled, sealed, primary container within a sealed, leakproof, unbreakable secondary container. Adequate absorbent material must be placed within the secondary container, around the primary container, to absorb all the solution in the event of a spill.

### ***Administration of MPTP***

1. Personal Protective Equipment (PPE): Two pairs of chemical-resistant gloves (e.g., nitrile), lab coat, wrist-guards, and mucous membrane protection (e.g., chemical goggles and surgical mask) must be worn before commencing this task.
2. Animals must be chemically or physically restrained prior to starting the procedure.
3. Administration of MPTP to rodents must be conducted in a certified chemical fume hood or at a certified down draft table. Both the personnel administering the MPTP, and those in the immediate vicinity of the procedure must wear appropriate PPE.
4. All work surfaces, except the down-draft table, must be covered with absorbent, plastic-backed, disposable bench paper.
5. Use only safety engineered needles. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated before disposal. After the completion of each injection, immediately place the syringe-needle unit in a sharps disposal container.

### **Animal Housing**

#### ***For the first 72 hours post-injection:***

1. Personal Protective Equipment (PPE): Wear a disposable jump suit, wrist-guards, head and foot covering, two pair chemical-resistant gloves (e.g., nitrile), N-95 disposable respirator, and chemical goggles. The door to each MPTP-animal room must be worn before entering a room with or handling MPTP-treated animals.
2. Signage should be posted in such a manner that it is clear that the room contains MPTP-treated animals (see Appendix). The animal cages must also be properly labeled (see Appendix).
3. For rodents, use disposable cages; cover the cages with filter bonnets; place cages in a certified chemical fume hood or ventilated rack.
4. For large animals (Non-Human Primates and Dogs):
  - a) To minimize the creation of aerosols, line the cage drop pans with disposable, plastic-backed, absorbent pads. Adequately wet excreta with water.



- b) Carefully remove the pads and place in a hazardous waste container for disposal. If pan liners are not used, adequately wet the bedding with water to keep the dust down. Place bedding in a hazardous waste container, using care not to aerosolize dust from the bedding.
5. Cage changes should not be performed for the first 72 hours post-injection.

***Beyond 72 hours after the final MPTP injection:***

1. Personal Protective Equipment (PPE): Wear a disposable jump suit, wrist-guards, head and foot covering, double chemical resistant gloves (e.g., nitrile), N-95 disposable respirator, and chemical goggles before changing cages/disposing cage waste.
2. The animals may be transferred to clean, standard caging and the MPTP door signs and cage tags may be removed.
3. Disposable rodent cages must be carefully placed into hazardous waste containers, taking care to avoid creation of dusts.
4. Cage change/dumping should be performed using certified local exhaust ventilation (e.g., biosafety cabinet or chemical fume hood). This local exhaust ventilation should be wiped down with 1% bleach solution, detergent and water after use.
5. Dirty cages and racks should be covered with a full drape and moved to the cage wash for immediate cleaning.
6. The use of special PPE is not required after the animals have been transferred to clean cages (beyond 72 hours post-injection).

**Emergency Response to Exposures and Spills:**

An exposure can result from:

- A. Skin injection with sharps contaminated with MPTP;
- B. Ingestion of MPTP;
- C. Absorption of MPTP through intact skin, broken skin or mucous membrane. Absorption can occur when there is direct contact with MPTP or contaminated materials (e.g. clothing);
- D. Inhalation of MPTP particulates or particles contaminated with MPTP.

***In the event of an exposure:***

1. Immediately ingest four (4) Selegiline Hydrochloride 5 mg capsules.
2. Flush body area for a minimum of 15 minutes:
  - a) Contaminated skin should be washed with copious amounts of soap and water;
  - b) Contaminated eyes and mucous membranes should be irrigated using normal saline or water.



3. Notify the supervisor, if immediately available. Supervisor to fill out an Accident Web form for employees or a Student/Contractor/Guest form for students and submit the form electronically (see [www.ehs.utoronto.ca](http://www.ehs.utoronto.ca)).
4. Seek medical attention as soon as possible.
5. If in doubt, call EHS Occupational Hygiene & Safety to determine further steps.

***In the event of a small spill:***

1. Only employees/students trained in the handling of MPTP should clean up spills.
2. Wear appropriate PPE.
3. If a spill occurs on linings and underpads, spray linings and underpads, allow to soak for 10 minutes.
4. If a liquid spill occurs on an unlined surface, clean the area with plastic-backed pads to prevent MPTP contaminating gloves. The area should be soaked with 1% bleach solution, rinsed with water, then washed with detergent, rinsed with water and dried with pads.
5. If a solid spill occurs on an unlined surface, cover the spill with disposable towel dampened with 1 % bleach solution.
6. Discard contaminated linings, underpads and materials in hazardous waste bags
7. If in doubt, call EHS Environmental Protection Services.

***In the event of a large spill:***

1. Evacuate people from the immediate area.
2. During business hours, call EHS Environmental Protection Services.
3. During off-hours, contact the Campus Police.



## References

Lau et al., 2005, MPTP treatment in mice does not transmit and cause Parkinsonian neurotoxicity in non-treated cagemates through close contact, *Neuroscience Research* 52: 371-378.

National Institutes of Health (August 15 2005), Procedures for Working with MPTP or MPTP-Treated Animals. Accessed at:  
[http://dohs.ors.od.nih.gov/pdf/Procedures\\_for\\_Working\\_with\\_MPTP\\_or\\_MPTP\\_Treated\\_Animals\\_july%202006.pdf](http://dohs.ors.od.nih.gov/pdf/Procedures_for_Working_with_MPTP_or_MPTP_Treated_Animals_july%202006.pdf). Viewed on February, 2010.

Przedborski et al., 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.



**Appendix A: MPTP Worker/Student Screening Form**

Parts A and B of this form are to be completed by the employee/student intending to work with MPTP or MPTP-treated animals.

**A: Worker/Student Information**

Last Name: \_\_\_\_\_ First Name: \_\_\_\_\_

Personnel/Student Number: \_\_\_\_\_

Department: \_\_\_\_\_

Title: \_\_\_\_\_

Telephone: (\_\_\_\_) \_\_\_\_\_ Fax: (\_\_\_\_) \_\_\_\_\_

Supervisor Name: \_\_\_\_\_

\_\_\_\_\_

**B: Work Conditions**

I have read the "Working with MPTP and MPTP-Treated Animals Procedure". I understand the hazards of working with MPTP or MPTP-treated animals and how to minimize the risks by following the safe work procedures.

YES       NO

Describe the capacity in which you will be working with MPTP or MPTP-treated animals

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



### C: Health Conditions/Restrictions – For Completion by Family Physician

After completion by your Family Physician, please submit this part to U of T Occupational Health Nurse/Physician. For medical office use and records only – please do not submit to EHS.

Note that any cost of testing charged by the physician should be reimbursed by the Principal Investigator.

Dear Doctor,

Your patient \_\_\_\_\_ is going to be working with a neurotoxin known as MPTP and may have to take Selegiline Hydrochloride as a post exposure prophylaxis in the event that he/she is exposed to the neurotoxin. Please tick yes if any of the following medications are used by the patient, or if any of the conditions listed are applicable. A final decision on clearance will be provided by U of T Occupational Health, possibly in consultation with you.

(a) Do any of the following conditions apply or are the following medications being taken?

- NO       YES (tick applicable conditions below)
- Meperidine (e.g., Demerol and other tradenames) and MAO inhibitors including selective MAO-B inhibitors
  - Dextromethorphan
  - Selegiline Products
  - Sympathomimetic medications (e.g. ephedrine)
  - Tricyclic Antidepressants and Selective Serotonin Reuptake Inhibitors
  - Levodopa/carbidopa
  - Cytochrome P450 Enzymes: CYP2B6 and CYP3A4 are involved in the metabolism of
  - selegiline. CYP2A6 may have a minor role in the metabolism of selegiline.
  - Drugs that induce CYP450 (e.g. phenytoin, carbamazepine, nafcillin, phenobarbital, and rifampin)
  - Other drug(s) that may affect your ability to work with MPTP or Selegiline hydrochloride:
  - Known hypersensitivity to MPTP or its metabolites





- Known hypersensitivity to selegiline hydrochloride or its ingredients
- Pregnancy (known or suspecting)
- Nursing
- Other condition(s) that may affect your ability to work with MPTP or Selegiline hydrochloride

(b) Has your patient shown any previous hypersensitivities to any medication?

- YES       NO

(c) Do you have any concerns about your patient having sensitivity to MPTP and its metabolites or selegiline hydrochloride and its ingredients?

- YES       NO

(If you have concerns, the matter will be further considered in consultation with U of T Occupational Health Dr. Gabor Lantos.)

Name of Family Physician: \_\_\_\_\_ Assessment Date: \_\_\_\_\_

Signature of Family Physician: \_\_\_\_\_



**Part D of this form to be completed by U of T Occupational Health Nurse, and returned to U of T EHS along with Part A.**

**D: Health Care Professional Primary Assessment (if required)**

Assessment Date: \_\_\_\_\_

- Working with MPTP and its metabolites:       Yes       No
- Use of Selegiline Hydrochloride permitted?       Yes       No
- Referred to further medical assessment       Yes       No

**Comments:**

Name of Health Care Professional (HCP): \_\_\_\_\_

Title: \_\_\_\_\_

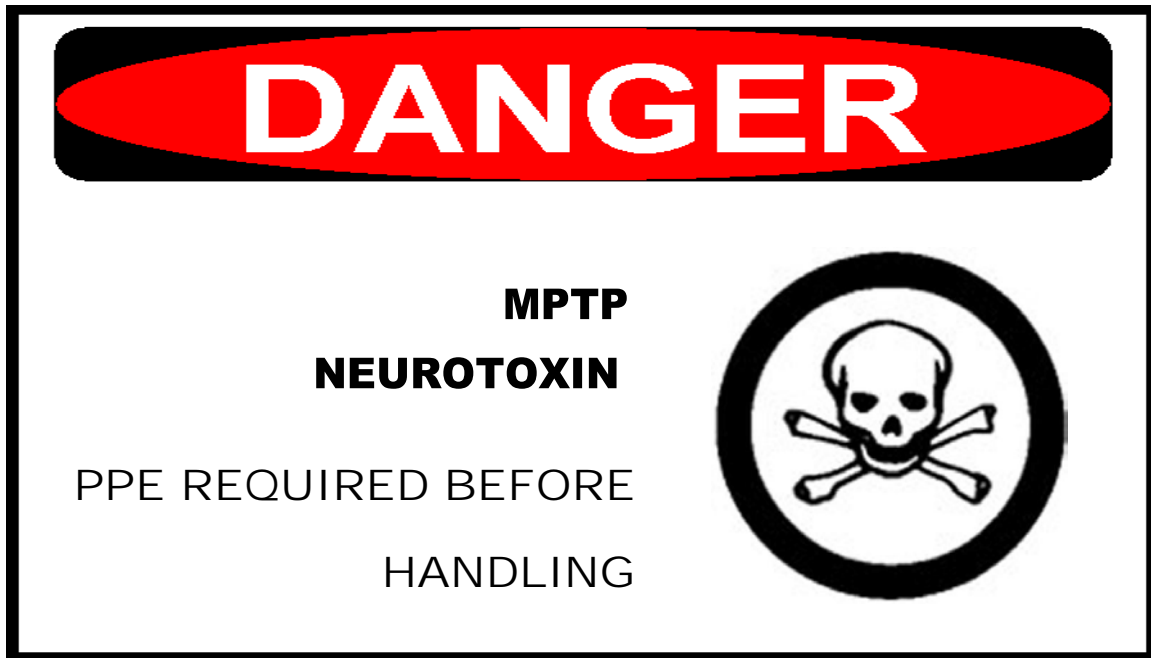
Signature of HCP: \_\_\_\_\_

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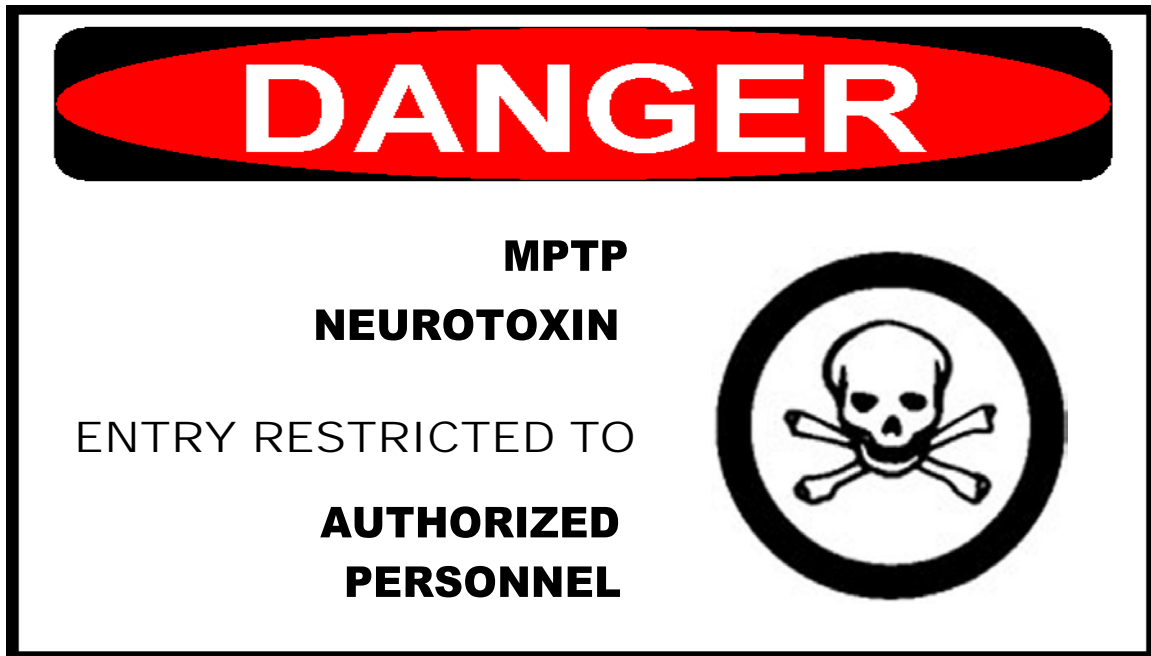


**Appendix B. General Signage for MPTP or MPTP-Contaminated Materials**





***Appendix C. General Signage for Rooms Where MPTP Is Used or Where MPTP-Treated Animals are Housed***





**Appendix D. General Signage for MPTP-Treated Animal Cages.**

