**General Use Standard Operating Procedure (SOP)**

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

*CAS-Nos. 28289-54-5 (free base form); 23007-85-4 (hydrochloride form)*

# **Purpose**

This document provides a comprehensive source for all matters covering the preparation of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its administration to small rodents. Specifically, it describes the procedures to be used to ensure a safe working environment while working with MPTP, including lab/technique specific safety practices. **The PI is required to develop a lab specific MPTP SOP (template can be obtained from OEHS Chemical Hygiene Officer) and submit it with the PI’s IACUC protocol.**

**NOTE**: This SOP is specific to the use of MPTP in small rodents (mice or rats). If the PI intends to use MPTP in another animal model or for another purpose, the PI must first consult with the Office of Environmental Health and Safety (OEHS) and Department of Laboratory Animal Research (DLAR) (where relevant).

# **Hazard Description and Background**

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin used to induce parkinsonism symptoms in experimental models of Parkinson’s disease (PD). MPTP can produce irreversible neurological damage indistinguishable from PD in humans and animals. The inappropriate handling of MPTP may result in exposure and irreversible neurological damage to research staff.

MPTP is lipophilic which freely and rapidly crosses the blood-brain barrier. It is metabolized to 1-methyl-4-phyenyl-2,3-dihydropyridinium (MPDP+) which is oxidized by the enzyme monoamine oxidase to the active toxic compound 1-methyl-4-phenylpyridinium (MPP+). Excretion of MPTP occurs mainly during first day post injection while MPTP metabolites (MPDP+ and MPP+) are excreted up to 5-days post injection. Excretion is mainly in the urine and feces. MPTP in urine is expected to be ionized and not volatile.

Exposure potential is greatest during preparation of MPTP, administration, and in the bedding during the 5-days post injection (prior to the first cage change).

**NOTE:** It is known that immediate adverse health effects can result from short term exposure to a high dose of MPTP and its metabolites (acute exposure, via ingestion, inhalation, or injection). Some research suggests adverse health effects may also occur from repeated long term exposure to low amounts of MPTP and its metabolites (chronic exposure). Therefore, appropriate protection is essential at all stages of this procedure, even when handling minute amounts of MPTP.

| **Hazard Pictograms** | **Hazard Statements** |
| --- | --- |
| **Globally Harmonized System pictogram Indicating a chemical is an Acute Toxic, capable of causing serious adverse health effects (i.e. lethality) after a single or short-term oral, dermal, or inhalation exposure to a substance or mixture.** | Acute toxicity: Oral (Category 3); Dermal (Category 3); Inhalation (Category 3) |
| **Globally Harmonized System pictogram Indicating a chemical is an health hazard, such as a carcinogen, mutagen, reproductive toxic, target organ toxic, or aspiration toxic.** | Specific target organ toxicity – single exposure (Category 1), Nervous system |
| **Globally Harmonized System pictogram Indicating a chemical is an Irritant chemical** | Causes skin irritation (Category 2); Causes serious eye irritation (Category 2A); May cause respiratory irritation (Category 3) |

LD50 oral (mouse) – 150 mg/kg

LD50 dermal (mouse) – 300 mg/kg

LD50 subcutaneous (mouse) – 54 mg/kg

LD50 intraperitoneal (rat) – 72 mg/kg

# **Routes of Potential Exposure**

SUBCUTANEOUS – Needlestick or other sharps injuries that would allow entry of the MPTP into the blood.

ORAL – Ingestion due to aerosol generation or when working with solid MPTP.

INHALATION – Due to aerosol generation, when working with solid MPTP, or when handling bedding from animals during the time period of 5-days post injection.

DERMAL – Direct contact with skin when handling solid or solutions of MPTP; when handling animals, the animal cage inner surfaces, and contaminated bedding material during the time period within 5-days post injection, before cage change.

EYE – From splashes or aerosol generation or when working with solid MPTP.

# **Selection and Purchase**

MPTP must be purchased in the form of non-volatile salt (hydrochloride or tartrate) form instead of as free base. The free base form of MPTP, a colorless crystalline solid (melting point 37-40°C), has a tendency to sublime and is volatile, increasing the risk of an inhalation hazard. Purchase pre-weighed MPTP in the smallest amount and quantity needed to perform the work.

# **Storage Requirements**

* Store the MPTP in a DLAR/lab room dedicated to the preparation and use of highly toxic chemicals.
* All MPTP primary containers MUST be tightly capped and labeled with the full chemical name (e.g., MPTP) and the appropriate hazard warning (e.g. neurotoxin).
* Store MPTP containers in a sealed shatter-proof, leak-proof, secure secondary container, ideally a lockbox tethered to the cupboard/drawer/freezer where stored. Only personnel who have been trained in the safe use of the toxin will have access to the storage container. The secondary container must be labeled with toxin name, hazard warnings, and contact information of trained, responsible laboratory staff.
* The storage location must be in a secure location (e.g., locked room with limited access). The storage location should be labeled with hazard warnings and contact information of trained, responsible laboratory staff.

# **Control of Hazards – General**

* Conduct a hazard assessment to identify proper use and handling techniques, fire safety, storage, and waste disposal issues specific to MPTP and the techniques being used.
* Develop a Lab Specific SOP for MPTP use and post it next to the area where MPTP will be used, for easy access, in case of an emergency.
* Preparation and administration of MPTP should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
* Work with MPTP must be conducted in an area designated for the preparation and use of highly hazardous chemicals.
* Limit access to areas where MPTP is actively being used to persons who:
	+ Have read and signed this SOP.
	+ Have been trained and are knowledgeable on the safe use of MPTP.
* Signs shall be posted on the room door and CFH when MPTP is in use, warning of its presence (e.g. “DANGER! MPTP NEUROTOXIN IN USE. AUTHORIZED PERSONNEL ONLY”). Include on the sign(s) the name and contact information of the responsible individual(s). See Appendices I and II for example signs.
* Avoid weighing powdered MPTP. Instead, reconstitute a whole vial of MPTP and dispose of excess.
* Verify your experimental set-up and procedure prior to use. Conduct a dry run if possible.
* Before starting work, ensure a yellow chemo-therapy drug type sharps container, a hazardous chemical waste container, and **freshly prepared 1% sodium hypochlorite** is next to the MPTP work area.
* Verify all required materials and equipment are available and ready to use before beginning MPTP work. Ideally all items should be available in the MPTP-work room to reduce the need to leave the room until after all MPTP work is done and decontamination has been completed.
* All procedures should be performed carefully to minimize the creation of splashes or aerosols.
* Change gloves at least every 2 hours, or immediately after contamination or signs of damage, and wash hands at time of glove change.
* DO NOT touch door handles or sink faucet handles with potentially contaminated gloved hands.
* Containers of MPTP should be removed from the ventilation control device only after the exterior of the closed container has been decontaminated with **freshly prepared 1% sodium hypochlorite for 10 minutes**.
* All waste and disposable items should be left in the CFH until contained in a bag or sealed container, which then can be placed into a hazardous chemical waste container. Closed vials/tubes of solid or liquid MPTP waste can be placed directly into a hazardous chemical waste container.
* Wipe down the surface of all non-disposable equipment used in manipulations (pipettors, etc.) with **freshly prepared 1% sodium hypochlorite and allow a contact time of 10 minutes** before removing from the CFH.
* A hand-washing sink must be readily available to all locations where MPTP is being used. Wash hands with soap and water when work with MPTP is completed and before leaving the work area.
* An eyewash station and safety shower must be easily accessed, and available within 10 seconds travel time (~55 ft.) for emergency use in locations where MPTP is being used.

# **Engineering/Ventilation Controls**

* Work with MPTP should be done in a certified chemical fume hood (CFH) ducted to the outdoors. This includes opening shipment packages. DO NOT OPEN A VIAL OF POWDERED MPTP OUTSIDE OF A CFH.
	+ Each person working with MPTP should review how to safely use a CFH ([CFH use](https://research.wayne.edu/oehs/chemical/fume-hood)).
	+ The user should verify the CFH has been certified by OEHS within the last 12 months.
	+ The user should verify the inward airflow before initiating work.
	+ The user should conduct work within the operationally effective zone of the CFH.
* Do not use a biological safety cabinet (BSC) unless it is a Class II B with 100% exhaust to the outdoors. The BSC must be certified within the last 12 months and used within the operational parameters. Review [Working Safely in the Biological Safety Cabinet](https://research.wayne.edu/oehs/bio-safety/cabinets#generalsuggest).
* If the ventilation control device is a shared resource, it is recommended that a sign-up calendar be implemented.
* Use a spill tray or plastic-backed absorbent material inside the ventilation control device.
* The interior of the CFH should be decontaminated periodically, at the end of the experiment or, at a minimum, the end of each day it is used. Decontaminate using **freshly prepared 1% sodium hypochlorite with 10-minute contact time**, followed by a water rinse.
* Until thoroughly decontaminated, the ventilation control device must be posted to indicate that toxins remain in use (e.g. DANGER! MPTP NEUROTOXIN IN USE. AUTHORIZED PERSONNEL ONLY), and access must be restricted. See Appendix I for example sign.
* DO NOT use vacuum lines with MPTP.
* If centrifuging materials containing MPTP, centrifuge safety cups or sealed rotors must be used and the outside surfaces routinely decontaminated. Open the sealed cups or rotors inside a CFH.
* Use disposable plastic ware whenever possible and dispose into a hazardous chemical waste container after use. Avoid the use of glass liquid transferring devices, such as pipettes or syringes, to minimize the exposure risk to MPTP-contaminated broken glass.
* Use safety engineered syringes, needles, and sharps to help protect against puncture or cut hazards. Examples of safety engineered devices include sliding sleeve syringes and protective sheath/retractable needles (e.g., BD safety-lok syringes and BD safety glide needles).
* For MPTP supplied in septum sealed vials, use a hands-free device to stabilize the vial to avoid an accidental needle stick when resuspending.
* Use filter tops with animal cages.

# **Personal Protective Equipment**

In addition to proper street clothing (long pants or equivalent that cover legs and ankles, close-toed non-perforated shoes that completely cover the feet), wear the following Personal Protective Equipment (PPE) when performing lab operations/tasks:

* Safety glasses (If splash potential exists, use goggles + face shield instead)
* Lab coat
* Rear closing disposable water proof gown.
* Nitrile gloves, double-gloved. Preferably extended cuff. No latex.
	+ Place the first pair of gloves over the cuff of the standard lab coat. Place the second pair of gloves over the cuff of the disposable gown.
* N95 disposable respirator for:
	+ All work with solid MPTP (even inside a ventilation device)
	+ Work outside of a CFH/exhausted BSC with animals which have been administered MPTP
	+ Cage changes and handling of bedding from animals which have been administered MPTP
	+ Use of an N95 respirator requires fit testing and training. Contact OEHS (7-1200) to schedule completion of training/fit testing.

PPE must be removed in a specific order to prevent exposure. Remove as follows:

1. Outer gloves
2. Disposable gown
3. Safety glasses (or face shield then goggles)
	1. Spray with **freshly prepared 1% sodium hypochlorite and allow a contact time of 10 minutes** before rinsing with water and drying.
4. N95 respirator (where applicable)
5. Lab coat
6. Inner gloves
7. Wash hands with soap and water

Disposable PPE must be discarded into a hazardous chemical waste container to be disposed by OEHS.

# **Special Handling Procedures**

**Dissolving MPTP**:

* During MPTP solution preparation, warn other lab members to keep their distance from the ventilation control device or restrict access to the room to only personnel immediately needed for preparation.
* It is highly recommended to dissolve an entire vial of MPTP instead of weighing out the solid. This will reduce the risk of particulates becoming airborne (inhalation hazard) and particulates contaminating surfaces that may occur from weighing MPTP. Current literature indicates solutions of MPTP are stable for up to 3 months at -20°C.
* Dissolve MPTP by injecting liquid through the rubber septum of the vial. After adding the liquid, the rubber septum will be carefully removed using forceps and a pipet will be used for additional mixing.

**Transporting MPTP:**

* Transport MPTP in a minimum of 2 layers of sturdy, leak-proof, non-breakable containers (e.g., plastic container with sealing lid as a secondary container). Line with absorbent material.
* The secondary container must be clearly labeled with the toxin name, PI name, and lab contact information.
* The use of a hand cart is recommended when transporting between buildings and between floors.
* Ideally, syringes should be loaded with MPTP at the end point location, not prior to transporting.

# **Considerations for Work with Animals**

* Follow procedures for safe use of sharps, and practice doing a “dry run” with less hazardous materials as needed. Use safety engineered needles/sharps whenever possible. A sharps container must be within arms-reach for safe sharps disposal.
* DO NOT RECAP NEEDLES. Use a fresh needle/syringe per animal.
* Syringes loaded with MPTP solution should be placed in a tray or holder with the needle protected or positioned away from personnel to minimize the risk of needlestick injury.
* Animals should be anesthetized or placed into a restraining apparatus during injection with MPTP.
* MPTP exposed animals are considered hazardous for up to 5-days post last injection of MPTP. All procedures performed during this time frame must either be completed in a CFH/BSC ducted to the outdoors or personnel must wear an N95 respirator.
* Disposable or micro-isolator filter top cages must be used during the 5-days post MPTP injection. Animal cages must be clearly labeled to indicate that the animals have been treated with MPTP.
* For the 5-days post last injection of MPTP, animals must be housed in a room designated by DLAR for use with animals treated with hazardous chemicals.
* After procedures are complete, the restraining apparatus, surrounding workstation, and any reusable equipment must be decontaminated using **freshly prepared 1% sodium hypochlorite for 10-minute contact time**, followed by a water rinse.
* Hands must be washed with soap and water after completion of the injections.
* Initial animal cage change should be conducted in a CFH/exhausted BSC by personnel who are fully trained in handling the toxin.
* Animal carcasses treated with MPTP must be placed in a black carcass bag with a label attached that includes the PI name, IACUC number, and the hazardous agent. Then place in the Waste Bin for incineration only.

# **Decontamination Procedures**

**NOTE: Undiluted household bleach typically contains 5-6% sodium hypochlorite. If using household bleach, be sure it is diluted properly to a final concentration of 1% sodium hypochlorite (not 1% bleach). Also, undiluted household bleach degrades over time and should be replaced after 1 year.**

* Most disinfecting agents will not inactivate MPTP and may adversely react with MPTP (e.g., AccelTB and Peroxigard).
* After MPTP injection and before first cage change, interior surfaces of the cage, the surfaces that the animals and/or their excreta could physically touch, including food and drinking bottle, are considered to be contaminated with MPTP and its metabolites.
* Work space surfaces must be decontaminated with **freshly prepared 1% sodium hypochlorite** **for a minimum of 10 minutes** at least once daily, during the length of the experiment. To prevent corrosion of metal surfaces rinse with water then 70% ethanol after using chlorine-based chemicals.
* Absorbent pads will be replaced daily. The used and contaminated absorbent pads, disposable PPE, etc. will be placed in a hazardous chemical solid waste container.
* Reusable surgical tools can be decontaminated by soaking in a solution of **freshly prepared 1% sodium hypochlorite** **for a minimum of 10 minutes**. The surface of the tools must be gently scrubbed if there are significant amounts of organic material on the surfaces. Following this treatment, the tools must be rinsed with water. The surgical tools must be autoclaved prior to re-use per DLAR requirements.

# **Waste Disposal**

* Unused MPTP (solid or liquid) that is to be disposed and any disposable materials/items contaminated with MPTP (e.g. disposable labware, spill absorbent pads, disposable PPE) must be collected and disposed as hazardous chemical waste. This includes the original product container.
* Discard closed disposable containers (e.g. Eppendorf tubes, conical tubes up to 50 ml) of excess MPTP solution directly into a hazardous chemical waste container (e.g. 5-gallon pail or carboy obtained from OEHS). Do not try to decant the excess MPTP solution.
* Avoid inhalation of bedding dust. At first cage change or any cage changes within the 5-days post last MPTP injection:
	+ For disposable cages, the disposable cages with the bedding material can be directly placed in a plastic bag, tied closed, and disposed of as hazardous chemical waste for incineration.
	+ For reusable micro-isolator cages, wet the bedding, the interior of the cage, and the outside of the water bottle with a solution of **freshly prepared 1% sodium hypochlorite** **and allow a minimum of 10-minute contact time.** The bedding then should be disposed of hazardous chemical waste. Re-wet the cage with **freshly prepared 1% sodium hypochlorite** **and allow a minimum of 10-minute contact time** before dumping in front of a Biobubble and disposing as chemical waste in yellow incinerate only bin.
* DO NOT autoclave any material contaminated with MPTP and its metabolites. This will aerosolize the chemical, creating an inhalation hazard.

Do not dispose of waste by dumping down a drain or discarding in regular trash containers, unless authorized in writing by OEHS. [Submit requests to OEHS](https://research.wayne.edu/oehs/forms/chem-waste) for waste containers, labels, and waste collection. Also, refer to the [OEHS Hazardous Waste Management web page](http://research.wayne.edu/oehs/hazardous/index.php) and [WSU Chemical Hygiene Plan](http://research.wayne.edu/oehs/pdf/chemical-hygiene-plan.pdf) for more information.

# **Spill procedures**

All spills should be reported to the Principal Investigator. For any spills involving broken glass or sharps, use forceps to remove any broken glass or other sharp items and place in a sharps container.

1. **Powder or Liquid Spills Outside of CFH**

For spills or releases that have impacted the environment (via the storm drain, soil, or air outside the building); or that cannot be cleaned up by local personnel due to size of spill or location of spill; or a powder spill outside of a CFH.

* 1. Evacuate the spill area of all personnel.
	2. Remove any contaminated PPE and place in a plastic bag for disposal.
	3. Call WSU Police (313) 577-2222. Available 24 hours a day, 7 days a week.
	4. Post someone or mark-off the hazardous area with tape and warning signs to keep other people from entering.
	5. Remain in the vicinity until emergency personnel arrive and provide them with information on the chemicals involved.
1. **Powder or Liquid Spills Inside of CFH**

To be cleaned by properly protected and trained personnel.

* 1. Alert personnel in the immediate area of spill and restrict access.
	2. Remove any contaminated PPE or clothing. Don fresh PPE.
	3. Personnel cleaning spill must be wearing a lab coat, disposable gown, safety glasses/goggles, two pairs of nitrile gloves, and N95 respirator.
	4. Dampen absorbent material with **freshly prepared 1% sodium hypochlorite** and gently place over the spill, to avoid raising dust.
	5. Apply to absorbent material **freshly prepared 1% sodium hypochlorite**, starting at the perimeter and working towards the center, **allowing 10-minute contact time to deactivate MPTP**.
	6. Collect spill cleanup materials using a scoop or other suitable tools and place in a tightly closed hazardous waste container.
	7. Clean the spill area with **freshly prepared 1% sodium hypochlorite** **allowing 10-minute contact time**, then soap and water.
	8. Allow CFH to run for at least 10 minutes before resuming work.
	9. Place all contaminated materials, including contaminated items such as gloves, in the hazardous chemical waste container.
	10. Wash hands thoroughly after completing any spill cleanup.
	11. Label waste container with completed hazardous waste tag (available from OEHS).
	12. Submit online [waste pickup request](https://research.wayne.edu/oehs/hazardous/chemical-waste.php) to OEHS.

# **Medical Consultation and Post-Exposure Prophylaxis**

Prior to initiating work with MPTP, OEHS recommends the PI and any lab personnel working with MPTP complete a neurological examination and medical consultation with a WSU approved Occupational Health Physician regarding exposure risks and treatment options in the event of an exposure. Exposure risks and treatment options can be affected by complicating factors such as personal health history, work history, lifestyle, and exposure to environmental contaminants. Physicians may consider the use of a non-selective inhibitor of monoamine oxidase. Contact the OEHS Occupational Health Nurse (7-1200) to receive a referral to a WSU approved medical provider for evaluation.

# **Emergency Procedures**

**\*\*If medical attention required, call WSU police (313-577-2222) immediately\*\***

* **Eyewash and Safety Shower** – An eyewash station and safety shower must be easily accessed, and available within 10 seconds travel time (~55 ft.) for emergency use. Instruct personnel on the locations of eyewashes and safety showers, and how to activate them, prior to an emergency. **NOTE**: The eyewash must be flushed on a weekly basis and documented using the [Emergency Eyewash Maintenance Log](https://research.wayne.edu/oehs/docs/eyewash-log-sheet.doc), which must be posted near the eyewash.
* **Sink** – A sink for handwashing must be available and accessible within the room(s) where MPTP work will be performed.
1. **Injuries and Exposures**

In case of an injury or exposure, a secondary person should be available to secure the MPTP and the area and to contact emergency personnel. Bring copies of the Safety Data Sheet for MPTP and this SOP to the occupational health clinic or hospital. After receiving treatment for injuries and exposures, call OEHS (313) 577-1200, to report the exposure. Complete the [Report of Injury](https://risk.wayne.edu/files/rofi.pdf) form and submit to WSU Enterprise Risk Management.

First aid measures for MPTP exposure.

* 1. **Needlestick or sharps injury involving MPTP.**
		1. Halt all work and immediately wash the wound with soap and water for at least 15 minutes.
		2. Call WSU Police (313) 577-2222.
	2. **Eye contact or dermal exposure.**
		1. Remove any contaminated clothing.
		2. Flush with copious amounts of water for at least 15 minutes using an eyewash/safety shower.
		3. Call WSU Police (313) 577-2222.
	3. **For oral (mouth) exposure or if MPTP has been swallowed and if the person is conscious.**
		1. Wash out mouth with water for at least 5 minutes.
		2. Call WSU Police (313) 577-2222.
	4. **For inhalation exposure.**
		1. Move the exposed individual from the area to fresh air.
		2. If breathing becomes difficult call WSU Police (313) 577-2222.
1. **Authorized Occupational Health Clinic and Emergency Rooms**

For injuries and exposures that are not considered serious or a medical emergency, visit:

Henry Ford Occupational Health – Harbortown

3300 East Jefferson, Suite 100

Detroit MI 48207

(313) 656-1618

Monday – Friday 8:00 AM to 6:30 PM

If Henry Ford Occupational Health Center is closed or for serious injuries, visit:

Henry Ford Hospital – Emergency Room

2799 W. Grand Blvd.

Detroit MI 48202

(313) 916-8742

OR

Detroit Receiving Hospital - Emergency Room

4201 St. Antoine St, Detroit, MI 48201

Phone: (313) 745-3000

# **Minimum Training Requirements**

1. **General Training:**
* [Laboratory Safety Training](https://research.wayne.edu/oehs/training/lab) (general lab and chemical safety issues).
	+ Online through the [Collaborative Institutional Training Initiative (CITI)](https://about.citiprogram.org/en/homepage/).
* [Laboratory-Specific Safety Training](https://research.wayne.edu/oehs/docs/lab-safety-training-checklist.doc)
* [HazCom and Global Harmonization System (GHS)](https://research.wayne.edu/oehs/health-safety/hazcom-ghs).
1. **Laboratory Specific Safety Training:**
* Review of Safety Data Sheet for MPTP.
* Review of this SOP.
* Review of lab specific SOP
* Review [WSU Hazardous Waste Management](https://research.wayne.edu/oehs/hazardous/chemical-waste) guidelines.

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APPENDIX I – CFH WARNING SIGN



**MPTP NEUROTOXIN IN USE**

**AUTHORIZED PERSONNEL ONLY**

PI/Responsible Person(s):

Contact Information:

APPENDIX II – ROOM WARNING SIGN



**MPTP NEUROTOXIN IN USE**

**ENTRY RESTRICTED TO AUTHORIZED PERSONNEL**

PI/Responsible Person(s):

Contact Information: