



## Standard Operating Procedure for

# ***Laboratory Handling and Animal Injections of Paraquat (Pesticide) and Maneb (Fungicide): A Mouse Parkinson Disease Model***

### 1.0 PURPOSE

Parkinson's disease (PD) is a well characterized disorder with several animal models. These animal models provide an outstanding opportunity to test the ability of novel therapies to treat, delay or cure the disease.

Paraquat dichloride tetrahydrate (PQ) is a known pesticide and Maneb (MB) is a known fungicide. Prolonged exposure (via I.P. injections) of PQ alone or combined with MB was shown to produce moderate Parkinsonism by affecting the dopaminergic neurons of the substantia nigra and may also affect the lung function. Epidemiological studies suggest that prolonged environmental exposure to PQ- like compounds may be one of the causes of PD. PQ can cause eye, skin and respiratory tract irritation, may be harmful if absorbed through the skin, inhaled or swallowed. Exposure can cause liver, kidney, cardiovascular and lung injury.

### 2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who will inject mice on with PQ and/or MB within the Stem Cells Engraftment and *in vivo* Analysis Core.

### 3.0 PROCEDURE- Preparation of Injection Solution

All chemical Handling is done under Chemical Hood. All personnel wears Double Nitrile gloves, Tyvek suit, N95 mask, plastic shoe covers and full face shield during drug preparation, injection and handling animals that have been or are injected with PQ+MB. These same precautions are required for handling of the cages and bedding of labeled cages

#### **Paraquat dichloride tetrahydrate (PQ)**

Ampule: 1770ug/mL in D.I. Water F1103AS 5mL (LD50= 57 mg/kg approx. 3 g/person)

Transfer total of 24.5 ml of PQ (from 5 ampules each 5ml, using plastic Pasteur pipette) into 50 ml dark glass bottle containing 11.6 ml of 2.8% NaCl. Keep this bottle it inside another container under chemical hood.

On the morning before injections withdraw approximately 6 ml/24 mice; or 12 ml for 48 mice into 15 ml Blue cap tube and bring inside another container to P2 facility.

	Inject i.p.
Dose mg/kg	per 30 g mouse
5	125 $\mu$ l = 0.151 mg
10	250 $\mu$ l = 0.302 mg

**Maneb (MB)**, ampule of 1000 mg (LD50 750 mg/kg, approx. 37.5 g/person)

Under chemical hood: Add approximately 45 mg MB/into 15 ml screw cup tube. Check the weight and store tube in a dark container under hood.

MB Injection solution- 4.5 mg/ml : Before injection dissolve MB in warm 0.9% saline (45 mg MB per 10 ml saline). Vortex and keep at 45-50C water beaker

## Dosing:

Per 1 kg:	<u>15 mg.</u>	<u>30 mg</u>
Per 30 g B.W. mouse	0.45mg	0.9 mg
Injection (i.p.) solution volume (ul):	100 ul	200 ul

### Equipment:

- pipeter for 5 to 10 ml disposable pipets
- Magnetic stirrer, smallest stir bar,
- Beaker with warm to keep MB bottle in while on magnetic stirrer
- Tube rack for 10 ml blue screw cap tubes labeled MB or PQ to keep syringes during injection
- Bench cover paper (diapers)
- I.P. Syringe – 1 ml for MB and 2 ml (if possible for PQ)

### Manipulations:

- Chemicals are prepared: PQ diluted; MB dissolved and kept on a magnetic stirrer.
- Mice (maximum 6 at a time) are brought to injection room in their housing cages
- MB and PQ are aspirated into their separate syringes (MB 1 ml, PQ 1ml)
- Three mice are injected first with PQ and then with MB
- Next three mice are injected first with PQ and then with MB
- Mice are returned to their home cages and labels placed on cages
- Disposable lab supplies (pipettes, tubes, ampules) in contact with PQ or MB are placed into a biohazard bags which are sealed and labeled with contents.

## 3.1 MICE INJECTIONS

- Animal Injections are done under chemical hood. Cage change will be done in the first 48 hours after any injection.
- Animals in this room that have chemical hazard tags on their cages have been injected with PQ+MB. Animals must be housed in static micro-isolator caging.
- Double Nitrile gloves, Tyvek suit, N95 mask, plastic shoe covers and goggles and/or full face shield are required for handling animals that have been injected with PQ+MB. These same precautions are required for handling of the cages and bedding of labeled cages.
- The investigator will place chemical hazard tags on animal cages after they receive an injection of PQ+MB.
- Laboratory technicians will remove the chemical hazard tags after the cage change, minimum 48 hours after the last injection of PQ+MB is given. The PQ+MB injections will be performed twice a week on Mondays and Wednesdays, or on Sundays and Tuesdays. The cage change will be performed once a week on Friday. Laboratory staff will be responsible for husbandry while the hazard tag is on the cage.
- Laboratory staff will be responsible for dumping and preparing cages for return to the LAF cage washing facility. Cages are placed in a BSC and are dumped into a biohazard bag. Cages are wiped with Spor Klenz the cloth or towel used will be placed in the biohazard bag with the bedding. The

cages should be returned to animal room with a note noting date of decontamination initialed by person who prepared the cages. The biohazard bag should be sealed and placed in the walk-in cooler, room 05, in the basement of the BEB. The bag should be labeled with contents, Bedding from BrdU, and the date.

- LAF staff will be responsible for incineration of the bag.

### 3.6 REFERENCES

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