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**Standard Operating Procedure  
Morphine Lethality**

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STANDARD OPERATING PROCEDURE  
**Morphine Lethality**

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<b>Area: CBA</b>	
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<b>Controls:</b>	
Superseded Document	N/A, New
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<b>Signatures:</b>	
Author	<p>I indicate that I have authored or updated this SOP according to applicable business requirements and our company procedure: Preparing and Updating Standard Operating Procedures.</p> <p>Name: - Signature: <u>Sylvia Caldwell</u> Date: -</p>
Approver	<p>I indicate that I have reviewed this SOP, and find it meets all applicable business requirements and that it reflects the procedure described. I approve it for use.</p> <p>Name: Jason Bubier Signature:  Date: 11/16/2020</p>

**Morphine Lethality**

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**1. PURPOSE**

This SOP addresses the routine procedures used for conducting the morphine lethality study with the Piezo Sleep system.

**2. SCOPE**

The SOP applies to laboratories within the JAX Chesler Lab

**3. RESPONSIBILITIES**

3.1. Laboratory Staff

3.1.1. Remain up to date in training with this SOP

3.1.2. Comply with this SOP

3.2. Principal Investigator

3.2.1. Ensures that all personnel involved running this SOP are trained to comply with this SOP

**4. GLOSSARY/DEFINITIONS**

**4.1. Definitions**

<b>Item</b>	<b>Definition</b>
PVDF (polyvinylidene fluoride)	Sensor pads for bottom of each cage
USB DAQ	Universal serial bus data acquisition module

#### 4.2. Procedure Inputs

<b>Name</b>	<b>Description</b>	<b>Format</b>
Weight pre test	g	Int
Light level	Lux	Int
Test duration	min	Int
Start date/time		(YYMMDD)/time
Stop date/time		(YYMMDD)/time
Cleaning agent		Text
Equipment ID		Text
Equipment manufacturer		Text
Equipment model		Text
Software ID		Text
Software manufacturer		Text
Software model		Text
Date Software Calibrated		(dd/mm/yyyy)/time

#### 4.3. Procedure Outputs

<b>Name</b>	<b>Description</b>	<b>Format</b>
Data Confidence Level		Int
Sleep Daily Percent		Int
Sleep Light Phase Percent		Int
Sleep Dark Phase Percent		Int
Sleep Bout Lengths Mean		Int
Sleep Bout Lengths Standard Deviation		Int
Light Sleep Bout Lengths mean		Int
Light Sleep Bout Lengths Standard Deviation		Int

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Dark Sleep Bout Lengths Mean		Int
Dark Sleep Bout Lengths Standard Deviation		Int
Diurnal Wake Ratio Mean		Int
Peak Wake wrt Dark Onset Median		Int
Light Onset Wake Median		Int
Breath Rate During Sleep Mean		Int
Breath Rate During Sleep Standard Deviation		Int
Start Date/time		Date/time

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Test Duration		Int
Hobo data – Temp	F	Int
Hobo data – humidity	%	Int
Hobo data- light intensity	Lum/ft 2	Int
Sleep %	%	Int
Sleep bouts		int

## **5. MATERIALS**

### 5.1 Piezo Sleep Mouse Behavior Tracking System (Signal Solutions, LLC Lexington, KY)

- Computer
- NI box (National Instruments)
- 72-Polycarbonate Cage Wall Units with 302 Stainless Steel Feeding Grates
- Polycarbonate cage lids (3000 Series Aluminum Vents)
- Acetate floors
- PVDF Sensor mats
- 150 ml water bottles with long sippers
- Required feed (6% grain)
- Scale (0.1g accuracy)
- Acidified clean water
- Pine shavings
- Alpha-dri
- Haemo-sol
- 70% ethanol
- Paper towels
- HOBO Environment Tracker
- Forceps (if desired for mouse handling and transfer)

## **6. PROCEDURE**

### 6.1 Subjects

#### 6.1.1 Species. Mice

6.1.1.1 Study specific animals (e.g, strain, sex, date of birth) ordered or bred and documented.

6.1.1.2 Receipt of animals logged (e.g., date of arrival)

6.1.2 Sex. Males or females

6.1.3 Age. The test is validated for mice 8 weeks and 5 months of age.

6.1.4 Housing. Subjects are group housed prior to testing. During testing, mice go into individual chambers for monitoring. Food and water are always available ad libitum.

6.1.5 Subject Identification. Mice are assigned an identification number as they are group housed upon arrival.



6.1.6 Subjects are pre-assigned a test chamber.

6.2 Cage Set-up

6.2.1 In each quad, place a rubber pad securely beneath each corner. Secure it beneath the edge/corner of the quad.

6.2.2 Next, each PVDF sensor pad is placed over the rubber pads, ensuring that the correct pad is laid onto its corresponding mat.

6.2.3 Place an acetate floor over each sensor pad. This will act as the cage floor and hold the bedding. The circle cut-out directs the placement of the floor on the pads.

6.2.4 Next, place a cage wall unit on top of each floor setup. Make sure it is fit into the acetate floor lines.

6.2.5 Add roughly a ½ cup of pre-mixed pine shavings and alpha-dri to each chamber.

6.2.6 Fill each cage feeder about halfway with food. Amount of food is based on study length.

6.2.7 Fill water

6.2.7.1 150 ml water bottles will need to be filled with the clean supply acidified water.

6.2.7.2 After filling each bottle, make sure metal sipper is secure.

6.2.7.3 Place a water bottle in each cage; sliding it down the edge of the cage to ensure no water reaches the cords or pads on the bottom of the quad.

- 6.3 System Computer Setup. Prior to placing subjects into the arena, the software and input files must be prepared.
  - 6.3.1 Create a folder for the run on the desktop. This is where the runsheet, ID template, and data will be stored.
  - 6.3.2 ID Template. This is an excel sheet that contains only one column of information-the mouse IDs. Any empty chamber can be filled with "BLANK" or "EMPTY."
  - 6.3.3 Your runsheet should include all relevant mouse identification information, such as sex, strain, and date of birth, as well as any relevant notes to be taken: subject start weights, behavioral notes, injection times, etc.
  - 6.3.4 Open Piezo Sleep software on desktop.
  - 6.3.5 You will be prompted to select number of DAQs. Type 1; click ok.
  - 6.3.6 Next, select DAQ device. There should only be one option.
  - 6.3.7 Type number of active channels for the test – 76 (or the number being used)
  - 6.3.8 You will then be asked if you wish to import IDs from an excel spreadsheet.
    - 6.3.8.1 For testing the system, click "no" and it will auto-fill a previous ID template. These IDs will not matter, as you will automatically delete any data recorded to test the functionality of the system.
      - 6.3.8.1.1 Name the file "test" so you can delete this data afterward.
      - 6.3.8.1.2 You can test that each box is recording properly by gently running a hand along each cage and monitoring the signal. Make sure nothing is obstruction the sensor pads. Once testing is complete, you can hit "stop" on the computer and delete the test data.
    - 6.3.8.2 For an actual test in which you wish to save data, click "yes" and select your ID template file. Import.
  - 6.3.9 You will be prompted to enter a file name for your data. Name with date of run and make sure this saves to your desktop folder. Click ok.

6.3.10 There will be a summary screen depicting the system inputs and all selections you've just made. There is an option to edit each section on the right. If everything looks as it should, click "ok," and then "start". Data saves automatically.

6.4 Testing: Day One

6.4.1 Each mouse is individually weighed and then loaded into its preassigned chamber.

6.4.2 A cage lid is placed on immediately after loading each mouse.

6.4.3 After each mouse is loaded, follow steps of 6.3.

6.4.4 Start test. Be careful to not touch or interrupt the system once the test is started.

6.5 Testing: Day Two

6.5.1.1 Drug Preparation.

6.5.1.1.1 Dose and injection amount are predetermined and based on body weight. Mice are tested on a probe dose. If lethality >50% for a particular strain, the strain will be retested at a lower dose. If lethality <50%, the dose will be raised to flank the LD<sub>50</sub> for each strain.

6.5.1.1.2 Morphine is carefully weighed on a scale and then mixed into sterile saline.

6.5.1.2 24 hours after the start of test, mice are given a subcutaneous injection of morphine and placed back into their chambers for continued monitoring. Injection time for each mouse is recorded on the runsheet.

6.5.1.3 Mice are monitored for 30 minutes post injection and any relevant notes taken.

6.6 Testing: Day Three

6.6.1 Hit "stop" on the computer.

6.6.2 Mice found dead are highlighted red on the runsheet.

6.6.3 Surviving mice are put into cull boxes for euthanasia via CO<sub>2</sub>.

6.6.4 Save runsheet and data to desired location (CSNA phenotype share drive).

- 6.6.5 Delete folder from desktop.
- 6.6.6 Once mice are out of the room and euthanized, begin clean up.
- 6.6.7 Plug HOBO environment tracker back into computer and export and save the data to its software folder.
- 6.7 Cleaning.
  - 6.7.1 Remove cage lids and water bottles and stack on cart.
  - 6.7.2 Dump the food from each cage into a used feed disposal bag.
  - 6.7.3 Wipe cage with haemosol wipe and load onto cart.
  - 6.7.4 Dump bedding from acetate sheets and collect.
  - 6.7.5 Remove rubber mats.
  - 6.7.6 Once all pieces are taken off, quads can be vacuumed and then gently wiped down with 70% ethanol.
  - 6.7.7 Gently wipe sensor pads with 70% ethanol.
  - 6.7.8 Vacuum room.
  - 6.7.9 After dumping water down the sink, cage lids, bottles, and sippers can be washed in the dishwasher with a tablespoon of haemosol.
  - 6.7.10 Cages wall units, rubber mats, and acetate sheets can be washed in the sink with it ½ full of warm water and a tablespoon haemosol. All are spread out on racks to dry.
- 6.8 Data Analysis
  - 6.8.1 Breath rates are estimated from 4-s intervals in which animal activity dropped low (i.e. during sleep and brief rest periods and pauses during wake), and averaged over 24-min overlapping intervals to provide an average respiratory rate every 12 min. The respiratory rate baseline consisted of the average respiratory rate over the first 24 h, which included both sleep and wake periods. Respiratory rate is then measured in the same way after injection of opioid. These measures were then used to determine thresholds for obtaining the recovery time or survival time.

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