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| **SOP#: 002** | Date Issued: 8/03/21 | Date Revised: 7/08/2021 |
| **TITLE:** | **TSE Metabolic Chambers** | |
| **SCOPE:** | Research Personnel | |
| **RESPONSIBILITY:** | BORC staff | |
| **PURPOSE:** | To outline the proper procedures for use and maintenance of the TSE Metabolic Chambers. | |

## 1 PURPOSE

This SOP explains how to apply TSE Metabolic Chambers in biomedical research such as animal behavior, metabolic and eating drinking study.

## 2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

## 3 BEFORE CONDUCTING YOUR EXPERIMENT

3.1 All users have to be trained and approved by BORC staff before they use the equipment.

3.2 All user need to notify Life Science Annex facility and BORC staff one day before use the equipment.

3.3 All investigators must have “metabolic cage housing” listed in their existing IACUC protocol prior to use.

3.4 While using the TSE system, the investigator/investigator’s personnel will be responsible for checking the animals at least twice per day. The investigator will also be responsible for replenishing/replacing all food, water and bedding as needed and insuring that the system is functioning properly. No maintenance will be provided by BORC staff.

3.5 The investigator and/or investigator’s personnel are responsible for assembly/disassembly and cleaning of the system. Parts of the system are delicate and expensive; please exercise extreme caution! Make sure o-rings on feeding/ drinking sensors have a thin layer of silicone grease and are in tracks. Use a gentle twisting motion while pushing straight down or pulling straight up – do not angle or torque sensor or the plastic housing will crack.

## 4 PROCEDURES

### A week before

All animals should be individually housed, as stress of separation may eventually modify their body composition. (At the time of the experiment animals should have recover their initial body weight)

Animals should be carefully weighted and if available their body composition monitored by a non-invasive methods (Dual X-ray) at this time and prior running the calorimetric analysis.

It is recommended that the animals are trained on the special drinking nipple before the first test is carried out. This can be done by using a training bottle equipped with the special nipple that can be inserted in the feed rack of the standard home cage or the CaloCage.

### Before Day 1:

### 4.1 System warm up

4.1.1 Turn on the system

Turn on power switch on surge protector to turn the module on. Turn on computer (Behind door with lock, bottom of the monitor control tower) and start the software PhenoMaster by clicking the icon on Desktop)

The system of calorimetry must be switch ON to warm up all the following module:

- Air pump
- Calorimetry Process control Master and Slave unit
- Air Drying unit.
- High Speed Sensor Unit.
- Sample switch unit.
- MoTil2 process control unit.

4.1.2 Check the tubs of AUD without water inside. So follow them from the front of ADU, sample out, back to the control unit, and the bottles. Verify there is no water in any of these tubes or bottles.


**4.2 Feeding & Drinking Calibration (every 3 month if need to analyze feeding & drinking activity)**

4.2.1 Hang the empty food and drink bottle under the sensors that need to be calibrated.

4.2.2 Click Calibrate Batch Calibrate

4.2.3 Under presets tab, task box, select calibration

4.2.4 Under presets tab, select sensors box, select drink and feed

4.2.5 Under presets tab, select boxes, box, set all

4.2.6 Click Calibration values

4.2.7 Enter the calibration weight in grams (g)

4.2.8 Press OK

4.2.9 First do a zero reading for all sensors, click on auto catch, wait for all box show "1" (in square)

4.2.10 Put weight on first sensor

4.2.11 Clear Buffer

4.2.12 Use Auto Catch, waiting until the one with weight turn into "check" mark.

4.2.13 Repeat steps 10-12 for all of the sensors.

Notes: 24 Number Sensors

0 g ---> 90g Cal Weights

89.9675 exact weight (written on ziplock bag)

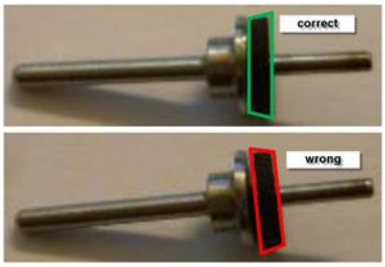
***AutoCatch: Max Drift 3, Max Variance 10

Red (auto catch) ---> Yellow (Calculate) --->Green (Save)

**Day 1:**

**4.3 Set up cages**

4.4.1 Fill all the bottles and food baskets




4.4.2 Add new bedding to the cages, make sure bedding doesn't interfere with food and drink basket or the activity frames.

**4.4 Gas Calibration**

120 minutes before the start of the calibration of the $O_2$ and $CO_2$ sensor switch on the calorimetry control unit and start the program.

4.4.1. Open all three gas valves all the way of calibration gas tanks and purge the sensor with the calibration gas for at least 30 minutes.:

20.95% Oxygen, 0.05% Carbon Dioxide

20% Oxygen, 0.95% Carbon Dioxide

~100% Nitrogen

4.4.2. Click **Calibrate/Gas Measurement/Auto Cal** (takes about 15 minutes)

***If a box appears saying it cannot calibrate click **No/Continue**

4.4.3. Shut all three gas valves all the way when done!

Put mice in metabolic cages with food and water for 3 days acclimation

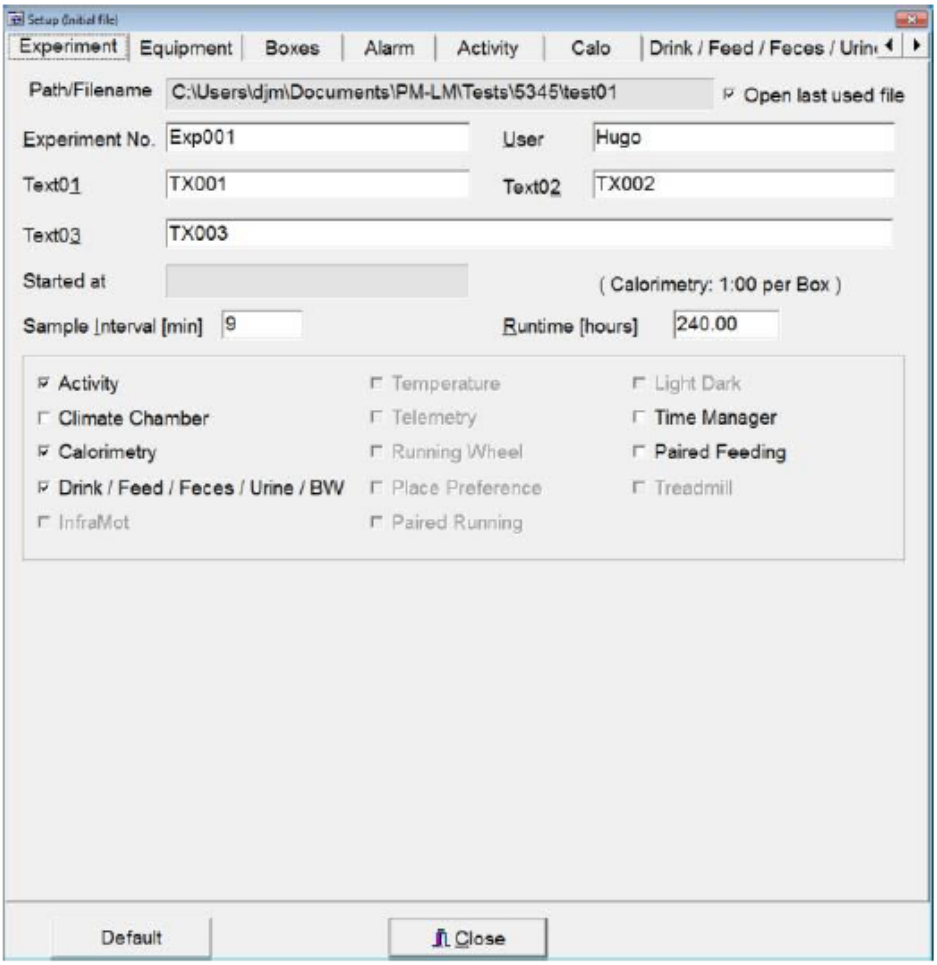
**4.5 Set parameters**

4.5.1 Create new file for the experiment by clicking menu File-New

4.5.2 Fill information of experiment.

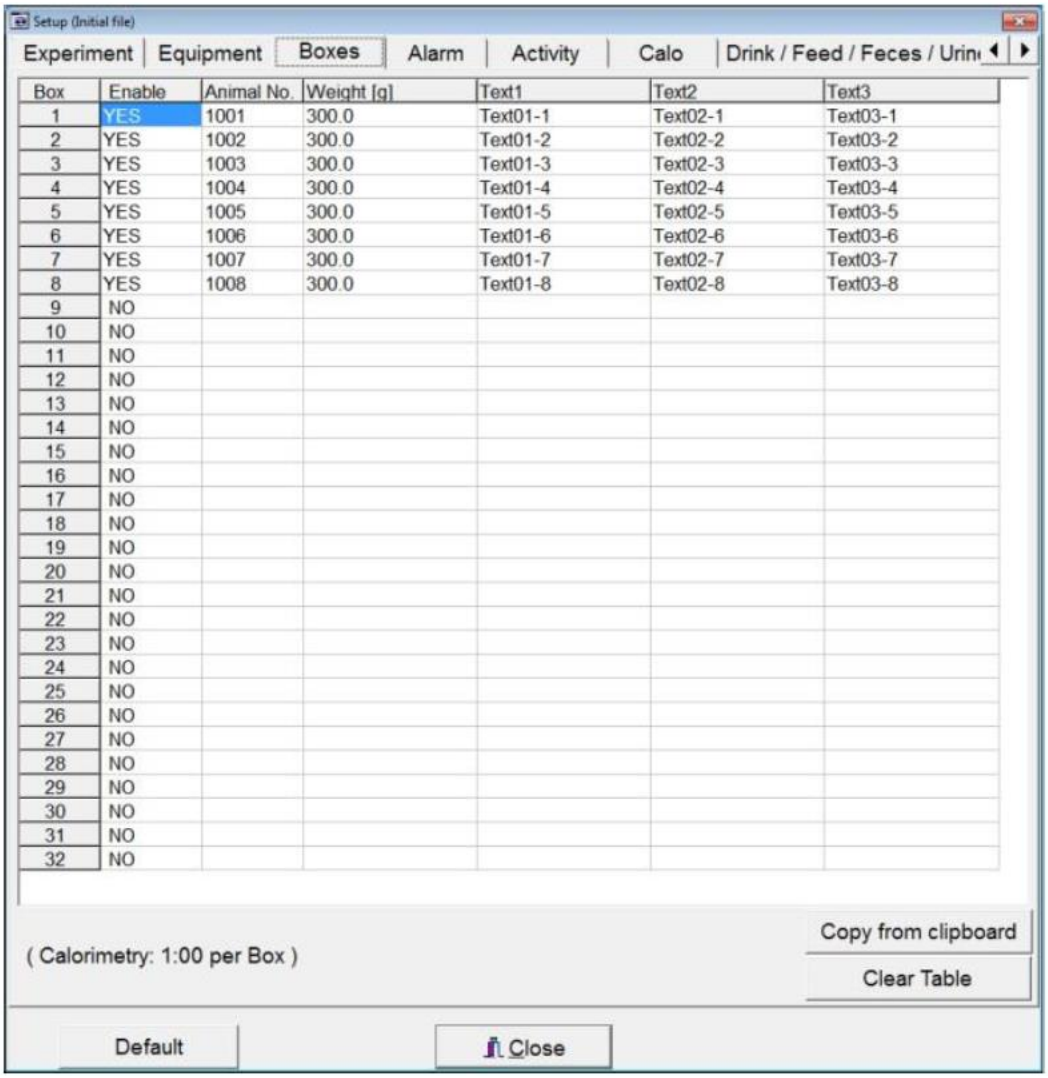
- Fill the blank space with the name of the user, name of the experiment, type of animals, the date.
- Fill the Runtime [hours] Entry in hours for Auto-Stop. The measurement can be stopped at any time, set 240h.

- Fill the Sample Interval [min], (it will depends of the number of cages being used + the cage reference, the O2/CO2 module, the distance of the cage to the module). The time per Box will be automatically calculated.
- Check Boxes - Activity, Calorimetry, Drink/Feed/Body Weight, Time Manager, Paired Feeding, etc... Checkboxes for activating the required measurements.




4.5.2 Enter animal information

- Enable the number of cages being used (Yes).
- Filled the animal number.
- Enter the weight (it is needed to calculate the Heat production/Energy expenditure or H(1) in [kcal/h/kg body weight].
- Filled the blank (text 1,2,3) for maximum information.




| Box | Enable | Animal No. | Weight [g] | Text1 | Text2 | Text3 |
|---|---|---|---|---|---|---|
| 1 | YES | 1001 | 300.0 | Text01-1 | Text02-1 | Text03-1 |
| 2 | YES | 1002 | 300.0 | Text01-2 | Text02-2 | Text03-2 |
| 3 | YES | 1003 | 300.0 | Text01-3 | Text02-3 | Text03-3 |
| 4 | YES | 1004 | 300.0 | Text01-4 | Text02-4 | Text03-4 |
| 5 | YES | 1005 | 300.0 | Text01-5 | Text02-5 | Text03-5 |
| 6 | YES | 1006 | 300.0 | Text01-6 | Text02-6 | Text03-6 |
| 7 | YES | 1007 | 300.0 | Text01-7 | Text02-7 | Text03-7 |
| 8 | YES | 1008 | 300.0 | Text01-8 | Text02-8 | Text03-8 |
| 9 | NO | | | | | |
| 10 | NO | | | | | |
| 11 | NO | | | | | |
| 12 | NO | | | | | |
| 13 | NO | | | | | |
| 14 | NO | | | | | |
| 15 | NO | | | | | |
| 16 | NO | | | | | |
| 17 | NO | | | | | |
| 18 | NO | | | | | |
| 19 | NO | | | | | |
| 20 | NO | | | | | |
| 21 | NO | | | | | |
| 22 | NO | | | | | |
| 23 | NO | | | | | |
| 24 | NO | | | | | |
| 25 | NO | | | | | |
| 26 | NO | | | | | |
| 27 | NO | | | | | |
| 28 | NO | | | | | |
| 29 | NO | | | | | |
| 30 | NO | | | | | |
| 31 | NO | | | | | |
| 32 | NO | | | | | |

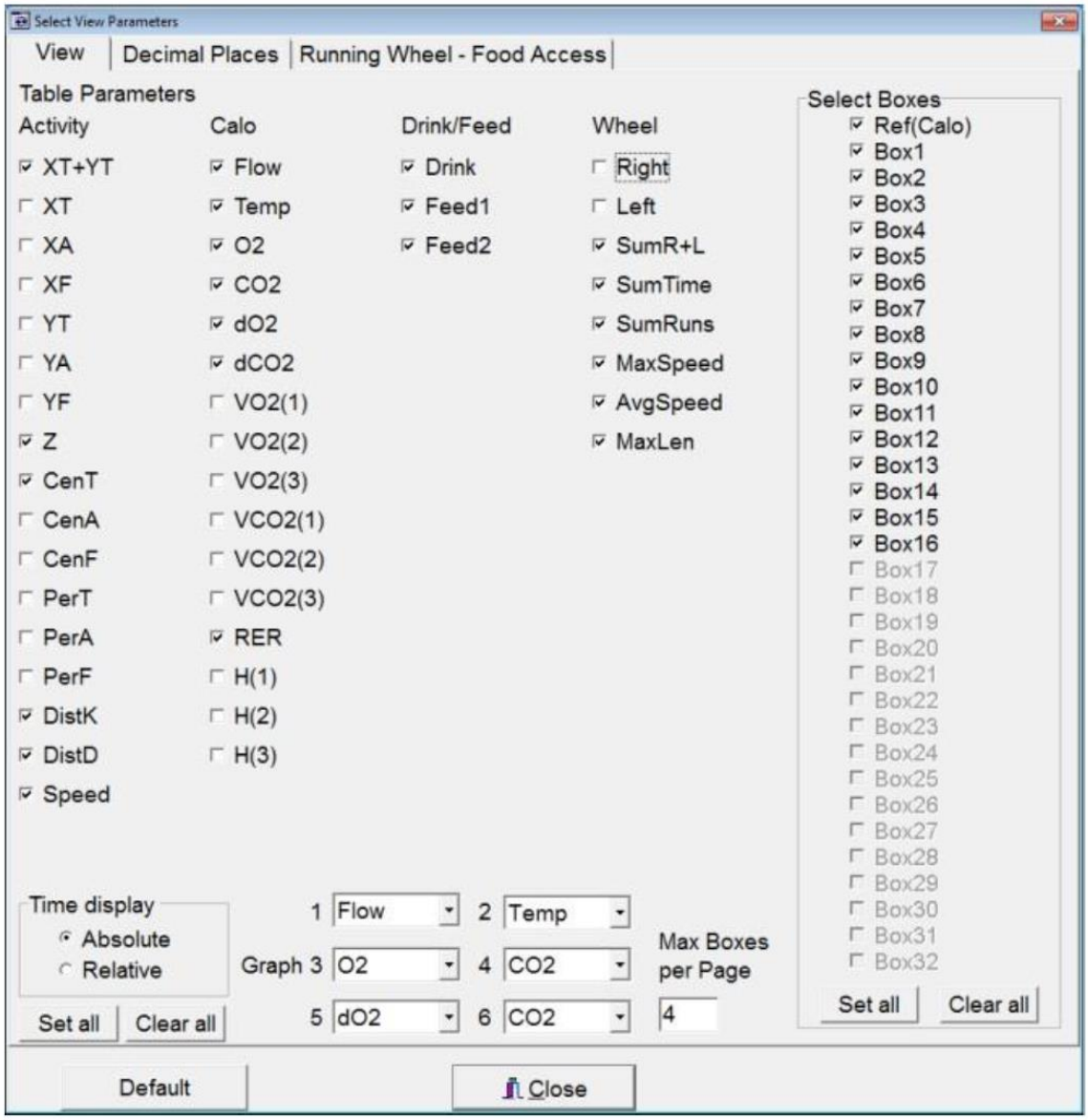
## 4.6 Start measurement

4.6.1 Start measurement.

Click Measurement / Start. While the experiment is running, the parameters of the experiment can be view in real-time.

4.6.2 View status

In the "View" menu, those parameters can be selected which, e.g., are to be shown on the "Table" or "Graph". Measured values of those parameters of the program part that have been selected in "Setup" can be shown only, as otherwise the checkboxes are disabled (shown in gray).




### 4.7 Stop experiment

At the end of the experiments: Click Measurements/ Stop. Airflow is still ON, but the measurements stopped, no parameters are being recorded.

4.7.1 Hook all cover.
4.7.2 Remove the animals, we recommend that all animals should be weighed and scanned for their body weight composition ( DEXA).
4.7.3 Disconnect all Samples in/out.
4.7.4 Removed food and drink baskets.
-**CAUTION**- *Laid the sensors on their side not on their hook.*

4.7.5 Empty the bottle below the air-drying unit
4.7.6 Stopped the software (End/ Exit Program? / Yes)
4.7.8 In the case that no other experiment are being run within the next 48h, we recommend that you switch off all equipment and close the compressed dry air bottle

### 4.8 Cleaning TSE System After Experiment

4.8.1 Water Bottles and Drinking Nipples – glass bottles should be cleaned with mild soapy water, rinsed thoroughly with warm water, and air dried on racks. BE CAREFUL! The screw caps and the drinking nipple components can be dismantled into their individual components, cleaned with mild soapy water, rinsed with warm water and air dried. Lysol wipes may also be used. No cleaning products containing bleach, alcohol, or hydrogen peroxide should be used.

4.8.2 Food Baskets – food baskets should be cleaned with mild soapy water, rinsed thoroughly with warm water, and air dried on racks. Lysol wipes may also be used. No cleaning products containing bleach, alcohol, or hydrogen peroxide should be used.

4.8.3 Metabolic Cage tops – disinfect underside of cage top by wiping down with a soapy cloth or Lysol wipe, followed quickly by a second cloth moistened with water only to rinse disinfectant from plastic. No cleaning products containing bleach, alcohol, or hydrogen peroxide should be used.

4.8.4 Cage Bottoms - disinfect cage bottoms by wiping down with a soapy cloth or Lysol wipe, followed quickly by a second cloth moistened with water only to rinse disinfectant from plastic. After cleaning and drying, the lower part of the cage can be autoclaved. No cleaning products containing bleach, alcohol, or hydrogen peroxide should be used.

## 5 MAINTENANCE

### 5.1 General

To carry out bio-decontamination efficiently, all adhering dirt should first be removed by a preliminary cleaning process, as otherwise microorganisms could survive in the contaminated areas. If detergents or chemicals are used on the devices, these should be completely removed before further decontamination steps, e.g., autoclaving, are carried out. There should be no residues on the devices. Ensure that all noxious and odorous substances can evaporate sufficiently so that the animals are not subjected to any irritations.

H2O2 Gas Plasma Sterilization

- Gassing must be done according to guidelines and protection measures provided by the manufacturer of the decontamination unit.
- Gassing type must be adjusted in accordance with the guidelines for gassing of laboratory devices including sensitive electronic equipment.

- After gassing, allow for a outgassing time >=24h.
- Systems that contain porous materials (e.g. foamed material, insulation, plastics) should be allowed an elongated outgassing time (>=72h) to ensure that no traces of H2O2 remain. Otherwise, there is a risk that animals could be impaired by H2O2 residues. Please Do NOT touch equipment without wearing protective gloves before outgassing time has been elapsed.
- Prior to gassing, all air inlets and air outlets of Calorimetry Control Units / High-Speed Sensor Units must be sealed to protect O2/CO2 sensors from exposure to gas.
- All system devices must be powered OFF (Exception: Intense gassing of climate chambers. In that case, fans may keep running.)

**5.2 Cage**

The lids should be wiped off with a moistened soft, lint-free cloth or tissue to remove any adhering dirt. A pH-neutral detergent solution can also be used for moistening. Detergent residues should then be wiped off.

**5.3 Control Unit, PC, Monitor, and Keyboard**

Organic solvents must not be used for cleaning purposes. Avoid the use of cleaning sprays, spray disinfectants, or strong detergents, as they could damage the device. If necessary, the Process Control Units, PC, monitor, and keyboard can be wiped clean with a clean soft lint-free cloth, which can, if required, be moistened with water or a pH-neutral detergent. Detergent residues should then be wiped off. After cleaning the above-mentioned devices, they can be decontaminated with H2O2 vapor before they are brought into the test laboratory. Clean dust filter of PC every three months.

**5.4 Cable**

Organic solvents must not be used for cleaning purposes. Avoid the use of cleaning sprays, spray disinfectants, or strong detergents, as they could damage the cables. The cables can be wiped clean with a clean soft lint-free cloth, which can, if required, be moistened with water or a pH-neutral detergent. Detergent residues should then be wiped off. The cables can be decontaminated with H2O2 vapor before they are brought into the test laboratory.

**5.5. Rack**

Racks should be wiped off, if required, with a moistened soft, lint-free cloth or tissue to remove any adhering dirt. A stainless steel cleaning agent can also be used to remove any stubborn dirt. Detergent residues should then be wiped off. After cleaning, the shelves can be decontaminated with H2O2 vapor.

**5.7 Capsule and pump filter**

Capsule and pump filter, for heavy use, change every 6 months; for light use, change at lease every year

### 5.8 Gas

The N2 gas can be kept until finished. But the good and bad air have to change at lease every year. The Gas calibration, cal. with 3 gases. N2 is only need for CO2. and every time after changing to new tank, remember to update the information from the tank QA sheet in the software, then click “Save data”

## 6 REFERENCES

Refer to the manufacturer’s manual for additional information.

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