

## **WVU IACUC- APPROVED STANDARD OPERATING PROCEDURE (SOP): Inhalation Facility**

### **1. Overview**

The purpose of the WVU Inhalation Facility is to generate artificial atmospheres of inhalable toxicants under tightly controlled conditions and expose laboratory animals. The goal of the exposure(s) is to replicate the personal, environmental and occupational inhalation exposures that humans experience, so that the subsequent health effects may be studied. Information gained from these studies will be critical to the prevention/treatment of the consequences of inhalation exposures, identify safe exposure limits, generate fundamental dose-response relationships for Federal agencies and identify safe materials for use in diverse human activities. The Inhalation Facility is comprised of four rooms within 4040 Health Sciences Center- North (HSC-N). 4040B is a designated animal housing room, constructed to match existing animal housing facilities located in the HSC Vivarium. 4040B will comply with all regulatory standards established by the *Guide for Care and Use of Laboratory Animals*, the *WVU IACUC* and the *Office of Laboratory Animal Resources (OLAR)* and is considered to be an extension of the HSC Vivarium. 4040C is designated for combustion engine emission exposures. 4040D is designated for complex pollutant exposures such as: field collections from mountain top mining, fracking or heavy vehicular traffic areas; printing emissions; cigarette smoking and e-cigarette vaping. 4040E is designated for nanoparticle and advanced material exposures. The atmospheric pressures in each of these exposure rooms is negative relative to the common space immediately outside it. In each exposure room, two large walk-in hoods contain all exposure equipment. These hoods can draw up to 100 cubic feet per minute of air (with open sashes), and therefore, each exposure system is fully contained in each hood (redundant engineering controls). The aerosols generated in these exposure chambers are ventilated through a multi-stage filtration system prior to being emitted into the general atmosphere directly above the HSC.

Rats and mice are currently approved for use in the Inhalation Facility. Animals may be housed in 4040B for  $\geq 24$  hours. All experimental procedures will be performed by the Inhalation Facility staff; Animal care and husbandry will be performed by OLAR staff. Only currently approved WVU IACUC protocols can be used to request animal housing and exposures in the Inhalation Facility. The Principal Investigator (PI) is responsible for procuring the animals, providing the proper information (including, but not limited to number of animals/groups, and exposures to be conducted) in the protocol, as well as confirming that this SOP is referenced. It is recommended that the PI consult with the Inhalation Facility staff and the Attending Veterinarian (AV) prior to including any procedures in the protocol. All appropriate Inhalation Facility staff members **must** be included in any protocol and the Inhalation Facility SOP **must** be followed. After approval, an OLAR Animal Transfer Form should be completed in order to have animals transferred to the Inhalation Facility. Once in the facility, the animals will remain housed there for the duration of the exposure(s). After the exposure(s) is complete, the PI **must** take the proper steps to reclaim his/her animals. While the animals are housed in the Inhalation Facility for the duration of the experiment(s), they will remain on the PI's approved protocol and applicable per diem charges will apply.

### **Pain and Distress Category**

All exposures are **Category C** procedures, as no experimental or invasive manipulations are being performed on animals while they are housed in the Inhalation Facility. Animals may be momentarily uncomfortable during initial habituation/training. For whole body inhalation exposures, animals are free to move about in exposure cages. For nose-only exposures, animals are secured in Allay<sup>TM</sup> restraints (Data Sciences International, St. Paul, MN). Allay restraints secure the animal without compressing its thoracic cavity or otherwise affecting the

animal's respiration and the animals are habituated as described below prior to the initiation of exposure experiments. The PI **must** include the appropriate Appendix H in their protocol for nose-only exposures.

## **2. Exposures and Procedures**

### **A. Xenobiotic Particle Aerosol and Gaseous Toxicant Exposures**

Many artificial atmospheres can be created in the Inhalation Facility. Five broad categories of aerosolized toxicants are generated:

- *Engineered nanomaterial aerosols* (e.g., metals, metal oxides, carbon nanotubes, graphene, nanospheres, nanowires, nanobelts)
- *Particulate matter air pollution aerosols* (e.g., concentrated ambient particles from the environment, fracking platforms, mountain-top mining operations, road traffic, fires)
- *Cigarette/E-Cigarette/vaping aerosols* (e.g., traditional cigarettes, vapes, propylene glycol, vegetable glycerin)
- *Gases* (e.g., ozone, carbon monoxide, nitrogen oxides, sulfur dioxide)
- *Emission mixtures* (e.g., laser/3D printers, on- and off-road engines, 2- and 4-stroke, diesel, hybrid, oil/fuel alternatives, combustion)

The goals of these exposures are based on either toxicological and/or pharmaceutical assessments, therefore, the calculation of dose is critical. Not all exposure doses are based on the same metric. The most commonly measured/calculated doses are aerosol concentration (e.g., < 40 mg/m<sup>3</sup>), exposure time (e.g., < 6 hours), pulmonary deposition (e.g., < 1mg/animal/exposure), and number of exposures/unit time (e.g., < 6 exposures/week). The specific/target dose will be identified for each xenobiotic particle aerosol or gaseous toxicant prior to any exposure. The PI **must** include the appropriate details of each exposure material to be used in Appendix C in their protocol.

### **B. Inhalation Exposure Procedures**

**B.1. Whole-Body Inhalation Exposure:** Animals will be placed in inhalation chamber cages for up to 6 hours. Inhalable xenobiotic particles and/or gases will be aerosolized and delivered to exposure chambers. An online feedback system controls toxicant concentration in the exposure chamber. This constant feedback control is achieved by numerous aerosol monitors that sample air from the exposure chamber. The signal is processed by computers interfaced with the aerosol monitors that adjust the diluent air to maintain a constant toxicant concentration and ambient temperature/humidity. Temperature, relative humidity and air pressure are recorded during exposures. Any animal that displays abnormal behavior (e.g., erratic breathing, distress) will be immediately removed from the exposure chamber and reported to the OLAR veterinary staff.

**B.2. Nose-Only Inhalation Exposure:** Animals will be progressively trained in the Allay™ restraint tubes for up to two weeks prior to exposures. Initially, this is achieved by inducing a light plane of anesthesia (1-3% isoflurane) and placing the animals in the tubes. Food rewards are provided throughout training. Restrained animals are connected to the inhalation tower for up to 6 hours. Toxicants will be aerosolized and delivered to the inner core of the inhalation tower. Only the animals' noses are exposed to aerosols. An online feedback system controls toxicant concentration in the exposure chamber. This constant feedback control is achieved by numerous aerosol monitors that sample air from the exposure chamber. The signal is processed by computers interfaced with the aerosol monitors that adjust the diluent air to maintain a constant toxicant concentration, and ambient temperature/humidity. Temperature, relative humidity and air pressure are recorded during exposures. Any animal that displays abnormal behavior will be removed from chamber and immediately reported to the OLAR veterinary staff. Appendix H **must** be completed in individual protocols.

**B.3 Pulmonary Function Assessments:** After training (typically 3-5 sessions, 15-30 minutes each), animals are singly placed in whole-body plethysmography chambers. Animals are conscious and unrestrained. Fresh, filtered air is continuously delivered to the chamber. Pressure transducers connected to the chamber via sensitive diaphragms are used to measure breathing frequency, pressure and volume changes. These data are used to calculate a battery of pulmonary function volumes.

### **3. Animal Housing**

Animal housing will be in room 4040B. All standard housing policies and procedures in place for the WVU HSC vivarium are practiced in the Inhalation Facility. Lighting is automatically controlled on a 12/12 hour on/off schedule. Temperature and humidity logs are maintained electronically. Data points are logged every 15 minutes per day. Spreadsheets contain one week of data collection/logging and are maintained on the WVU HSC server. This data is also displayed in real-time, on computer screens outside each room of the Inhalation Facility. If room airflow, temperature and/or humidity exceed the HVAC standards established by the *Guide for Care and Use of Laboratory Animals*, a visual and audible alarm occurs through these displays. Inhalation Facility and OLAR staff check these alarms and reset the unit if the error has been autocorrected by the system (this regularly happens as the HVAC system is a computer-controlled feedback loop that requires nominal error signals). If the alarm indicates an error/deviation of those standards established by the *Guide for Care and Use of Laboratory Animals*, WVU HSC Facilities and OLAR Veterinary staff will be immediately contacted to rectify the HVAC error and be advised on appropriate animal housing actions.

Any animal that displays abnormal behavior will be immediately reported to the OLAR veterinary staff. Upon completion of the final inhalation exposure, the PI will promptly transfer animals from the Inhalation Facility.

### **4. Personal Protective Equipment (PPE)**

The PPE policies and procedures in place for the WVU HSC vivarium are practiced in the animal holding room (4040B) of the Inhalation Facility.

### **5. Cleaning Procedures**

The standard policies and procedures in place for the WVU HSC vivarium are practiced in the Inhalation Facility.

### **6. Non-Clinical Injections, Exogenous Treatments and Non-Surgical Procedures**

These are not applicable as they will not be performed while animals are in the Inhalation Facility. If an exception arises, the PI ***must*** amend their active protocol to reflect the injection/exogenous treatment/non-surgical procedure. If necessary, Inhalation Facility personnel ***must*** be added to the indicated protocol prior to performing any injection/exogenous treatment/non-surgical procedure.

### **7. Animal Identification**

Animals will be identified via cage cards and data logs in the Inhalation Facility with the following information:

- PI name and contact information
- Protocol number
- Species/strain
- Date of birth
- Date of entry into Inhalation Facility
- Exposure details
- Date of exit from the Inhalation Facility

## 8. **Inhalation Facility Contacts**

### a) **Director**

Timothy R. Nurkiewicz, Ph.D.  
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### b) **Engineer**

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### c) **Head Technician**

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## 9. **OLAR Contact Information**

G-186 HSC-N, Morgantown, WV 26506  
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### a) **OLAR Director**

Tara Cotroneo, DVM  
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### b) **OLAR Operations Manager**

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c) **OLAR Operations Coordinator**

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d) **OLAR Office Administrator**

Minnie Mahajan

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e) **OLAR Veterinary Contact information**

**A. For Veterinary Emergencies:**

i. During normal business hours (M-F 8:00a-4:00p)

1. 304-293-3737

2. If no answer, page 829 (call 304-293-3595; enter pager # 829; enter in call-back number; hang up)

ii. After hours, weekends and University holidays

1. 304-276-1314

2. If no response within 15 minutes, contact Dr. Jessica Pinckard at 204-290-0783 or Dr. Rebecca Jernigan at 304-376-1306 or Dr. Tara Cotroneo at 304-288-0130

**B. For Non-Emergencies:**

i. Call 304-293-3737 (leave a message) or send email to [olarvetstaff@hsc.wvu.edu](mailto:olarvetstaff@hsc.wvu.edu)