**WVU IACUC SOP:**
**Inhalation Facility**

1. **Overview**

The purpose of the WVU HSC Inhalation Facility is to generate artificial atmospheres of inhalable toxicants, under tightly controlled conditions, and expose laboratory animals. The goal of the exposures 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: prevent/treat the health 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). 4040B is a designated animal holding room, constructed to match existing animal holding facilities in the HSC Vivarium, and comply with the heating, ventilation and air conditioning (HVAC) standards established by the *Guide for Care and Use of Laboratory Animals*. Because of these engineering standards, 4040B is considered part 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 the animal species used in the Inhalation Facility. The Inhalation Facility is recognized by the WVU IACUC as part of the HSC vivarium. As such, animals are housed in the Inhalation Facility for ≥ 24 hours. Inhalation Facility staff perform all experimental procedures. Facility staff and OLAR staff provide animal care and husbandry. Only current, approved WVU IACUC protocols can be used to request animal housing and exposures in the Inhalation Facility. The Principal Investigator (PI) is responsible for providing the rationale for the animal model, number of animals/groups, and exposures to be conducted. All appropriate Inhalation Facility staff members must be included in any protocol using the Inhalation Facility SOP. After approval, animals are transferred to the Inhalation Facility, and housed there for the duration of the exposure(s). After exposures, it is the responsibility of the PI to reclaim his/her animals. Despite being housed and exposed in the Inhalation Facility, animals are purchased by individual investigators and remain on their active protocols, *which must reference this SOP*.

2. **Pain and Distress Category**

All inhalation 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™ 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 habitualized as described below prior to the initiation of exposure experiments.

3. 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 fracking platforms, mountain-top mining operations, road traffic, fires)
- Cigarette/E-Cigarette/vaping aerosols (e.g. traditional cigarettes, vapes, hookah)
- 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)

Because the goals of these exposures are based on either toxicological and/or pharmaceutical assessments, 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/m³), exposure time (e.g. <6 hours), pulmonary deposition (e.g. <1 mg/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.

4. Inhalation Exposure Procedures

4.1. 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 will be immediately referred/reported to the WVU veterinary staff.

4.2. 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 immediately referred/reported to the WVU veterinary staff.

5. 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.

6. 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 though 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 greater than 20% 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.

During inhalation exposures, animals will be singly housed, but returned to 4040B after exposures. Any animal that displays abnormal behavior will be immediately referred/reported to the WVU veterinary staff. Upon completion of the final inhalation exposure, the PI will promptly transfer animals from the Inhalation Facility.

7. 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.

8. Cleaning Procedures

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

9. 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.

10. 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
11. Inhalation Facility Contacts

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