APPENDIX 7

The Center for Inhalation Toxicology (iTox Center)

A description of the development of the inhalation toxicology center, including figures illustrating the current facility

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Creation of the Center for Inhalation Toxicology

The original Inhalation Facility, while very successful, did make some faculty anxious as poor engineering controls were in place, the generators were noisy, the floor frequently leaked, and the toxic aerosols created were receiving considerable attention across the University, region, state and country. By 2012, the Inhalation Facility was operating at maximum capacity and while smaller scale systems were incorporated (e.g. nose-only exposure towers) to provide additional animals to collaborators, the space was physically full of equipment and no further growth could occur. Dr. Tim Nurkiewicz spent the following five years advocating for an expanded facility, but was not successful.

Dr. Vince Castranova retired from NIOSH in 2014. As a long-time adjunct professor to multiple HSC departments, he took a part-time faculty position in the Department of Pharmaceutical Sciences in 2015. When Dr. Laura Gibson replaced Dr. Glenn Dillon as Senior Associate Vice President for Research and Graduate Education in 2016, she transitioned Dr. Castranova into an Assistant Dean position in this Office. In 2016, Vince and Tim proposed an expansion to Dr. Gibson, and this was approved. ~ $3M dollars were allocated from the Office of Research and Graduate Education and the West Virginia Clinical and Translational Institute. Dr. Christopher Cuff was the "Core Czar" at this time and oversaw the creation of what was known as

Much had been learned from the limitations of the first Inhalation Facility, and the general "uneasiness" it created among its neighbors. The top floor (fourth) of the HSC-N was ultimately selected as space for Inhalation Facility 2.0, as many novel aerosols and toxicant generation systems were envisioned and exhaust systems needed to be not only short (to keep costs down), but easily accessible. Outside walls were also desirable as options to bring in outside air (for ambient sampling) and from engines were needed. Engineering controls needed to be robust and redundant to ensure safety and convincingly demonstrate amble containment of toxicants. Once more, Tom Fortuna drafted the original designs for the new Inhalation Facility. However, this time, a team of WVU employees from Environmental Health and Safety, Design and Construction (the project leader was Zenaba Qadeer), Facilities Management, IACUC and outside consultants (architects and engineers) were heavily involved in the design and ultimate development of the new Inhalation Facility. Dr. Yi, Carroll McBride (Tim's long-time research assistant) and Tim oversaw production and the collective team worked well together. Planning started in 2016, and the new Facility was completed by the end of 2017. William "Travis" Goldsmith was hired from NIOSH during this period, as the Inhalation Engineer for the new Inhalation Facility after Dr. Yi's abrupt departure during the construction process. Rooms 4037, 4038,

4039, 4041A, 4041B and 4043 were gutted and the second Inhalation Facility was built from scratch. The new Facility, designated as rooms 4040, 4040A, 4040B, 4040C, 4040D, and 4040E HSN is comprised of ~1200 square feet (a more detailed description is provided below). Three exposure rooms and six walk-in hoods create the heart of the Facility. A positive air pressure differential exists from the outside hall of the Inhalation Facility, into each exposure hood. This guarantees that any fugitive particles or toxicants that may escape an exposure system are contained and exhausted from the top of the HSC (after filtration). An animal holding room was also created in the Facility to decrease the stress associated with the daily transport of animals from the main Vivarium on the ground floor of the HSC to the 4th floor. The entire Inhalation Facility was recognized by the IACUC as part of the HSC Vivarium. This avoided the limitations associated with "satellite" housing designations. Finally, the water, HVAC and power circuits are independent of the original HSC systems. All the independent systems are contained in a machine room designated as the "Penthouse Suite" above the Inhalation Facility. This brown enclosure and two large exhaust stacks can easily be observed from the Loading Dock area of the HSC and the South Tower.

The new Inhalation Facility has become a common core element of the Center for Inhalation Toxicology, wherein investigators across the tri-state area interact and collaborate. The prominent aerosols regularly generated in the Inhalation Facility include diverse nanomaterials, ozone, chlorine, electronic cigarettes, and emissions from a military burn pit surrogate generator. Six faculty members from the Department of Physiology and Pharmacology regularly use the Inhalation Facility, and over twenty students across all the HSC graduate programs (including Cellular and Integrated Physiology) perform experiments daily in the Facility. Faculty across the campus (including Engineering, Psychology, Chemistry and even the College of Business and Economics) and NIOSH are regularly engaged in research activities in the new Inhalation Facility. The Facility came "online" in 2017, and by 2021 novel extramural awards from these faculty exceeded the initial investment of $3M. Exposures were briefly stopped during 2019 during the SARS-CoV-2 pandemic, but operations were quickly restarted to support the Statewide response. Activities at this time were focused on testing the efficiency of various masks, materials, detection equipment and even generating artificial atmospheres in the same size range as COVID droplets. Another major contribution of the Inhalation Facility during the pandemic was the generation of educational videos for masking and droplet transmissions.

At the time of this writing, a second expansion of the Inhalation Facility was approved by Dr. Gibson and the WVU Administration. In 2022, the HSC Morgue was relocated to renovated facilities on the ground floor. The approved second expansion of the Inhalation Facility would incorporate the old Morgue space (4032, 4032A 4033, 4034, 4034A, 4035, 4035A and 4036 HSN), labs 4042 HSN, 4044 HSN

and expand onto the roof of the HSC. The expansion would add ~2500 square feet to the Inhalation Facility, two new exposure rooms and four new exposure hood systems. Construction on this expansion began in the Summer of 2022 with demolition and remediation of the old Morgue space. The original Inhalation Facility was used for tinkering and small "non-animal" projects for many years. In 2021, the Department Space Committee proposed to convert that space into offices as new faculty hires had exceeded the existing office space. Those renovations were slated to begin in 2023.

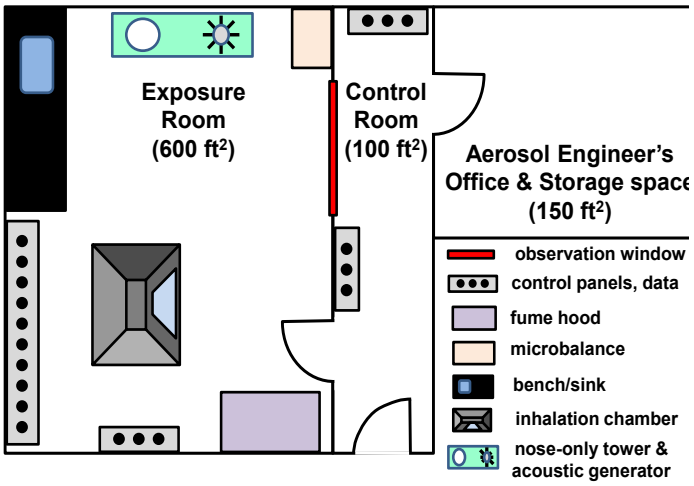

FIGURE A: Inhalation Facility (2008) floor plan. The volume of the whole-body inhalation chamber is 500 liters, and can house ~16 rats or ~50 mice.

WVU Health Sciences Center – INHALATION FACILITIES

The WVU Health Sciences Center houses two Inhalation Facilities, for which Dr. Nurkiewicz serves as the Director. The purpose of these facilities is to expose rodents (rats and mice) to xenobiotic aerosols, to directly assess systemic toxicities. The original facility was designed/built in 2008 (**FIGURE A**). The

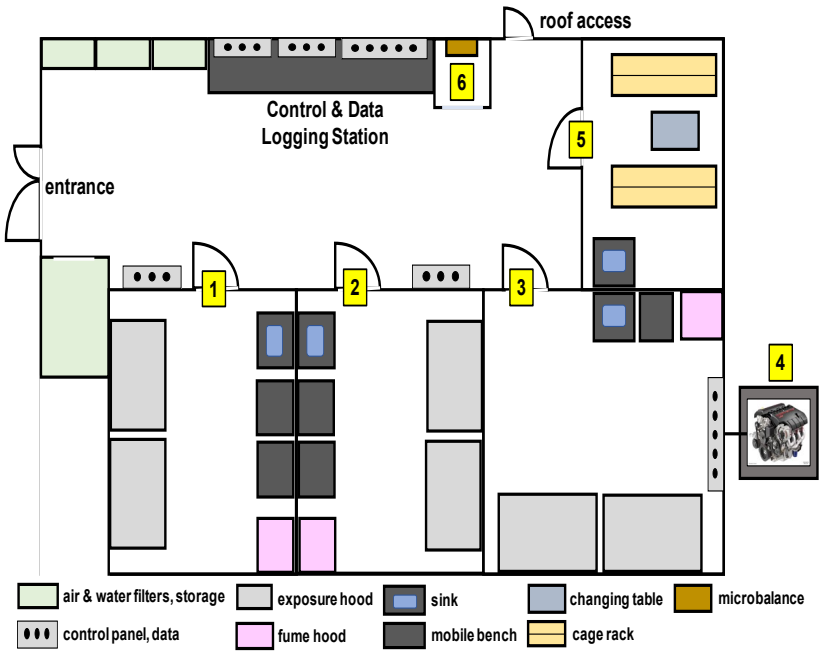

FIGURE B: WVU Inhalation Facility 2.0 (2017) floor plan. Construction of the facility was completed in September 2017. Six inhalation exposure systems are in place for diverse xenobiotic particle toxicity assessments. **1)** Nanomaterial epxosure room. **2)** e-cigarrette/vaping exposure room. **3)** engine emission exposure room. **4)** combustion engine placement (outside main facility). **5)** Animal holding room. **6)** Microbalance room. *This room is not ventilated, and tighly sealed in order to reduce air and thermal influences on microbalance measurements*. The collective rooms/space have WVU IACUC designation as a component of the animal vivarium (is not a "satellite") and houses animals independently of the main vivarium. WVU is an AAALAC accredited institution.

entire facility is 700 square feet. The exposure room is ~600 square feet. An insulated wall separates the exposure room from the Control room where exposures are monitored through an observation window and electronically/computer controlled. This Inhalation Facility is connected to a dedicated high-volume air compressor and vacuum in the machine room (one floor directly over the Facility).

FIGURE C: Inhalation Facility 2.0 Exposure Hoods in the nanomaterial exposure room. Walk in hoods have an airflow pull rate of up to 150 CFM, and can be purged directly to the filtered exhaut system of the facility's HVAC system. Internal aerosol generation/characterization equipment is networked and can be controlled immediately in the room or remotely over the HSC network..

Adjacent to the Control room, is a 150-square foot office. This space houses the Facility operator, an Aerosol Engineer with >15 years of experience with rodent inhalation exposures. This position has been committed by the West Virginia University Health Sciences Center Vice-President's office to Dr. Nurkiewicz's research program since 2008. *As such, the Aerosol Engineer will be available to support and perform these exposures for the duration of this proposal*.

The West Virginia University Health Sciences Center invested in a major infrastructure expansion for a second Inhalation Facility (**FIGURE B**). The footprint of the expanded facility is 2200 square feet. Inhalation Facility 2.0 represents a significant expansion not only in size, but also capacity. The six exposure hoods represent the collective capacity to expose up to 140 rats or >400 mice. This allows for the simultaneous execution of multiple research projects and contracts. Despite being physically housed in the Health Sciences Center, the facility is "free-standing" as it operates independently with dedicated HVAC, electrical, emergency power, and plumbing systems. A negative pressure gradient exists from the main control area, to individual rooms, and ultimately to the individual exposure hoods (**FIGURE C**). In **FIGURE D**, the military burn pit

FIGURE D: Military burn pit surrogate generator and aerosol exposure system in the WVU iTOX Inhalation Facility.

surrogate generator described in this proposal is presented.

All Dr. Nurkiewicz's laboratories have ample local area network (CAT6) and data (USB 3.0) connectivity jacks and, as well as power (standard 110V, and 220V, as well as emergency power outlets), compressed air, vacuum and RO water ports. Finally, all laboratories and Inhalation Facilities are secured by identification card swipe access.

Aerosol Generation Systems

Rodents are exposed to various aerosols in whole-body exposure chambers or nose-only exposure systems. Aerosols are generated by patented devices developed at West Virginia University (*Nanoparticle Aerosol Generator: U.S. Patent #8,881,997. 2014*), and the National Institute for Occupational Safety and Health (*Acoustical Generator: U.S. Patent, #8,875,702. 2014*). The method of exposure is dependent upon the amount of bulk materials available for aerosolization. Exhaust from the facility is piped through a five-stage low pressure filter also housed with this equipment. *Aerosols are characterized in real-time, and tightly controlled to ensure rigor and reproducibility*.

Major Equipment

The major equipment used in the Inhalation Facilities to characterize aerosols generated in real-time and measure physiologic responses include:

1) Nanoparticle aerosol generators (6): these are fabricated in house as research needs develop.
2) Acoustic generators (4).
3) Mettler, Six-digit ultra-microbalances (2).
4) TSI, Scanning mobility particle sizers + long differential mobility analyzers (2).
5) TSI, Aerodynamic particle sizer.
6) TSI, Nano micro-orifice uniform deposit impactors (2).
7) TSI, Dust Track (2).
8) TSI, Condensation particle counters (3).
9) Dekati, Electrical low-pressure impactors (2).
10) DSI, Whole body plethysmograph (rat and mouse chambers).
11) DSI, Rodent telemetry systems (blood pressure, EKG, nerve activity, temperature, movement).
12) ABB, Realtime/Continuous gas analyzer (sulfur dioxide, nitrite/nitrate, carbon monoxide, carbon dioxide, methane).
13) Agilent, Gas chromatograph and mass spectrometer

Xenobiotic Particles Aerosolized

Because of the collective technical capabilities present in the Inhalation Facility, most any particle may be aerosolized. The ultimate factor that dictates the conditions of generation is the amount of bulk material available for aerosolization. Up to six agents/exposures can be tested at a single time.

1) Nano-titanium dioxide.
2) Fine-titanium dioxide.
3) Titanium dioxide nanowires.
4) Titanium dioxide nanobelts.
5) Cerium dioxide.
6) Carbon black.
7) Single-walled carbon nanotubes.
8) Multi-walled carbon nanotubes.
9) Graphene.
10) Carbon fullerenes.
11) Silica.
12) Silver nanoparticles.
13) Vehicular engine emissions (conventional combustion and diesel engine).
14) Mountain-top mining particle pollution.
15) Hydraulic fracturing platform particle pollution.
16) Conventional cigarette smoke.
17) Electronic cigarette vapor- individual constituents and mixtures (vegetable glycerin, propylene glycol, artificial flavors)
18) Laser printer emissions.
19) Three-dimensional printer (additive) emissions.
20) Military Burn Pit Mixtures

    Note: a pellet maker compresses custom mixtures of the following components:

    - carbon black
    - rubber
    - naphthalene
    - nylon
    - plastic
    - wood, paper

    Note: +/- JP8/JAA drip (aviation/military grade jet fuel drip during combustion)
21) COVID Droplet Surrogates (plant phages)