Appendix A – Protocol, Protocol Amendment and Protocol Deviation

PROTOCOL

1. **STUDY TITLE:** **Inhalation Dose Range-Finding Study of APN01 in Beagle Dogs**

2. **IDENTIFICATION:** IITRI Project No.: 285700300101
Contract No. 75N91019D00013
Task Order No. 75N91020F00002

3. **SPONSOR:** Chemopreventive Agent Development Research Group
Division of Cancer Prevention
National Cancer Institute
9609 Medical Center Drive
Rockville, MD 20850

CONTRACTING OFFICER'S REPRESENTATIVE (COR): Robert Shoemaker, Ph.D.
Phone: 240-276-7077
e-mail: robert.shoemaker@nih.gov

4. **TESTING FACILITY:** IIT Research Institute (IITRI)
10 West 35th Street
Chicago, Illinois 60616

5. **OBJECTIVE:**

The objective of this study is to provide a preliminary characterization of the toxicity of inhaled APN01 in beagle dogs in order to support dose selection for inhalation toxicity studies of longer duration.

6. **IITRI SUPERVISORY STUDY PERSONNEL:**

a. **STUDY DIRECTOR:** Jeffrey Richig, D.V.M.
Phone: (312) 567-4904
e-mail: jrichig@iitri.org

b. **CONTRIBUTING INHALATION TOXICOLOGIST:** Sridhar Jaligama, Ph.D., D.A.B.T.
Phone: (312) 567-4285
e-mail: sjaligama@iitri.org

c. **PRINCIPAL INVESTIGATOR AND PRESIDENT:** David L. McCormick, Ph.D., D.A.B.T.
Phone: (312) 567-4972
e-mail: dmccormick@iitri.org

7. **PROPOSED SCHEDULE:**

a. **FIRST DAY OF EXPOSURE:** October 12, 2020

b. **COMPLETION OF IN-LIFE STUDY:** October 16, October 20, or October 23, 2020, depending on number of doses administered.

c. **DRAFT REPORT SUBMISSION:** November 25, 2020

8. **DURATION OF IN-LIFE STUDY:** Approximately 5 to 14 Days (+ quarantine), depending on number of doses administered.

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

9. **TEST AND CONTROL ARTICLES:**

   a. **IDENTIFICATION:**

   The test article is identified by the Sponsor as APN01, and will be supplied by the Sponsor and/or by Apeiron Biologics, Vienna, Austria. The test article will be supplied as a clinical formulation, and will be used without further purification.

   b. **HAZARDS TO PERSONNEL:**

   Routine safety procedures used for handling of hazardous or potentially hazardous chemicals will be followed to ensure the health and safety of personnel handling the test article.

   c. **TEST ARTICLE CHARACTERIZATION:**

   A Certificate of Analysis that includes the identity and concentration of the test article in the clinical formulation will be provided by or on behalf of the Sponsor. All characterization data (including method of synthesis, fabrication, or derivation of the test article) are maintained by the Sponsor or Sponsor's designee.

   d. **STORAGE:**

   Formulated test article will be stored refrigerated (at approximately 2-8 °C) in its original vials.

   e. **SAMPLE DISPOSITION:**

   All quantities of the test article that are dispensed will be documented.

   f. **BASIS FOR SELECTION OF TEST ARTICLE DOSES:**

   Test atmosphere concentrations to be used in the study will be selected based on the maximum feasible concentration (MFC) of APN01 that can be generated in a respirable aerosol. The 30 minute exposure time was selected based on its expected use in the twice daily administration regimen to be used in toxicity studies of longer duration.

   g. **ROUTE OF ADMINISTRATION:**

   The test article will be administered to dogs by oronasal inhalation. Inhalation is an intended clinical route of APN01 administration in humans.

   h. **DISPOSITION OF TEST ARTICLE:**

   Upon completion of the study, any remaining APN01 will be retained for use in additional studies to be performed as part of this program.

10. **EXPERIMENTAL DESIGN:** Two groups of four dogs each (2 per sex) will be used in this escalating dose range-finding study. APN01 will be administered in test atmospheres generated using Pari LC Plus nebulizers; exposures will be 30 minutes in duration. Exposures to APN01 will be alternated between groups; after the first exposure, test atmosphere concentrations of APN01 will be selected on the basis of toxicity seen after the previous exposure.

Group	Number of Dogs (M + F)	Agent	Route of Administration	Exposure Time (minutes)	Test Atmosphere Concentration (1st Exposure, mg/L)	Test Atmosphere Concentration (2nd Exposure, mg/L)
1	2 + 2	APN01	inhalation	30	0.5 MFD	TBD*
2	2 + 2	APN01	inhalation	30	MFD or 0.25 MFD*	TBD*

* Test atmosphere concentration (higher or lower) to be selected based on toxicity seen in the group receiving the previous exposure (see below).

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

In the initial exposure, dogs in Group 1 will receive a single 30 minute inhalation exposure to a test aerosol containing APN01 at half of the Maximum Feasible Dose (0.5 MFD).

- If no evidence of limiting toxicity is seen in Group 1 dogs after the initial exposure, dogs in Group 2 will receive a single 30 minute inhalation exposure to a test aerosol containing APN01 at the MFD.
- If limiting toxicity is seen in dogs exposed to APN01 at 0.5 MFD, dogs in Group 2 will receive a single 30 minute inhalation exposure to a test aerosol containing APN01 at 0.25 MFD.

After a washout period of at least three days, dogs may be re-exposed to test atmospheres containing higher or lower concentrations of APN01 for 30 minutes until a Maximum Tolerated Dose (MTD) or a Maximum Feasible Dose (MFD) is identified.

11. TEST SYSTEM:

a. TEST ANIMALS:

Four male and four female beagle dogs (Ridglan Farms, Mt. Horeb, WI) were purchased for use in this study. Prior to shipment, dogs were immunized by the supplier against distemper, hepatitis, rabies, parvovirus, canine adenovirus type 2, parainfluenza and Bordetella bronchiseptica. Dogs were also treated with a coccidiostat. Dogs are a minimum of five months old and will weigh approximately 6 to 10 kg on the first day of APN01 administration.

b. JUSTIFICATION OF SPECIES SELECTED:

The beagle dog is a standard non-rodent species that is accepted by the U.S. Food and Drug Administration (FDA) for studies of compounds used or intended for use in humans.

c. JUSTIFICATION FOR THE NUMBER OF ANIMALS:

The number of animals used is the minimum necessary to satisfy scientific principles. To the knowledge of the Sponsor and the Study Director, conduct of this study will result in no unnecessary duplication of existing data with regard to species, test article, dose(s), routes, and duration of administration.

d. HOUSING:

Except for periods of inhalation exposure, dogs will be housed in pairs (by sex) in pens equipped with automatic watering systems. Pens will be cleaned daily. Dogs will be housed in accordance with U.S. Department of Agriculture Animal Welfare Standards (Title 9, *Code of Federal Regulations*, Part 3, 1991 Revision) and standards set forth in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011).

e. FOOD:

Teklad Certified Canine Diet #2021C (Teklad Laboratory Animal Diets, Envigo, Madison WI). Approximately 400 g of food will be made available to each dog daily for a minimum of 1 hour. Each lot of diet is analyzed for contaminants to ensure that none are present at concentrations which would be expected to interfere with the conduct or purpose of this study. Analytical data from the lots of diet to be used in the study will be retained on file at IITRI.

f. WATER:

Coarse-filtered City of Chicago water will be provided *ad libitum* to all animals using an automatic watering system. Water is analyzed periodically for bacterial contamination and chemical composition (*e.g.*, electrolytes, metals, etc.). Water analysis records are retained on file at IITRI. No contaminants expected to interfere with the study are known to be present in the water.

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

g. ANIMAL IDENTIFICATION:

Each dog will be identified by a USDA tattoo number in the right or left ear. Each dog will also be assigned a unique number within the study. All pens will be identified by IITRI Project Number, Animal Number, and Sex.

h. ENVIRONMENTAL CONTROL:

Temperature and relative humidity in the animal room will be recorded manually each day. A 12-hour light/dark cycle (maintained with an automatic timer) will be used. Animal rooms will be generally held within temperature and relative humidity ranges as recommended by IITRI Standard Operating Procedures (SOPs).

12. METHOD:

a. QUARANTINE:

Each dog will be quarantined for a minimum of two weeks prior to test article exposure. Prior to study start, each dog will receive a detailed physical examination to ensure its suitability for use as a test animal. Animals will be released for use in the study by the IITRI Veterinarian.

b. ACCLIMATIZATION:

To condition animals to placement and restraint in the oronasal inhalation exposure systems, and to reduce stress during the exposure phase, each dog will be acclimated to the restraining sling and oronasal mask during pretreatment. Each dog will be acclimated sequentially for periods of 10 minutes, 20 minutes, and 30 minutes per day, and then returned to its run.

c. ADMINISTRATION:

At each dose level, APN01 will be administered as a single dose by oronasal inhalation using a mask-based delivery system that was custom-fabricated at IITRI.

1) **Test Atmosphere Generation:** APN01 will be administered as a liquid aerosol that is generated by nebulization of the clinical formulation using [redacted] that are integrated into the canine mask oronasal aerosol delivery system.

Redacted Per Agreement

2) **Test Atmosphere Monitoring:** The aerosol mass concentration in the oronasal inhalation exposure system will be determined by collecting the aerosol on 47 mm diameter glass-fiber filters placed in closed-face filter holders at one of the exposure system ports. Samples will be collected at a constant flow rate equal to the port flow of the delivery tube, and the total volume of air sampled will be measured by a dry gas meter. At least one aerosol sample will be collected during each exposure. Filter-collected samples will be weighed and selected filters will be extracted and analyzed by HPLC to quantitate APN01. In addition, the aerosol concentration in the oronasal inhalation exposure system will be monitored using an aerosol sensor. The aerosol sensor will serve as real-time indicator of short-term concentration changes and will be used in guiding laboratory personnel if excursions from target concentrations are encountered. Aerosol particle size distribution will be monitored twice per each group during the study using a Quartz Crystal Microbalance (QCM) based cascade impactor (California Measurements, Sierra Madre, CA).

Temperature, relative humidity, and airflow rate (for each animal at least 3.5 L /min) in the inhalation exposure system will be recorded at least once during each 30 minute exposure. To the maximum extent possible, the oronasal inhalation exposure chamber atmosphere temperature and relative humidity will be maintained in the ranges of 20 to 26°C and 30 to 70% RH. Airflow may be adjusted as a means of controlling test atmosphere concentration.

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

d. MORTALITY/MORIBUNDITY OBSERVATIONS:

Throughout the quarantine and exposure periods, all surviving animals will be observed at least twice daily for mortality or evidence of moribundity and to assess their general health. Any abnormal clinical signs will be recorded. Moribundity/mortality checks will be separated by a minimum of four hours.

e. MORIBUND ANIMALS:

During regularly scheduled mortality/moribundity observations, any animal judged not likely to survive until the next scheduled observation will, upon consent of the Attending Veterinarian and/or Study Director, be removed from the study, euthanized, and necropsied. These animals will be recorded in the study notebook as being euthanized *in extremis* and will be counted as a death for that day. Dead animals will be immediately removed for necropsy and the death will be recorded in the study notebook; the COR will be notified within 48 hours of any deaths or moribund sacrifices. If any death occurs overnight, the carcass will be stored refrigerated until the next morning when the necropsy procedure can be performed.

f. INJURED OR DISEASED ANIMALS:

Animals on test will be treated for disease or any injury according to procedures used in normal veterinary medical practice. A complete record of the circumstances and the disposition of any affected animals will be made in the study notebook. Any animals that pose a potential infectious threat to other study animals will be isolated.

g. PHYSICAL EXAMINATIONS AND CLINICAL OBSERVATIONS:

Detailed physical examinations, clinical observations, and measurements of blood pressure will be performed on all surviving dogs during pretest, prior to each exposure, and least once daily for three days after each exposure.

h. BODY WEIGHT MEASUREMENTS:

All surviving dogs will be weighed during pretest and on each day of dosing.

i. CLINICAL PATHOLOGY:

Blood for clinical pathology will be drawn from all dogs during quarantine (pretest) and from each exposed dog on the day after inhalation exposure. Approximately 5 ml of blood will be collected from the jugular or cephalic vein at each scheduled time point. The following clinical pathology tests will be performed:

1) CLINICAL CHEMISTRY:

Blood urea nitrogen	Triglycerides	Total bilirubin
Creatinine	Cholesterol	Sodium
Urea nitrogen/creatinine ratio (calculated)	Creatine kinase	Potassium
Alkaline phosphatase	Total protein	Chloride
Alanine aminotransferase	Albumin (A)	Calcium
Aspartate aminotransferase	Globulin (G)	Phosphorus (inorganic)
Glucose	A/B ratio (calculated)	

2) HEMATOLOGY:

Erythrocyte count	Mean corpuscular hemoglobin
Erythrocyte morphology	Mean corpuscular hemoglobin concentration
Platelet count	Reticulocyte count (absolute and relative)
Hematocrit	Total white blood cell count
Hemoglobin	Differential white blood cell count (absolute and relative)
Mean corpuscular volume	

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

3) **COAGULATION:**

Fibrinogen Prothrombin time Activated partial thromboplastin time

j. RESPIRATORY FUNCTION:

Respiration rate and tidal volume will be measured for each study animal during exposure, and a minute volume will be calculated on the basis of these measurements. In addition, oxygen saturation of the blood will be measured by pulse oximetry and blood pO_2 will be measured by pO2 electrode at the end of each exposure.

k. STUDY TERMINATION:

On the day after exposure to the MTD (or MFD), dogs in that exposure group will be humanely euthanized by barbiturate overdose and necropsied. The other four dogs will be transferred to the IITRI colony. At necropsy, eyes with optic nerves will be fixed in Davidson's solution and testes and epididymides will be fixed in modified Davidson's solution. All other tissues and organs will be fixed in 10% neutral buffered formalin. The dog identification will be retained with tissues upon histologic processing.

Animal identification (ear with tattoo)	Heart	Small intestine, duodenum
Artery, aorta	Kidneys (paired)	Small intestine, ileum
Bone, femur (with epiphyseal plate of head)	Large intestine, cecum	Small intestine, jejunum
Bone marrow, sternum	Large intestine, colon	Spinal cord, cervical
Brain	Large intestine, rectum	Spinal cord, lumbar
Cervix	Liver	Spinal cord, thoracic
Epididymis (paired)	Lung	Spleen
Esophagus	Lymph node, bronchial	Stomach
Eye (paired)	Lymph node, mandibular	Testis (paired)
Gall bladder	Lymph node, mesenteric	Thymus
Gland, adrenal (paired)	Muscle, skeletal	Tongue
Gland, mammary gland	Nerve, optic (paired)	Tonsil (paired)
Gland, parathyroid (paired)	Nerve, sciatic	Trachea
Gland, pituitary	Ovary (paired)	Urinary bladder
Gland, prostate	Pancreas	Ureter
Gland, salivary gland	Skin, ventral abdomen	Uterus
Gland, thyroid (paired)	Lymph node, mesenteric	Gross Lesions (if any)

l. HISTOPATHOLOGIC EVALUATION:

Up to four gross lesions from each dog undergoing necropsy will be processed histologically and evaluated microscopically to evaluate APN01 toxicity. Additional gross lesions (above four per animal) may be processed at the direction of the Contracting Officer and Contracting Officer's Representative (at additional cost).

If no gross lesions are identified at necropsy, histologic processing and microscopic evaluations will be performed on the lungs of all dogs undergoing necropsy.

13. STATISTICAL ANALYSIS:

Because of the small group size, no statistical analyses will be performed on toxicology data.

14. STUDY RECORDS:

Data captured electronically using ToxData® (*e.g.*, daily mortality/moribundity data, environmental data, survival data, body weight data, clinical and physical observation data, etc.) will be maintained within the computer system database. Electronic copies of the ToxData® .htm files will be backed-up onto CD(s) and the disc(s) will be maintained with the raw data.

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

All original paper data will be maintained in loose-leaf notebooks. Paper data to be maintained in loose-leaf notebooks will include, but not necessarily be limited to, the following:

- the original signed protocol and any amendments and/or deviations;
- animal care records;
- test article data;
- exposure data;
- necropsy data.

15. ALTERATION OF DESIGN:

Alterations in the Protocol may be made as the study progresses. No changes in the Protocol will be made without the specific written consent of the Sponsor.

16. REGULATORY STANDARDS AND COMPLIANCE:

Due to its exploratory nature, this study will not be conducted in strict compliance with U.S. FDA Good Laboratory Practices for Nonclinical Laboratory Studies (21 CFR Part 58).

17. ANIMAL WELFARE COMPLIANCE STATEMENT:

This study will comply with all applicable sections of the Animal Welfare Act (Title 9, *Code of Federal Regulations*), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Office of Laboratory Animal Welfare, 2015), and the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). The Sponsor's signature on the study protocol documents, for the Study Director, the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments, and that no known acceptable non-animal alternatives were available. Wherever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress, and pain to animals. All methods to be used are described in this study protocol or in written laboratory standard operating procedures.

The Institutional Animal Care and Use Committee (IACUC) at IITRI has reviewed this protocol and deems its study design appropriate to meet the objectives of the study, while minimizing both pain and distress to the test animals (IITRI IACUC Approval No. 19-009).

18. REPORTS:

A draft letter report will be prepared and submitted to the Sponsor for review. Information in the report will include, but not necessarily be limited to, the following:

- copy of the approved protocol, including any amendments and/or deviations;
- species and strain of animal used;
- date of death during the study or whether animals survived to termination;
- body weight and clinical observation data;
- clinical pathology data;
- exposure data (including aerosol generation and aerosol monitoring data); and
- detailed description of results.

Following Sponsor review of the draft report, a final report will be submitted to the Sponsor. The final report will contain the signatures of the IITRI Study Director and Principal Investigator.

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

19. PROPOSED MAJOR COMPUTER SYSTEMS:

Major computer systems to be used by IITRI personnel will include, but may not be limited to:

- ToxData® data collection system (PDS Pathology Data Systems, Basel, Switzerland; version 3.0 or latest version) to randomize animals and collect/calculate in-life toxicology data (*e.g.*, dose administration, moribundity/mortality observations, body weights, body weight changes, animal room environmental data, etc.).
- QCMSIZE to determine the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) from the quartz crystal microbalance.

All other computer systems used at IITRI will be documented in the study records and final report.

20. DATA RETENTION:

All raw data generated during this study, specimens (as applicable), and a copy of the final report from the study will be archived at IITRI for a period of at least one year from the date of completion of the study. The Sponsor will be responsible for all costs associated with continued storage of the archival materials in the IITRI archives or for the shipment of these materials to another storage facility. The IITRI QAU will maintain a complete record of the disposition of all archival materials.

21. PERSONNEL:

Curricula vitae for all personnel involved in the execution of the study are on file at IITRI.

22. PROTOCOL APPROVAL:

This protocol complies with the specific requirements of the Sponsor.

Study Director: Jeffrey W. Richig — 2020-10-08
Jeffrey Richig, D.V.M. — Date:

Principal Investigator and Director: David L. McCormick, Ph.D., D.A.B.T.
Digitally signed by David L. McCormick, Ph.D., D.A.B.T.
DN: cn=David L. McCormick, Ph.D., D.A.B.T., o=IIT Research Institute, ou, email=dmccormick@iitri.org, c=US
Date: 2020.10.08 14:37:50 -05'00'
David L. McCormick, Ph.D., D.A.B.T. — Date:

Contracting Officer's Technical Representative: Robert M. Shoemaker — 10/8/2020
Robert Shoemaker, Ph.D. — Date:

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

PROTOCOL AMENDMENT

Study Title: **Inhalation Dose Range-Finding Study of APN01 in Beagle Dogs**

IITRI Project No.: **285700300101**

Protocol Amendment No.: **1**

Section: **10. Experimental Design**

Protocol states:

Group	Number of Dogs (M + F)	Agent	Route of Administration	Exposure Time (minutes)	Test Atmosphere Concentration (1st Dose, mg/L)	Test Atmosphere Concentration (2nd Dose, mg/L)
1	2 + 2	APN01	inhalation	30	0.5 MFD	TBD*
2	2 + 2	APN01	inhalation	30	MFD or 0.25 MFD*	TBD*

* Dose (higher or lower) to be selected on the basis of toxicity of previous dose.

Amendment:

Add:

Group	Number of Dogs (M + F)	Agent	Route of Administration	Exposure Time (minutes)	Test Atmosphere Concentration (1st Dose, mg/L)[a]	Test Atmosphere Concentration (2nd Dose, mg/L)
1	2 + 2	APN01	inhalation	60		MFD

Reason for Amendment: **To add an additional exposure of 60 minutes at the MFD to Group 1.**

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

Section: **12.j. Respiratory Function**

Protocol states:

In addition, oxygen saturation of the blood will be measured by pulse oximetry and blood pO_2 will be measured by pO2 electrode at the end of each exposure.

Change to state:

In addition, oxygen saturation of the blood will be measured by pulse oximetry and blood pO_2 will be measured by pO2 electrode pre-exposure and at the end of the 60 minute exposure for Group 1.

Section 13. Methods

Add Section 13.m. Plasma Drug Level Analysis and Toxicokinetics

Blood samples (approximately 3 mL collected from the jugular or cephalic vein) for determination of plasma levels of APN01 will be obtained from Group 1 at nine time points [0 (pre-exposure), immediately at the end of the 60 minute exposure, 1, 2, 4, 6, 24, 48 and 72 hours post-exposure]. K_2EDTA will be used as the anticoagulant. Blood will be centrifuged; plasma will be collected and then frozen at approximately -70°C until analyzed at IITRI using a validated bioanalytical method.

Protocol Amendment Approval:

Study Director: Jeffrey W. Richig
Jeffrey Richig, D.V.M. Date: 2020-10-21

Principal Investigator and President/Director:
David L. McCormick, Ph.D., D.A.B.T. Date: 2020-10-21

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

PROTOCOL DEVIATION NO. 1

Study Title:	**Inhalation Dose Range-Finding Study of APN01 in Beagle Dogs**
Date/Period of Deviation:	October 22, 2020
Section:	**13. m. Plasma Drug Level Analysis and Toxicokinetics**
Nature of Deviation:	Protocol Amendment No. 1 states: "Blood samples (approximately 3 mL collected from the jugular or cephalic vein) for determination of plasma levels of APN01 will be obtained from Group 1 at nine time points [0 (pre-exposure), immediately at the end of the 60 minute exposure, 1, 2, 4, 6, 24, 48 and 72 hours post-exposure]. K_2EDTA will be used as the anticoagulant. Blood will be centrifuged; plasma will be collected and then frozen at approximately -70°C until analyzed at IITRI using a validated bioanalytical method." Serum samples were obtained instead of plasma samples using CAT Serum Sep Clot Activator tubes.
Effect on Study:	This deviation is not expected to affect the integrity of the study.

Date/Period of Deviation:	September 23, 2020
Section:	**11.a. Test Animals:**
Nature of Deviation:	The protocol states: "Prior to shipment dogs were immunized by the supplier against distemper, hepatitis, rabies, parvovirus, canine adenovirus type 2, parainfluenza and Bordetella bronchiseptica." The dogs were not immunized by the supplier against hepatitis.
Effect on Study:	This deviation is not expected to affect the integrity of the study.

Appendix A – Protocol, Protocol Amendment and Protocol Deviation

Date/Period of Deviation:	October 12-18, 2020 and October 22-25, 2020
Section:	**12.g. Physical Examinations and Clinical Observations:**
Nature of Deviation:	The protocol states: "Detailed physical examinations, clinical observations, and measurements of blood pressure will be performed on all surviving dogs during pretest, prior to each exposure, and least once daily for three days after each exposure." The detailed physical examinations were not performed after the pretest examinations since the clinical observations were deemed sufficient enough to establish the health status of the dogs.
Effect on Study:	This deviation is not expected to affect the integrity of the study.

Date/Period of Deviation:	October 12, 15 and 22, 2020
Section:	**12.c. (2) Test Atmosphere Monitoring:**
Nature of Deviation:	The protocol states: "In addition, the aerosol concentration in the oronasal inhalation exposure system will be monitored using an aerosol sensor. The aerosol sensor will serve as real-time indicator of short-term concentration changes and will be used in guiding laboratory personnel if excursions from target concentrations are encountered. Aerosol particle size distribution will be monitored twice per each group during the study using a Quartz Crystal Microbalance (QCM) based cascade impactor (California Measurements, Sierra Madre, CA)." An aerosol sensor was not used during this study. Aerosol particle size was monitored once per exposure.
Effect on Study:	This deviation is not expected to affect the integrity of the study.

Study Director:

Jeffrey W. Richig — 2020-12-04

Jeffrey Richig, D.V.M.
Study Director

Date

IIT RESEARCH INSTITUTE