MatTek Corporation

July 26, 2018

To Whom It May Concern:

With regards to the Nutriair inhalable nutritional supplements, the short term in vitro toxicity studies conducted by MatTek Corporation did not show any negative effects from the Nutriair products. The effects from the exposures to the Nutriair products were comparable to exposures to clean air.

Sincerely,

Patrick J. Hayden, PhD

Vice president for Scientific Affairs

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Final Report

Title: Evaluation of Toxicity Responses of EpiAirway™ to Nutriair® Product Exposures

MatTek Study Number: 012-18

Sponsor:
Joshua Matzkin
NV Nutrition LLC
3226 Bennett Street North
St. Petersburg, FL 33713

Study Director: _____
Anna Maione, Ph.D.

Date: April 4, 2018

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Quality Assurance Statement

I have reviewed the procedures and data presented in this report.

The AIR-100-PC12 tissues (Lot # 28024) and reagents used within this study were produced or obtained in accordance with MatTek's SOPs. The tissue production and test protocols were performed by qualified laboratory personnel.

Based on the results of standardized Quality Control testing, all tissues used in this study fulfill the acceptance criteria for the tissue models.

I hereby certify that the data contained herein are in agreement with the raw data collected during the performance of the study.

Paul Kearney

Paul Kearney
Quality Assurance Director

April 4, 2018

Date

2. Executive Summary

The current study was conducted to compare the toxicity response of EpiAirway™ tissues to aerosol from Nutriair Energy (20 puffs) and Nutriair Sleep (5 puffs), along with vehicle controls (pure 50:50 PG/VG, Energy vehicle + flavor and Sleep vehicle + flavor). Tissues were exposed in triplicate to aerosol and control air using a Vitrocell VC1 smoking machine. Untreated, incubator control tissues and Triton X-100 treatment control tissues were also included. Aerosol was generated using the CRM81 regime under a 0.5 L/min dilution rate and 20 mL/min vacuum rate. Twenty four hours following exposure, tissue viability was assessed by the LDH assay, barrier function was assessed by TEER and tissue morphology was assessed by H&E staining of tissue cross-sections. All tissues remained viable following exposure to either the full formulation Energy or Sleep aerosol. Likewise, tissue viability was unaffected by exposure to any of the vehicle controls. Similarly, tissue barrier function remained intact after all test article exposures. Histological assessment of tissue sections demonstrated that tissue morphology was unaffected by test article exposure, with all tissues exhibiting a pseudostratified and well-differentiated epithelial layer, stereotypical of the human respiratory epithelium. Taken together, these data indicate that exposure to Nutriair Energy Inhaler or Nutriair Sleep Inhaler did not negatively impact the viability, barrier function or morphology of EpiAirway tissues under the conditions tested. Follow-up experiments could be conducted to confirm and further expand on these results.

3. Experimental Methods

The EpiAirway™ model (AIR-100-PC12) is a highly differentiated *in vitro* human airway culture derived from primary human tracheal/bronchial epithelial cells. The human airway epithelial cells have been cultured to form a 3-dimensional model of human airway epithelial tissue. Morphologically, EpiAirway™ is of uniform thickness and is very similar to native nasal and tracheal epithelial tissue in that it exhibits a pseudostratified morphology and contains both ciliated and mucin producing cells. EpiAirway™ tissues possess *in vivo*-like barrier properties due to the formation of functional tight junctions between adjacent epithelial cells. EpiAirway™ cultures also express numerous *in vivo*-like drug metabolizing capabilities including P450, UDP-glucuronosyltrasferase, glutathione S-transferase and alkaline phosphatase activities (See MatTek Technical References #456 and 566 and internal data). The EpiAirway™ PC12 tissues (surface 1.12 cm^2) are cultured on collagen coated microporous membrane cell culture inserts and are grown at the air liquid interface, which allows direct apical application of test materials (similar to *in vivo* exposure). These inserts are the correct size for aerosol exposure using the Vitrocell VC1 smoking machine and 6 well exposure chamber. The model system is shown schematically in **Figure 1**. H&E stained paraffin sections of the EpiAirway™ cultures compared to excised human bronchial epithelium is shown in **Figure 2**. A transmission electron micrograph showing cilia and tight junctions at the apical surface the EpiAirway™ model is shown in **Figure 3**.

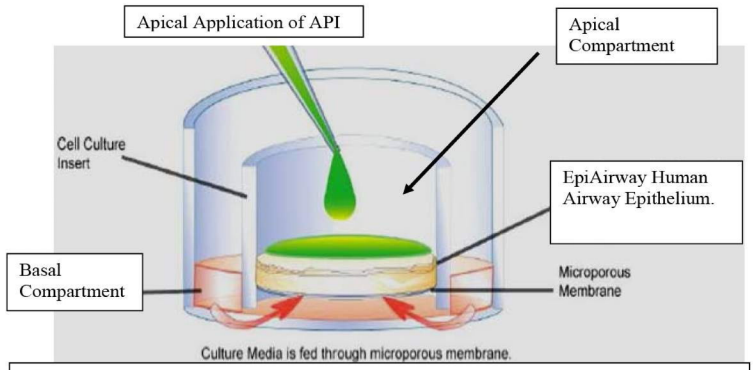


Figure 1. The EpiAirway Model System showing apical compartment, epithelial tissue, microporous membrane and basal (receiver) compartment.

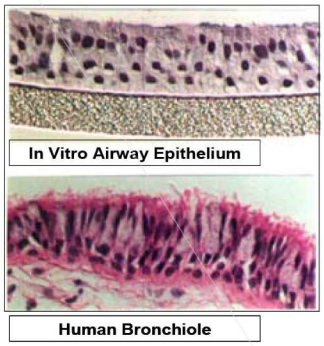


Figure 2. Pseudostratified muco-ciliary morphology of the EpiAirway tissue compared to excised human bronchial epithelium.

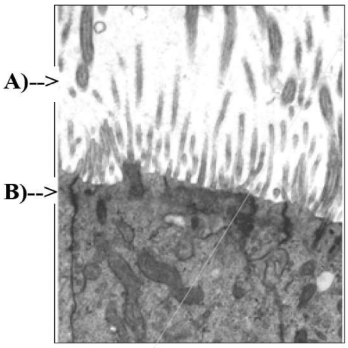


Figure 3. Transmission electron micrograph of EpiAirway showing cilia (A) and tight junctions (B).

A detailed protocol is found in **Appendix B**. Briefly, the apical surfaces of EpiAirway™ tissues were rinsed with PBS prior to exposure. Triplicate tissues were exposed to 20 puffs of Energy Inhaler, 20 puffs of Energy Inhaler flavor vehicle control, 5 puffs of Sleep Inhaler, 5 puffs of Sleep Inhaler flavor vehicle control or 20 puffs of PG/VG vehicle control in parallel with exposure to clean control air. All aerosol was generated by a Vitrocell VC1 smoking machine using the CORESTA Recommended Method Number 81(CRM81) regime (square-wave profile, 55 mL puff volume, 3s duration, 8s exhaust and 30s interval) under a 0.5 L/min dilution rate and 20 mL/min vacuum rate. Additional controls, performed in triplicate on each day of Inhaler exposure, included untreated incubator control tissues (negative control) and tissues treated apically for three hours with 0.5% Triton X-100 (positive control for tissue death).

EpiAirway™ tissues were analyzed for barrier function using trans-epithelial electrical resistance (TEER) before and after exposure. Tissue viability was assessed after exposure using the LDH assay and tissue morphology was assessed by Hemotoxylin and Eosin (H&E) staining of tissue sections. In addition, conditioned media were collected from all tissue cultures for possible future cytokine analysis. The mean ± standard deviation of TEER values (expressed as percent of pre-exposure value) and percent viability were calculated relative to matched controls. Additional analyses are available upon request.

4. Test Articles

Test articles were supplied by the Sponsor. Triton X-100 was supplied by MatTek. Air used for clean air controls was compressed breathing quality air composed of 76.5-80.5% nitrogen and 19.5-23.5% oxygen.

Additional components are outlined below:

Article	CAS #	Manufacturer/ Catalog #	Lot#
TritonX-100	9002-93-1	Sigma / X100	112217OOB
LDH cytotoxicity kit	N/A	Takara / MK401	AH4P029, AH4P028

Table 1. Test Articles.

Vehicle controls: 50:50 PG/VG, Energy Inhaler vehicle + flavor (Vfrost 50:50 PG/VG), Sleep Inhaler vehicle + flavor (Earl Grey 50:50 PG/VG)
Negative controls: Clean air, Incubator control
Positive controls: 0.5% Triton X-100 (positive control for cytotoxicity)

5. Quality Control

Baseline tissue barrier function by TEER:
Study QC acceptance criteria: >300 $\Omega \cdot cm^2$

Lot #	TEER ± s.d.
28024	755.9 ± 153.4

Table 2. QC TEER Data.

Conclusion: Based on the TEER results obtained from the standardized quality control tests, the EpiAirway™ tissues used in this study meet the QC acceptance criteria.

6. Results

6a. Macroscopic observations of EpiAirway™ tissues following exposure to test articles.

The apical surfaces of tissues exposed to test articles appeared similar to that of the matched control air-exposed tissues and to the untreated, incubator control tissues. Triton-treated tissues were leaky and media had seeped into the apical side of the insert.

6b. EpiAirway™ tissue viability determined by LDH following exposure to test articles.

Tissue viability following control treatments and aersol/air exposure was measured using an LDH assay. Viability was calculated relative to the untreated control (100% viable) and Triton X-100 treatment (kill control). All tissues were > 90% viable following treatment with either test articles or control air (**Figure 4a**). There was no difference in viability between the test article-exposed tissues and the matched control air-exposed tissues, indicating that the test articles did not induce any cytotoxicity (**Figure 4b**).

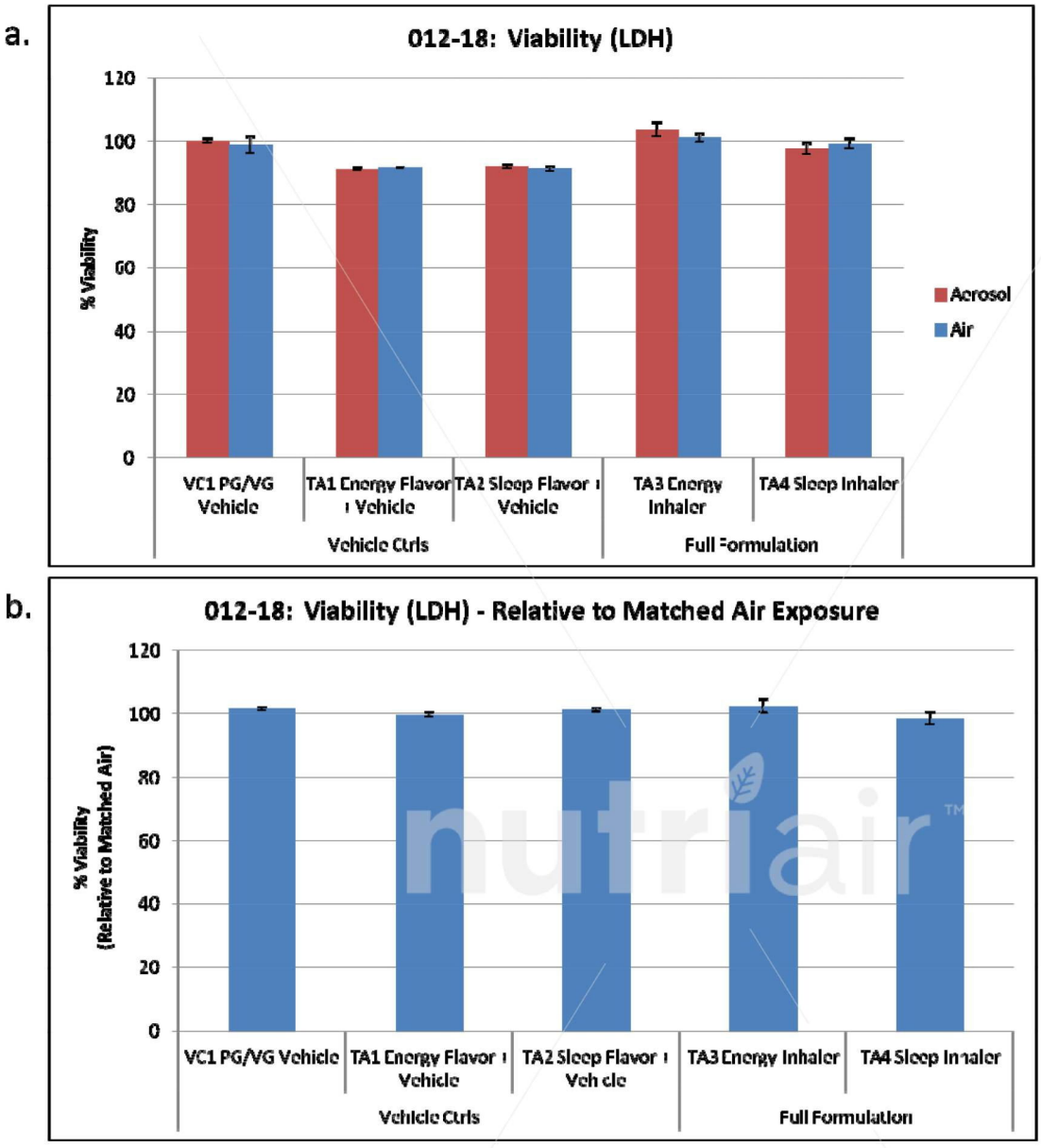


Figure 4. Tissue viability determined by LDH. Tissue viability following exposure to test article aerosol or matched control air was assessed using LDH. (a.) Tissue viability calculated relative to the untreated, incubator control (positive viability control) and the Triton X-100 control (cell death control) remained above 90% viable for all test article exposures and air exposures. (b.) Viability of test article-exposed tissues was expressed relative to the matched air-exposed tissues. Viability of tissues exposed to test articles remained ~100% of the matched air-exposed tissues. Mean viability ± s.d. is shown (n=3).

6c. Barrier function of EpiAirway™ tissues determined by TEER following exposure to test articles.

Barrier function was assessed by TEER following exposure to test article aerosol, air and control treatments. All tissues had pre-exposure TEER values >300 Ω*cm^2, indicative of an

intact epithelial barrier. Following exposure to Triton, the positive control for cell death, barrier function was completely lost (TEER values <1% of pre-exposure value, ≤3.4 Ω*cm^2). Untreated, incubator control tissues maintained barrier function with TEER values >300 Ω*cm^2. Likewise, all air-exposed and test article-exposed tissues maintained barrier function (TEER values >300 Ω*cm^2) after treatment (**Figure 5a**). Barrier function did not differ between tissues exposed to the test article aersol and tissues exposed to control air, suggesting that the test articles did not impact tissue barrier function (**Figure 5b**).

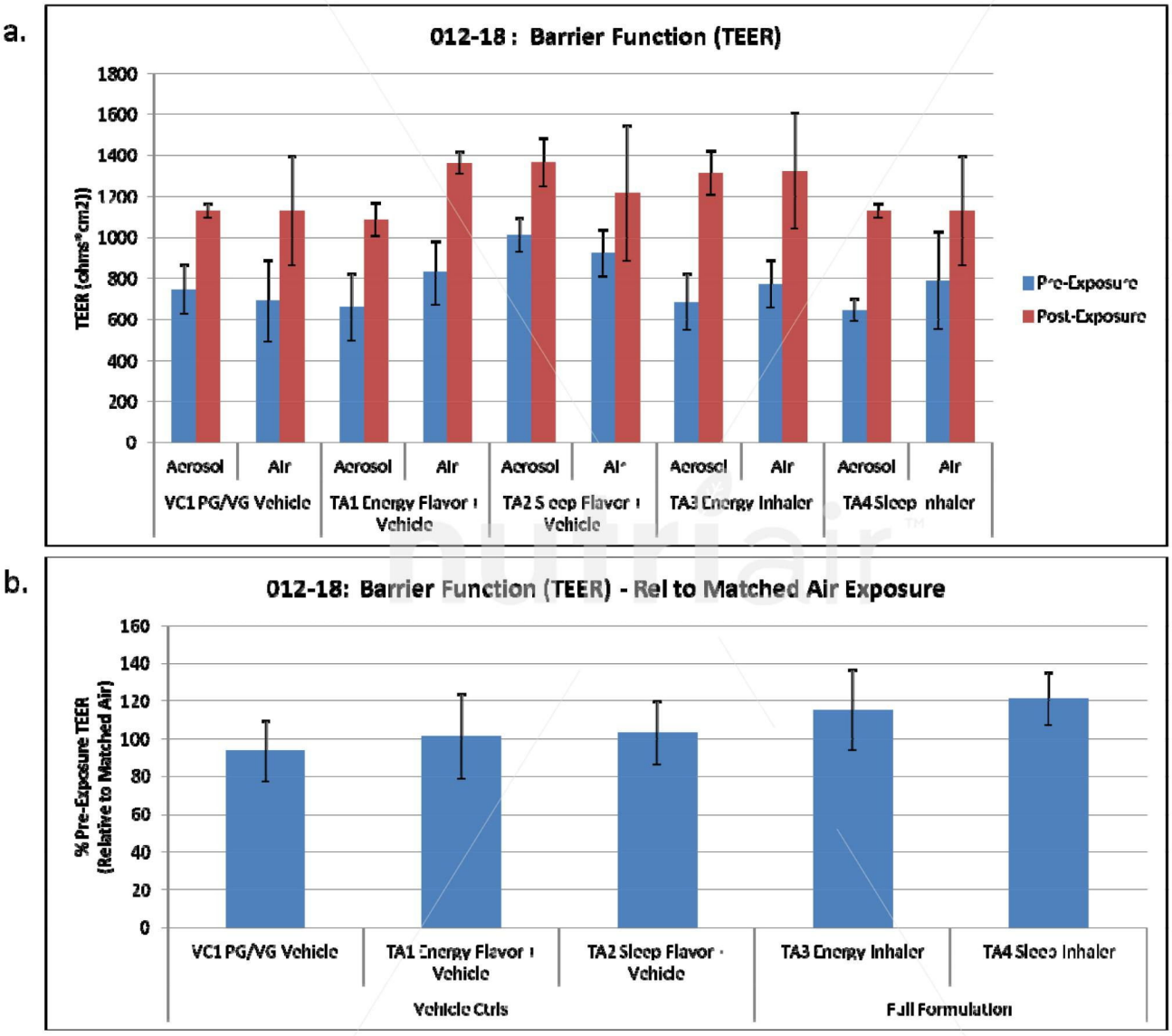
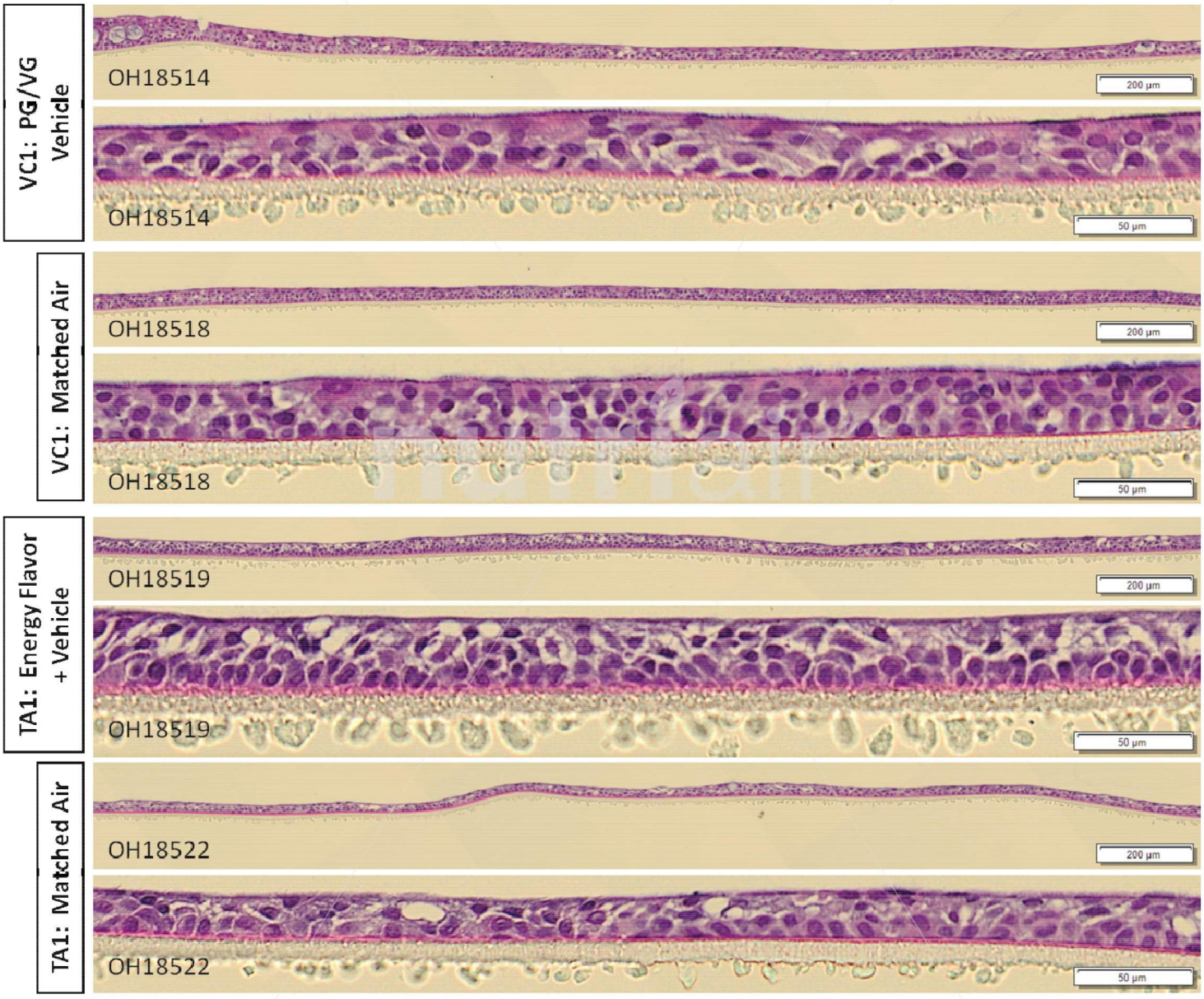
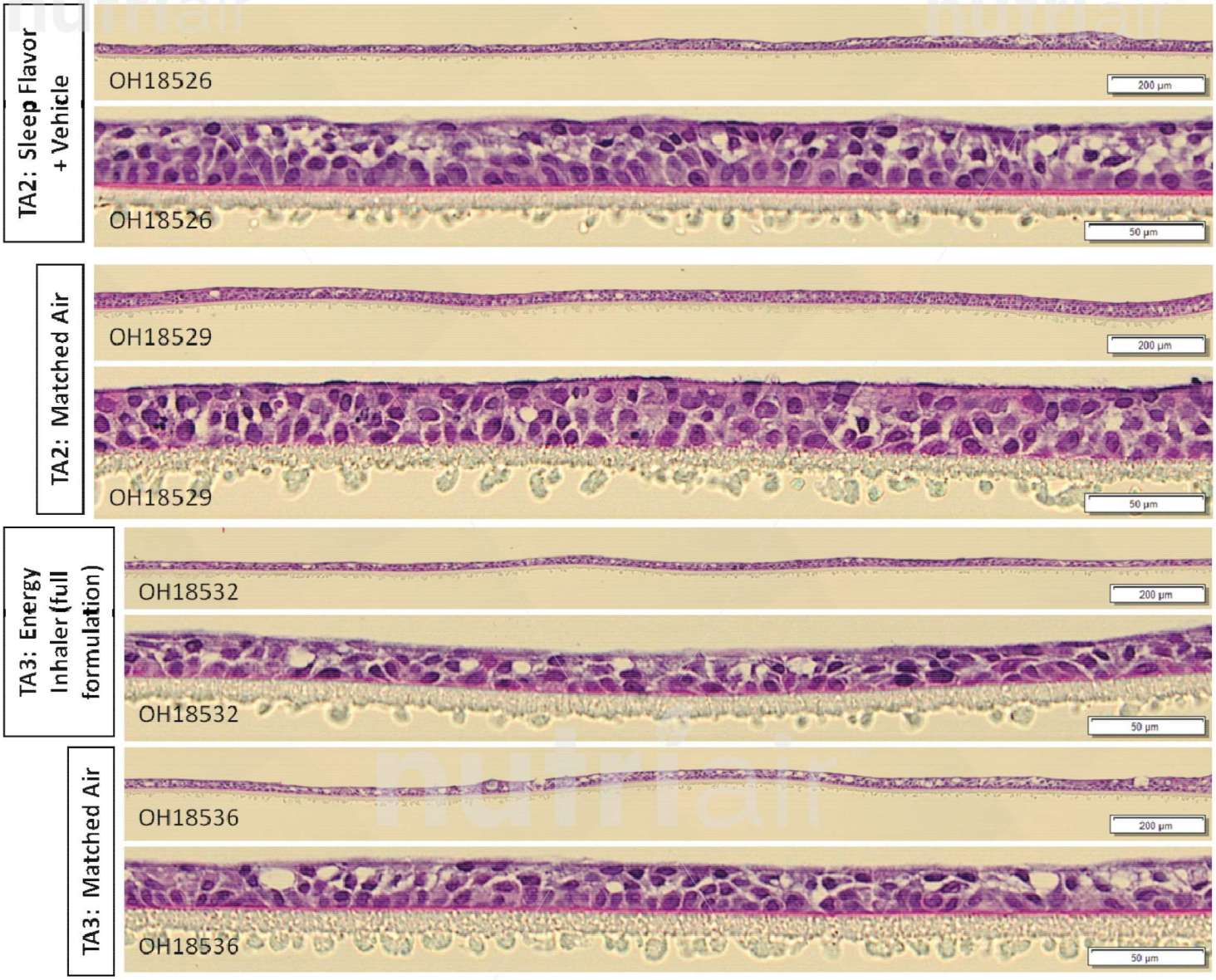


Figure 5. Barrier function of control-treated tissues determined by TEER. Barrier function following treatment was assessed by TEER. (a.) Mean ± s.d. absolute TEER values expressed in Ω*cm^2 are shown (n=3). All tissues exhibited pre-exposure TEER values ≥ 300 Ω*cm^2, indicative of an intact epithelial barrier. All post-exposure TEER values were ≥ 300 Ω*cm^2, demonstrating that none of the treatments impaired tissue barrier function. (b.) The % pre-exposure TEER values expressed relative to the matched air values show TEER was similar between treatments. Mean ± s.d. is shown (n=3).

6d. Effects of test articles on EpiAirway™ tissue morphology determined by histological assessment.

At the 24-hour time point, all tissues were formalin fixed, paraffin-embedded, sectioned and stained by H&E to assess overall tissue morphology. Untreated, incubator control tissues, air control-exposed tissues and test article-exposed tissues all showed comparable morphology that is typical of a healthy pseudostratified respiratory epithelium (**Figure 6**). No tissue damage or abnormalities were detected. The Triton-treated, negative control tissues were almost entirely destroyed, with only a few cells remaining attached to the insert membrane.




TA2: Sleep Flavor + Vehicle
OH18526
200 µm
OH18526
50 µm
TA2: Matched Air
OH18529
200 µm
OH18529
50 µm
TA3: Energy Inhaler (full formulation)
OH18532
200 µm
OH18532
50 µm
TA3: Matched Air
OH18536
200 µm
OH18536
50 µm

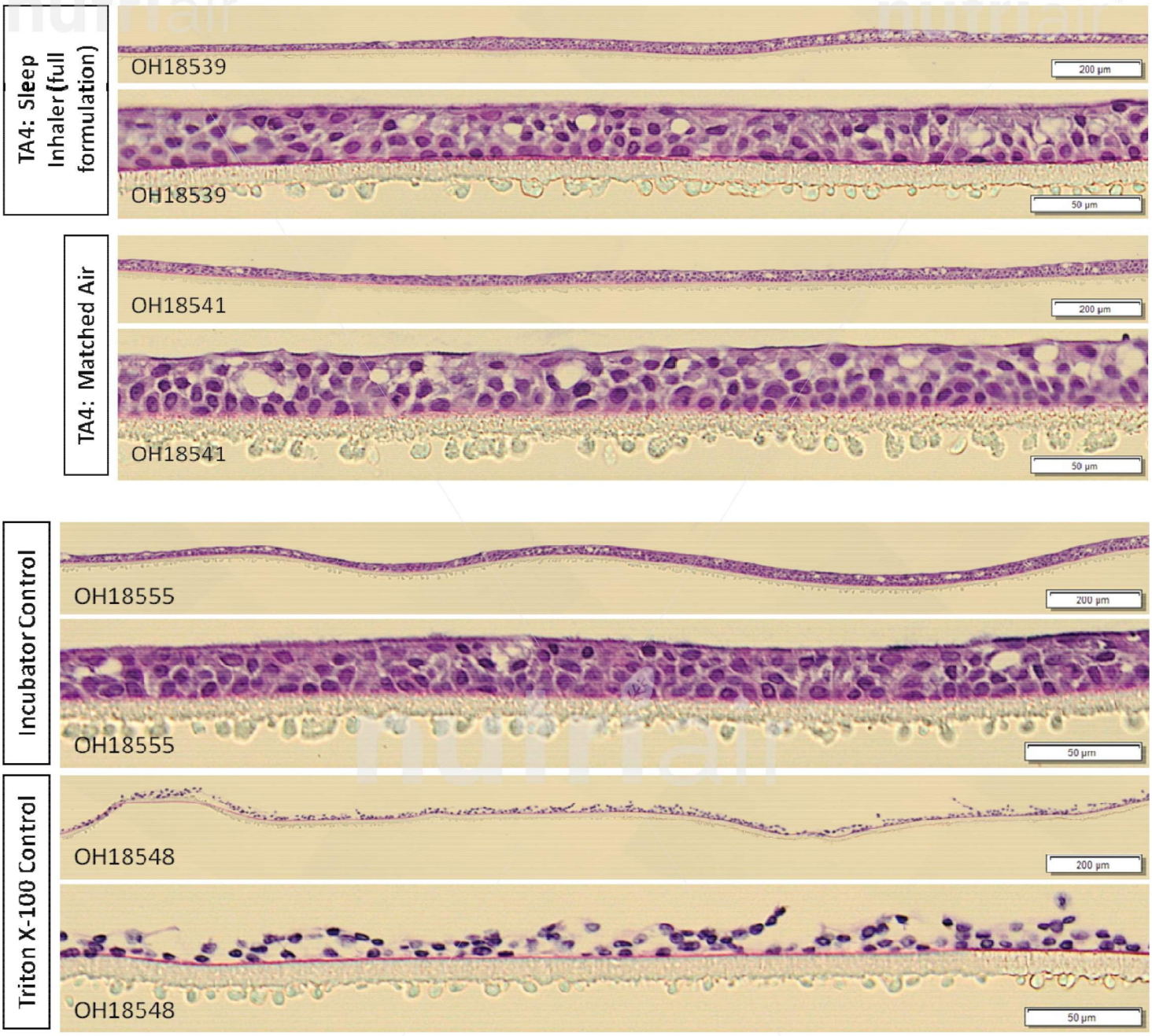


Figure 6. Morphology of EpiAirway™ tissues 24 hours post-treatment. Tissue morphology was assessed by H&E staining of tissue sections (n=3). One representative image from each treatment is shown at a low and high magnification. Images are labeled with the Slide ID number.

7. Conclusion

This study evaluated the effects of Nutriair Energy Inhaler and Nutriair Sleep Inhaler, along with the vehicle control aerosol (pure 50:50 PG/VG, Energy vehicle + flavor and Sleep vehicle + flavor) on EpiAirway tissue viability, epithelial barrier function and tissue architecture. Tissue viability, determined using the LDH assay, was unaffected by exposure to the fully formulated test articles and the vehicle control test articles compared to matched air exposure, indicating no cytotoxic effects of the test articles on the EpiAirway tissues at the doses tested. This result was further supported by the evaluation of epithelial tight junction integrity by TEER measurement. TEER showed that all tissues maintained barrier function following exposure to the test articles. Lastly, H&E stained tissue cross-sections demonstrated that the tissue structure and differentiation was similar between untreated tissues and tissues exposed to air, vehicle control aerosol and full formulation aersol. Follow-up experiments could be conducted to further confirm and extend these results. For example, additional functional endpoints like changes in gene expression or oxidative stress markers could be examined or higher doses could be evaluated to confirm that toxicity would not occur under extreme exposures. Conditioned cell culture media were collected and saved for possible future evaluation of cytokine secretion following test article exposure. In conclusion, the Nutriair Energy Inhaler and Sleep Inhaler did not impair EpiAirway tissue viability, barrier function or morphology under the conditions tested here. EpiAirway™ tissues are a valuable *in vitro* tool for screening and evaluating the effects aerosol products on human respiratory epithelial tissues at the air-liquid interface.

III. Application of Test Articles:

Tissues will be exposed to test material vapor or clean air via the Vitrocell exposure system according to the exposure conditions above. Three hour topical exposures to 200µl of 0.5% Triton X-100 will be utilized as positive controls for tissue death. Untreated and clean air treated tissues will be used as negative controls.

IV. Harvest Time:

Tissues will be harvested at 24 hrs post exposure and assessed for barrier function (TEER), tissue viability (LDH) and histological changes

V. Media and Reagents:

All medium, reagents, positive and negative controls, and tissues will be supplied by MatTek Corporation (MatTek). All items are covered under MatTek's STANDARD OPERATING PROCEDURES (SOPs) for producing these items for normal commercial sale.

VI. Tissue Quality Control:

As part of standard quality control (QC) procedures, mucus production and tissue thickness will be monitored during production. TEER will be measured and histological analysis will be performed on random tissues from the tissue lot to be used for the study. Results from these analyses will be used to determine whether the produced tissue lot is valid to perform the intended study

CRITERIA FOR DETERMINATION OF A VALID TEST. It is crucial that the tissue be reproducible so that assay results can be compared between tissue lots. Assay results will be accepted if the TEER for the QC testing is >300 $\Omega^{*}cm^{2}$ and H&E stained images of tissue cross sections show an organized epithelium.

VII. Quality Assurance:

MatTek's Quality Assurance department will review the results of standardized Quality Control testing for all tissues used in this study to insure that that it fulfills the acceptance criteria for the tissue. In addition, the final report will be reviewed to insure that all data are in agreement with the raw data collected during the performance of the study.

VIII. Deliverables and Agreements:

Preliminary Report including summary, methods and reagents, results, discussion, and raw data
Final Report upon Sponsor review of Preliminary Report

Payment Terms: Any payment terms will be applied at commitment of study. Net 30. 50% of the study cost is due prior to the initiation of the study. The final 50% of the study cost is due prior to the release of the final report.

Study Cost: $13,475.00

Agreed to and Accepted by:

Signature	Signature
CEO	DIRECTOR OF BUSINESS DEVELOPMENT
Title	Title
Joshua Matzkin	ALEX ARMENTO
Print Name	Print Name
12/18/17	12/18/17
Date	Date
Sponsor	**MatTek Corporation**

Appendix B: Study Protocol

MatTek CORPORATION

Study Protocol

Evaluation of Toxicity Responses of EpiAirway™ to Nutrovape® Product Exposures

Nutrovape Study #012-18

The Vitrocell VC1 smoking machine will be thoroughly cleaned before beginning the study, at the end of each day, between product changes and at the conclusion of the study. The following protocol will be conducted using EpiAirway standard donor tissues (AIR-100-PC12). All test articles and the vehicle control will be provided by the sponsor and stored at room temperature until use.

I. Preparation and exposure of EpiAirway tissues to Nutrovape vapor.

1. Gently rinse the apical surface of EpiAirway tissues twice by adding 500 µl of phosphate buffered saline (PBS) containing magnesium and calcium (referred to as "TEER buffer") to the culture insert and carefully aspirating to remove all liquid and mucus from the tissue surface. This process should remove all apical mucus. Prepare the necessary number of tissues for control and test article treatments in triplicate just prior to treatment/exposure.
2. Before beginning each exposure, measure the pre-exposure TEER for the tissues following Steps 4-10 in Part III.
3. **Control treatments (not vaped)**: Place the rinsed tissues into 12-well culture dishes containing 1 mL of assay media. Dose and record the time.
    a. **NC1 (Incubator control)**: Reserve three untreated tissues as incubator controls in the 12-well plate with media and harvest in parallel with the Triton control (PC1) after 24 hours.
    b. **PC1 (0.5% Triton X-100 in PBS)**: Dose the apical surface of three tissues with 200 µL Triton X-100 solution for three hours. After three hours, aspirate, rinse with 500 µL PBS, aspirate and move to a 12-well plate containing media. Harvest tissues 24 hours after exposure.
4. **Control & Test article treatments (vaped):** Before exposing tissues, condition the smoking machine by puffing 20 puffs of the control or test article before their respective exposures.
    a. Pipet 1.5 mL assay media into each well of the exposure chamber. Place six rinsed tissues into the Vitrocell exposure chambers.
    b. **NC2 (Clean air control):** Three of the tissues will be exposed to vapor, while three chambers are exposed to clean air (NC2) under the same conditions. The clean air control will be conducted in parallel with each vapor exposure and will be harvested 24 hours after exposure.
    c. Vapor from the following vehicle controls and test articles will be generated using the CORESTA Recommended Method Number 81 (CRM81) regime (square wave puff profile, 55 mL puff volume, 3s duration, 8s exhaust, 30s interval) under a 0.5 L/min dilution rate and 20 mL/min vacuum rate.
        i. **VC1 (Pure 50:50 PG/VG)**: Triplicate tissues will be exposed to **20 puffs** of VC1 vapor in parallel to 20 puffs of air.
        ii. **TA1 (Energy Vape vehicle + flavor; Vfrost 50:50 PG/VG):** Triplicate tissues will be exposed to **20 puffs** of TA1 vapor in parallel to 20 puffs of air.
        iii. **TA2 (Sleep Vape vehicle + flavor; Earl Grey 50:50 PG/VG):** Triplicate tissues will be exposed to **5 puffs** of TA2 vapor in parallel to 5 puffs of air.
        iv. **TA3 (Energy Vape; full formulation):** Triplicate tissues will be exposed to **20 puffs** of TA3 vapor in parallel to 20 puffs of air.

v. **TA4 (Sleep Vape; full formulation):** Triplicate tissues will be exposed to **5 puffs** of TA4 vapor in parallel to 5 puffs of air.

d. After each vapor/air exposure, move the tissues to a 12-well culture dish containing 1 mL of assay media and record the time. Tissues will be harvested 24 hours after exposure.

e. Following all tissue treatments and exposures, place the tissues in an incubator at 37°C, 5% CO_2 for 24 hours.

5. Tissues will be harvested after a 24 hour incubation period for the following endpoints and the exact time of harvest will be recorded.
   a. Barrier function: Transepithelial Electrical Resistance (TEER)
   b. Tissue viability: LDH activity
   c. Histology: Hemotoxylin & Eosin (H&E) staining
   d. Storage of culture media

II. Harvest of EpiAirway tissues for cilia, TEER, LDH, histology and storage of conditioned media.

1. At the time of harvest time, transfer the tissues to a new 12-well plate containing 1 mL TEER buffer for assessment by TEER (See Part III).
2. Retain the media from the post-exposure plate for assessment by the LDH assay (See Part IV).
3. Following TEER assessment, fix the tissues for histology (See Part V)
4. After the media for the LDH assay is removed, the remaining media will be saved for possible cytokine analysis.
   a. Divide the remaining media into two ~250 µL aliquots in capped Eppendorf tubes.
   b. Also aliquot non-exposed culture media to serve as a blank media control.
   c. Store at -80°C for at least 3 months.

III. Barrier function assessment by Transepithelial Electrical Resistance (TEER).

1. Move the tissues to a new 12-well plate containing 1 mL TEER buffer (PBS containing magnesium and calcium) to measure the post-exposure TEER.
2. Record any macroscopic observations of the tissues (leakiness, texture, etc.).
3. Rinse the apical surface of the tissue twice with 500 µL TEER buffer.
4. Equilibrate the EVOM2 voltmeter by filling the Endohm12 culture cup with TEER buffer and waiting at least 20 minutes.
5. Turn on the voltmeter and record the background ohms and mV readings of the culture cup filled with TEER buffer.
6. Remove all but ~2 mL TEER buffer from the culture cup.
7. Using forceps transfer each tissue insert to the culture cup.
8. Measure and record the ohms reading once it has stabilized.
9. Switch to the mV setting and record the mV reading once it has stabilized.
10. Replace the tissue in the 12-well plate and continue with the next tissue insert. Repeat until all tissues have been measured.
11. After all measurements are complete, continue to the LDH assay (Part IV) and histology (Part V).

IV. Tissue viability assessment by LDH assay.

1. At the time of harvest, transfer 25 µL of media from each tissue in triplicate into a clear 96-well plate.
2. Also transfer non-exposed culture media to serve as a blank media control.
3. Wrap the plate in parafilm and store at 4°C until analysis.

4. To perform the LDH assay for viability, allow the samples to warm to room temperature for at least 15 minutes.
5. Prepare the required amount of LDH reagent (LDH kit, Takara Cat # MK401) by mixing Solution A with Solution B at a ratio of 1:45 (A:B).
6. Add 25 µl of prepared LDH reagent to the wells containing media samples and incubate at room temperature, protected from light for 30 min.
7. After 30 minutes, add 25 µL of 1 N HCl to the wells to stop the LDH reaction.
8. Mix thoroughly by gently tapping the side of the plate.
9. Read the absorbance at 490nm/650nm and subtract the blank media reading.

V. Harvest of EpiAirway tissues for histology.

1. Following TEER assessment, aspirate the PBS from both the apical and basal compartments and fill each well with approximately 1 mL 10% formalin in the basal compartment and approximately 0.5 mL in the apical compartment to fix the tissues.
2. Fix at room temperature overnight. Then, remove the formalin and replace with PBS.
3. Using a scalpel, remove the tissue and insert membrane from the insert and place in a histology cassette.
4. The samples are then dehydrated, paraffin embedded and sectioned.
5. A single slide containing three serial sections will be stained by Hematoxylin & Eosin (H&E) to assess tissue morphology and imaged.

VI. Data analysis and statistics.

1. TEER analysis:
   a. Subtract background readings from raw numbers and multiply by insert surface area.
2. LDH analysis:
   a. Relative viability will be calculated by normalizing the absorbance reading for each tissue to total possible LDH release (Complete tissue death = Triton X-100 control) and baseline LDH release (Untreated tissue = Incubator control) using the following formula:
      % Viability = 100 – [Abs(X) - Abs(Inc)] / [Abs(Triton) – Abs(Inc)]*100
3. Histology analysis:
   a. A representative image of each tissue stained by H&E will be presented.
   b. Observations and interpretations of tissue morphology will be provided.
4. Statistics
   a. Calculate mean and standard deviation of the triplicate tissues in each treatment group.
   b. Statistically significant differences between groups will be calculated using Student's T test (comparing two groups) or 1-way ANOVA with appropriate post-hoc tests (comparing three or more groups). A difference will be considered statistically significant with a p-value ≤ 0.05.

Agreed to and accepted by:

Nutrovape		MatTek
Signature		Signature
Admin. Coordinator		Study Director
Title		Title
Kirby Drake		Anna Maione
Print Name		Print Name
1/15/18		1/15/18
Date		Date

Appendix C: Raw Data

Raw data will be provided as separate Microsoft Excel files.