Pulmonary Health Impacts of Golden Tobacco Flavored Vuse Alto Aerosols in Vulnerable Populations of Young Mice

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Introduction

- With ~ 2 million American youth using electronic nicotine delivery system (ENDS) devices, the health risks associated with electronic-cigarette use among young individuals are a growing public health concern in the U.S.
- The rationale for this study focuses on the prevalence of use of 4th generation ENDS in U.S. middle and high school students, what has been labeled "the youth vaping epidemic."
- The National Youth Tobacco Survey (NYTS) reported that 11.3% of high school students and 2.8% of middle school students currently used electronic-cigarette devices and that disposable, 4th-generation ENDS devices were the most commonly used. NYTS also demonstrated that 85.5% of high school users and 79.2% of middle school users reported using flavored ENDS products.
- 4th-generation ENDS devices, such as the popular Vuse Alto, have been on the market since 2019. They are often disposable devices that resemble a USB flash drive. They use nicotine salt-based formulas to deliver high doses of nicotine to the bloodstream. These products have been advertised as being a "safer" alternative to smoking and ad campaigns have openly targeted a younger demographic.
- Limited data is available regarding the pulmonary health impact and lung responses to flavored Vuse Alto aerosols. Therefore, It is imperative to investigate the pulmonary effects of these devices.

Research Objectives:

- Provide laboratory-based evidence on the pulmonary toxicity induced by golden tobacco Vuse Alto Aerosols on vulnerable populations of young mice.
  - Determine the effect of golden tobacco flavored Vuse Alto aerosol exposure on lung structure and function.
  - Assess biochemical changes to the lungs induced by golden tobacco flavored Vuse Alto aerosol exposure.

Hypothesis

Sub-acute exposure of juvenile 4-week-old mice to golden tobacco flavored Vuse Alto aerosols over a 3-month period will decrease lung function and down-regulate the expression of several lung genes related to immune responses.

Methods

Experimental Methods:

- 4-week-old BALB/c mice were exposed to either air (control group) or golden tobacco Vuse Alto aerosols via whole-body exposures in a 5 L chamber for 1 hour a day, 5 days a week, for 3 months.
- Vuse Alto aerosol exposures followed a standard vaping topography profile of 5 seconds puff duration, 55mL puff volume, every 30 seconds for 1 hour. The total particulate matter mass concentration in the chamber was determined gravimetrically, using filters placed in cassettes, and monitored continuously in real-time via a MicroDustPro device.
- Lung function was assessed via whole-body plethysmography.
- Lung structure was examined by histopathology of formalin-fixed lung samples and lung slides that were prepared to determine morphometric measurements such as mean linear intercept via Image ProPlus software.
- Lung biochemical changes were assessed via:
  - Bronchoalveolar lavage fluid (BALF) was examined for total & differential cell counts, and markers of oxidative stress.
  - Measurement of serum cotinine concentration via ELISA testing.
  - RNA extraction and qRT-PCR gene expression analysis of selected genes associated with inflammation, immune suppression, and asthmatic response.

Statistical Analysis:

- Results were analyzed using either a Student t-test for pairwise comparison or ANOVA followed by the Tukey's test for multiple comparisons. All outcomes are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 9 software. Results with a p-value < 0.05 were considered statistically significant.

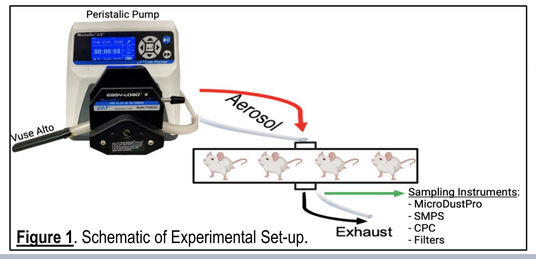

Figure 1. Schematic of Experimental Set-up.

Results

Table 1. Filtered-air and Vuse aerosol exposure characterization.

Group	Average aerosol concentration (mg/puff) ± SD	Exposure chamber average temperature (°C) ± SD	Exposure chamber average relative humidity (%RH) ± SD
Air		23.7 ± 1.4	44.0 ± 9.5
Vuse	0.39 ± 0.09	26.7 ± 2.1	58.4 ± 6.5
Air + Recovery		24.3 ± 1.4	41.7 ± 7.7
Vuse + Recovery	0.40 ± 0.11	26.9 ± 1.5	59.1 ± 6.4

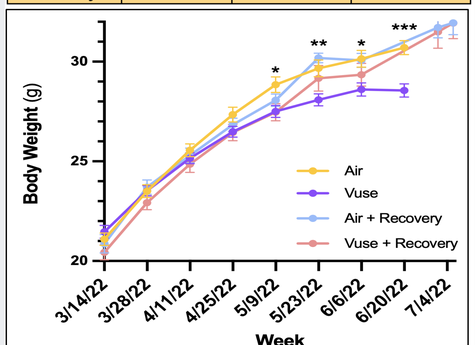

Figure 1. **Exposure to Vuse Alto aerosols significantly decreased body weight in exposed mice beginning around day 54.** Body weight was recorded for all 4 groups every 2 weeks beginning on 3/14/22 until the day of sacrifice. There proved to be a significant difference in weight between the Vuse and Air groups beginning on day 54 (5/9/22) until sacrifice (6/23/22).

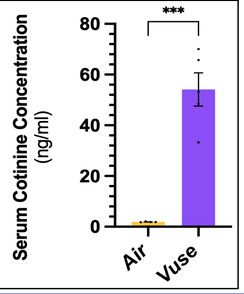

Figure 2. **Exposure to Vuse Alto aerosols significantly elevated levels of cotinine in the exposed mice.** Serum cotinine measured *via* ELISA testing. Student T-test with $\alpha = 0.05$ proved to be significantly different from the respective air control group.

Lung Structure

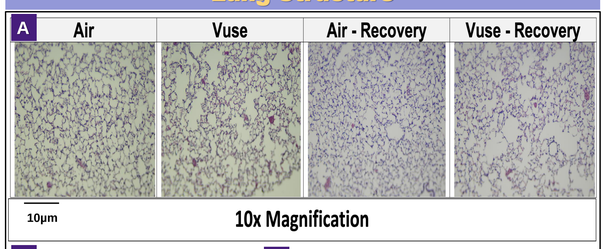

Figure 4. Exposure to Vuse Alto aerosols significantly alters lung structure. (A). Hematoxylin and eosin (H&E) stained lung tissue of mice exposed to either Vuse aerosols or filtered air and each group after a 2-week recovery period (10x magnification; scale bar = 10μm). **(B).** Mean Linear Intercept exposed mice and their respective air group, including comparison after a 2-week recovery period. **(C).** Morphometric analysis of lung tissue showing the surface area per unit volume.

Lung Function

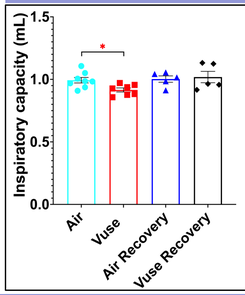

Figure 5.
Exposure to Vuse Alto aerosols significantly decreased Inspiratory Capacity (mL).

Lung Biochemical Assessment

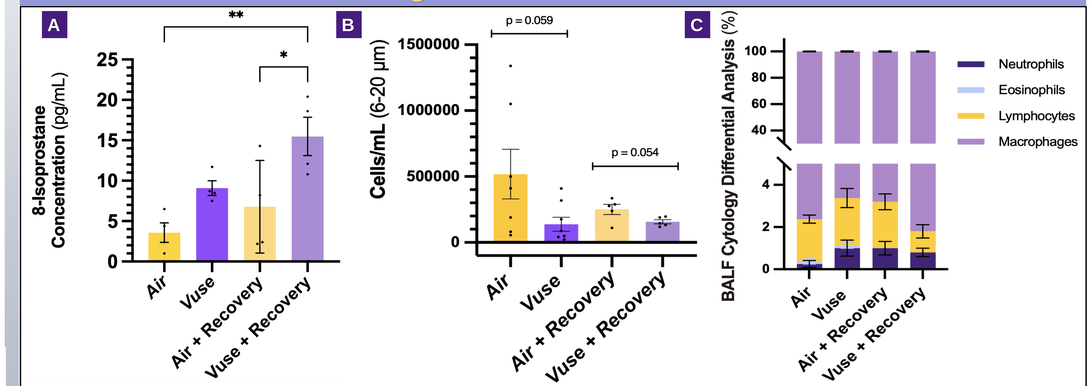

Figure 3. Exposure to Vuse Alto aerosols significantly elevated levels of 8-isoprostane, a biomarker of oxidative stress, in BALF of exposed mice. (A). BAL fluid 8-isoprostane levels were measured *via* ELISA testing. **Exposure to Vuse Alto aerosols altered cell count in exposed mice. (B).** broncho-alveolar lavage fluid was examined for total cell count for white blood cells between 6 to 20 μm in size using Bio-Rad TC10 Automated cell counter. **(C).** Broncho-alveolar lavage (BAL) fluid cytology revealed mostly macrophages upon examination.

Gene Expression

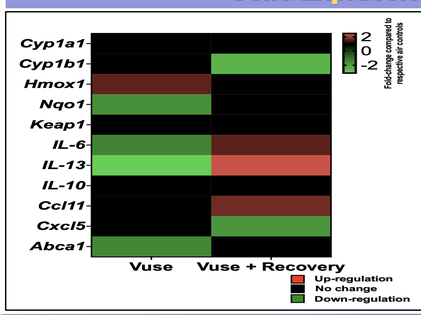

Figure 6. **Exposure to Vuse Alto aerosols significantly altered gene expression in exposed mice.** Heatmap displays the qPCR results expressed in fold-change compared to the respective air control group.

Discussion

- All mice exposed to Golden Tobacco Vuse aerosols were exposed via whole-body exposures at an average total particulate matter concentration of 0.39 to 0.40 mg/puff (**Table 1**).
- All mice exposed to Vuse Alto aerosols had significantly elevated serum cotinine levels (>33.2 ng/mL) compared to their respective air control group (<2.1 ng/mL) (**Figure 2**), confirming nicotine exposure from Vuse aerosols.
- Body weight monitoring of each group shows that beginning around day 54 (05/09/2022) until the end of the study, mice exposed to Vuse aerosols had significantly decreased body weight (27.5 g) compared to their respective air group (28.9 g) (**Figure 1**). This suggests a potential effect of nicotine exposure on weight gain.
- Significantly elevated levels of 8-isoprostane, a biomarker of oxidative stress, were found in broncho-alveolar lavage (BAL) fluid of Vuse exposed mice (9.1 pg/mL) compared to their respective air control group (3.6 pg/mL) (**Figure 3 A**). The significantly elevated levels of 8-isoprostane indicate increased oxidative stress in the lungs.
- Broncho-alveolar lavage (BAL) fluid total cell count proved to be insignificant based on an $\alpha = 0.05$ level of significance. Although insignificant, our data shows that there was decreased total cell count in both Vuse exposure groups compared to their respective air control groups (**Figure 3 B**).
- In all groups, examination of broncho-alveolar lavage (BAL) fluid cytology demonstrated that BAL fluid was composed of mostly macrophages (**Figure 3 C**).
- Histopathological assessment of mice lung tissue revealed that exposure to Vuse Alto aerosols significantly alters lung structure (**Figure 4**). Representative microscopic images of H & E-stained lung samples from both the exposed mice and their respective air control including each group after 2-weeks of recovery (**Figure 4 A**). Mice exposed to Vuse Alto aerosols demonstrated a significant decrease in Surface Area per Unit Volume (**Figure 4 C**) and a significant decrease in the Mean Linear Intercept (**Figure 4 B**). This enlargement of lung airspaces suggest a potential decrease in gas exchange in the lungs of exposed mice, an effect that does not return to control levels 2 weeks after vaping cessation (**Figure 4 B**).
- In terms of Vuse aerosol effects on lung function, using the *flexiVent* system, we found a slight but significant decrease in the inspiratory capacity of exposed mice compared to their respective air control group. However, this measure of lung function returned to baseline levels following a 2-week recovery period. (**Figure 5**).
- At the molecular level, we found that exposure to Vuse Alto aerosols altered the expression of genes associated with inflammation, immune suppression, and oxidative stress (**Figure 6**). Specifically, exposure to Vuse Alto aerosols suppresses the expression of *Il-6* and *Il-13*, biological factors that play a key role in lung maturation and acute inflammatory reactions.

Conclusions

- Overall, this study shows that exposure to Golden Tobacco flavored Vuse Alto aerosols in young mice results in lung structural, functional, and biochemical alterations, as well as dysregulation of genes associated with inflammation, immune suppression, and oxidative stress.
- This study provides laboratory-based evidence for future regulation of Vuse Alto products that are often used by young individuals.

Acknowledgements

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