

JUUL and Vape Pen Flavors Produce Reactive Oxygen Species, Potentially Eliciting Differential Cellular Oxidative Stress and Inflammatory Responses

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Disclosure

All authors

Relevant financial relationships with a commercial interest

No relevant commercial interests

JUUL AND EMERGING DEVICES AND FLAVORS



Background



- JUUL is made of an aluminum shell, lithium battery, circuit board and pressure sensor



- JUUL constituents include PG/VG, nicotine, benzoic acid, and flavoring chemicals
- Smoother hit even at high nicotine levels due to benzoic acid

Flavors and Flavorings (chemical groups) present in ENDS, cigarillos, and waterpipe flavorings for toxicity testing

Flavors

Tobacco



Menthol



Cherry



Fruit



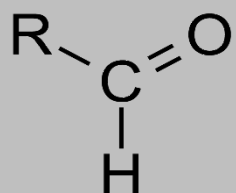
Chocolate



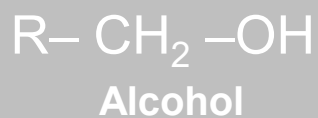
Alcoholic Beverages
Piña Colada



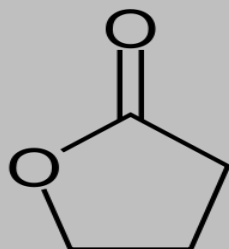
Flavorings



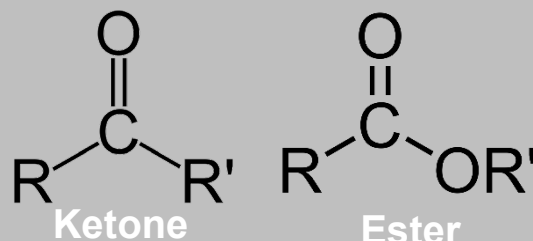
Aldehyde



Alcohol

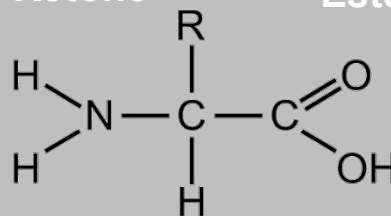


γ Lactone

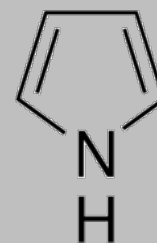


Ketone

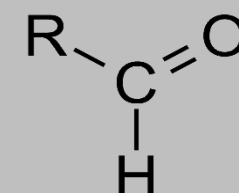
Ester



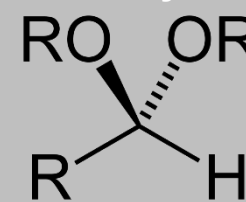
Amino acid



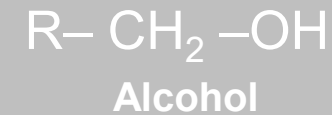
Pyrrole



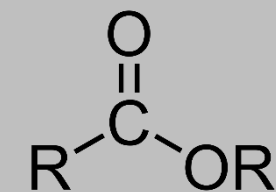
Aldehyde



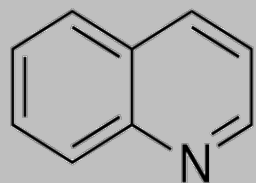
Acetal



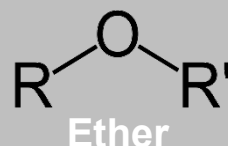
Alcohol



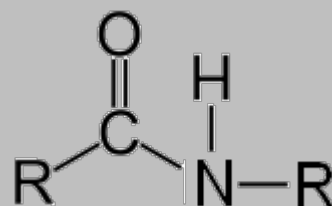
Ester



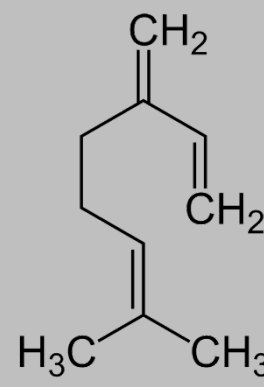
Quinoline



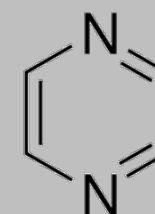
Ether



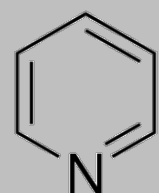
Peptide



Monoterpene



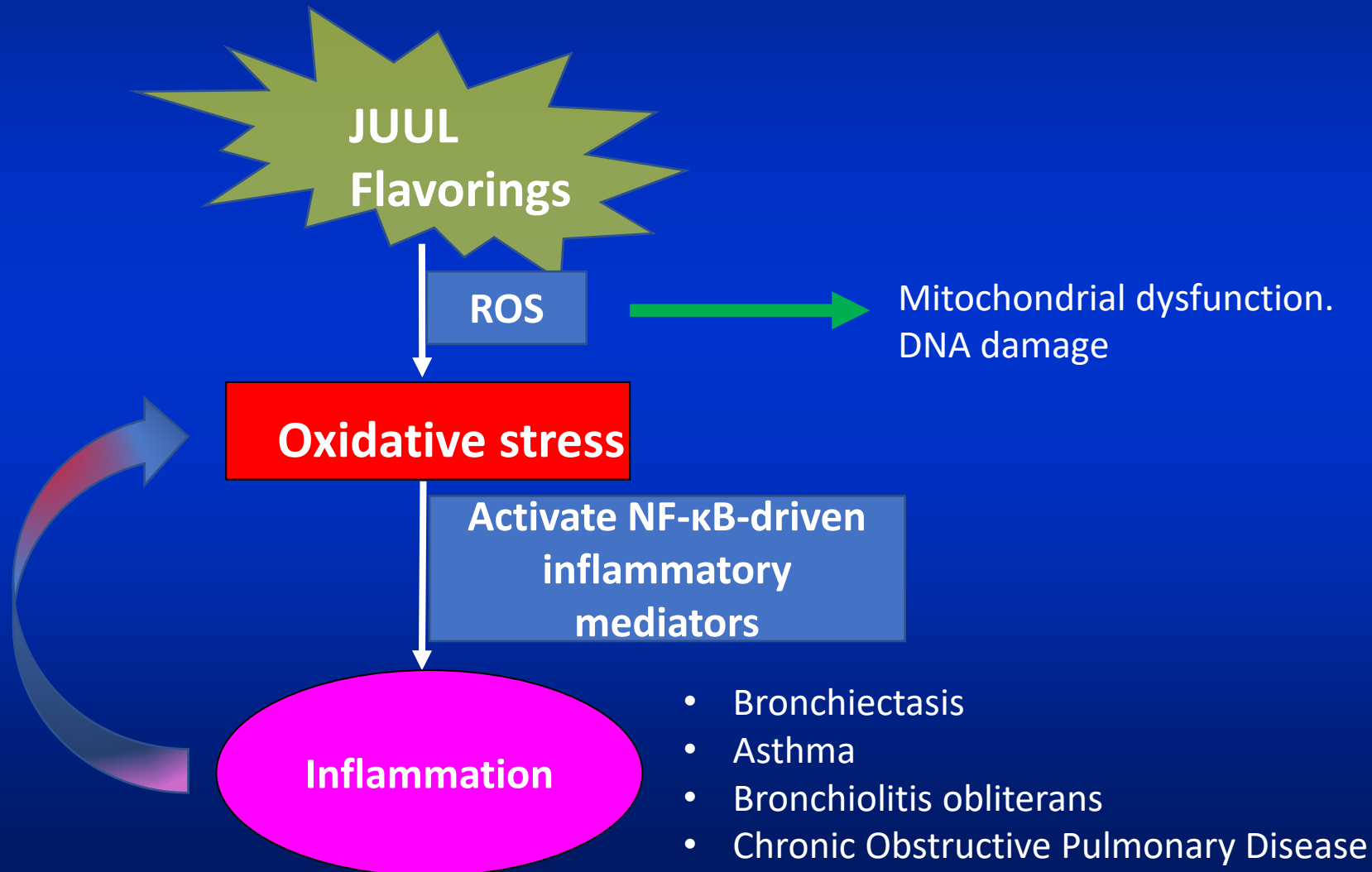
Pyrazine



Pyridine

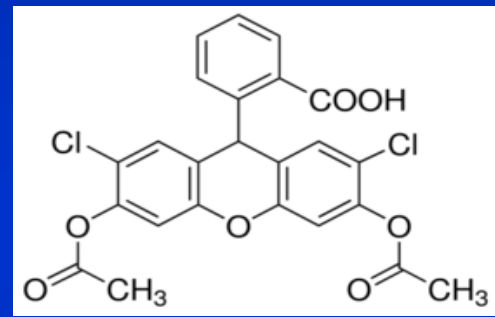
Hypothesis

JUUL flavors and flavoring chemicals produce reactive oxygen species (ROS) and the exposure to these flavors result in an inflammatory response.

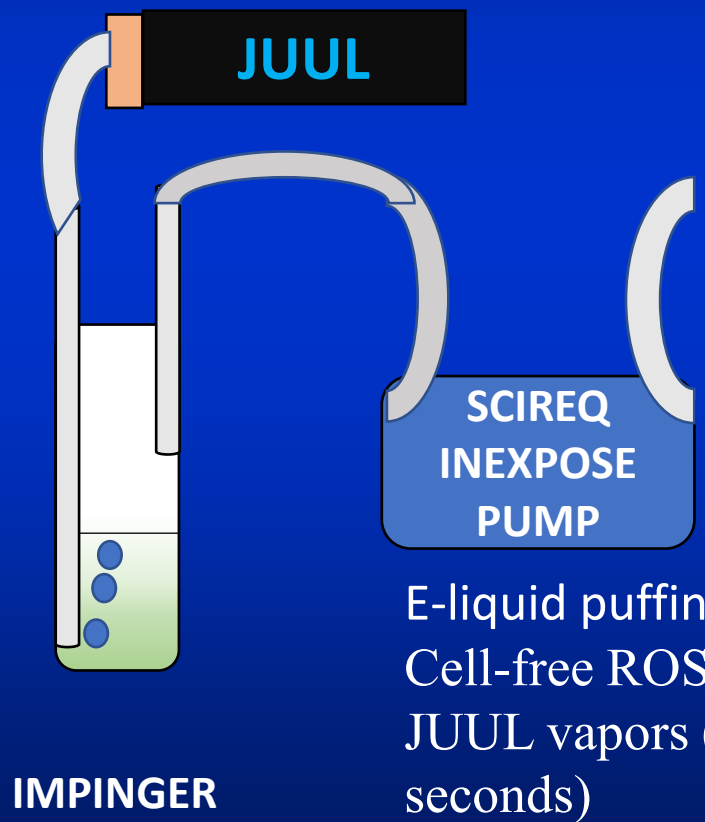


Cell-free ROS assessment in JUUL flavors

Principle: 2',7'-dichlorofluorescein diacetate (H₂ DCF-DA) fluorogenic probe.



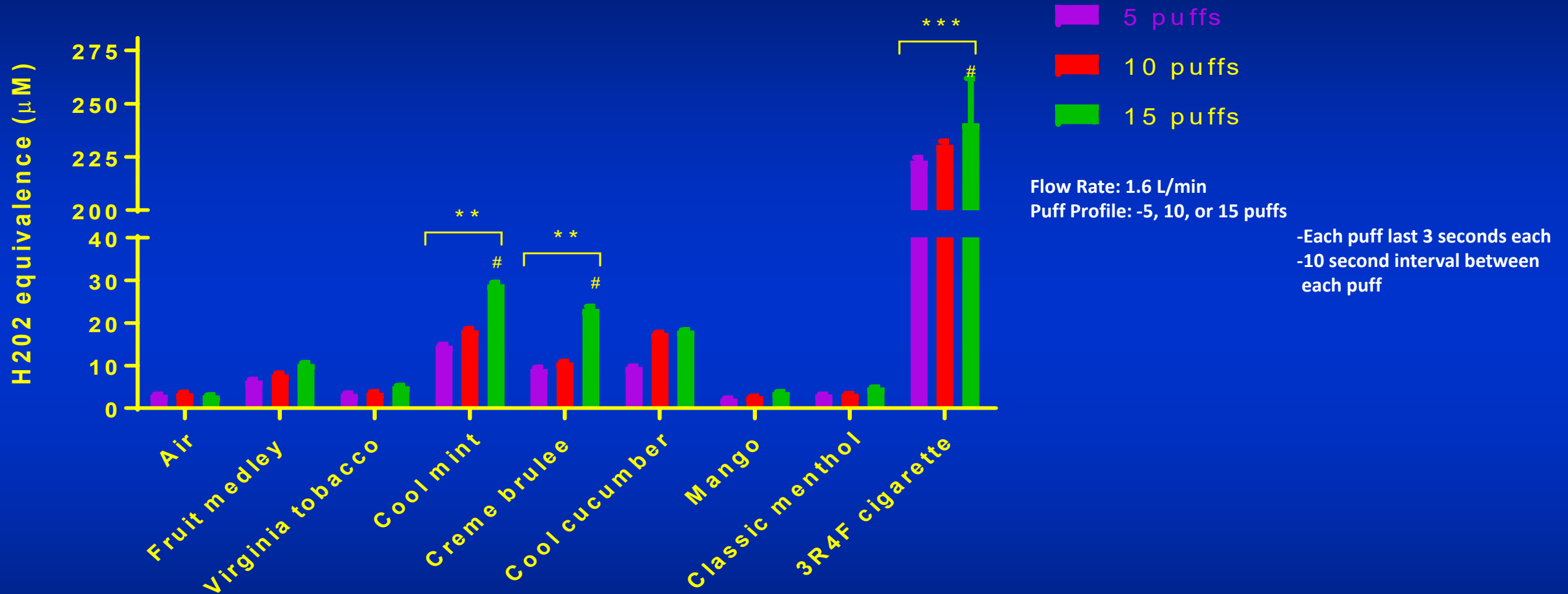
1. Prepare H₂O₂ standards (0 though 50 μM)
2. Bubbling JUUL aerosol (using SciReq InExpose through the DCFH solution).
3. Read fluorescence (ex 485 nm/em 535 nm)
4. Report OX/ROS as H₂O₂ equivalents.



E-liquid puffing was an adaptation of Behar profile: Cell-free ROS produced was measured by bubbling JUUL vapors (5,10, and 15 puffs, each puff lasting 3 seconds)

JUUL aerosols generate acellular reactive oxygen species (ROS)

(Virginia Tobacco, Cool Mint, Fruit Medley, and Crème Brûlée flavors with 5% nicotine strength)



- Dose-dependent increase in acellular ROS generation by JUUL.
- Cool mint, crème brûlée, and cool cucumber showed highest.
- Significantly less ROS levels generated compared to cigarettes.

Vaping pens with different flavors and products



Nutrovape Energy:
Instant Energy, No Crash

Ingredients: Vegetable Glycerin, Propylene Glycol, Caffeine (0.3%), Theanine, L-Theanine, and a proprietary blend of flavorings.

Nutrovape's Inhalable Energy Aid is a natural blend of caffeine, theanine, and amino acids that will invigorate your morning, leaving you feeling refreshed and alert throughout your entire workday. Nutrovape Energy provides you with instant energy for the days ahead with no crash - and it all fits right in your pocket! Improve mental performance, concentration, and focus, while combating fatigue.

How Should I Use My Nutrovape Energy?
Nutrovape Energy is intended for use whenever you need that extra boost to keep you going. DO NOT use Nutrovape Energy when you are attempting to go to sleep. Please do not exceed more than 20 inhalations in an hour.




Nutrovape Sleep:
Sleep Easily, Wake Up Refreshed

Ingredients: Vegetable Glycerin, Propylene Glycol, Melatonin, L-Theanine, natural passionflower extract and natural chamomile extract, and a proprietary blend of flavorings.

62% of American adults experience trouble either falling or staying asleep multiple nights per week. Nutrovape Inhalable Sleep Aid is a blend of Melatonin, L-Theanine, Natural Passionflower, and Natural Chamomile that will help you relax and sleep soundly throughout the night - with no morning hangover. A single Nutrovape should last you approximately a month, providing you with restful nights of sleep for the weeks ahead.

How Should I Use My Nutrovape Sleep?
Take 5 inhalations before you are ready to go to bed. Nutrovape is intended for use when you are ready to relax, unwind, and go to sleep. DO NOT operate heavy machinery while using Nutrovape Sleep.




Nutrovape Focus

Ingredients: Vegetable Glycerin, Propylene Glycol, Guarana Extract, Theobromine, L-Theanine, L-Theanine, Vitamin B-12.

It's easy to get overwhelmed, find yourself rushing from one thing to the next, and lose focus! With Nutrovape Focus your body is provided with the right natural ingredients and supplements that boost your ability to concentrate without the jittery feeling. Nutrovape Focus is a natural, invigorating blend of Guarana Extract, Theobromine, L-Theanine, L-Theanine, and Vitamin B-12. Each portable device gives you inhalations of sweet peppermint flavoring, so whether you are studying, at work, or just need to focus it will provide you the motivation to combat anything life throws your way!

How should I use my Nutrovape Focus?
Use whenever needed to be focused and alert. Do not exceed more than 20 servings in any given hour.

New Products




Nutrovape Recover:
Hangovers Are Over

Ingredients: Vegetable Glycerin, Propylene Glycol, Pickle Pear, Milk Thistle, Green Tea, L-Theanine, Glutathione, Sarsaparilla, and Vitamin B-12 and a proprietary blend of flavorings.

Nutrovape Recover is a natural blend of Pickle Pear, Milk Thistle, Green Tea, L-Theanine, Glutathione, Lutein, Root, and Vitamin B-12 that will give you a fun taste of vanilla, licorice that pairs well with almost everything! Aids in reducing hangover symptoms.

How Should I Use My Nutrovape Recover?
Adults ages 21 and over. Take 10 inhalations before your first alcoholic beverage. Continue to use throughout alcohol consumption. Works best when combined with 2 glasses of water after your last alcoholic beverage.



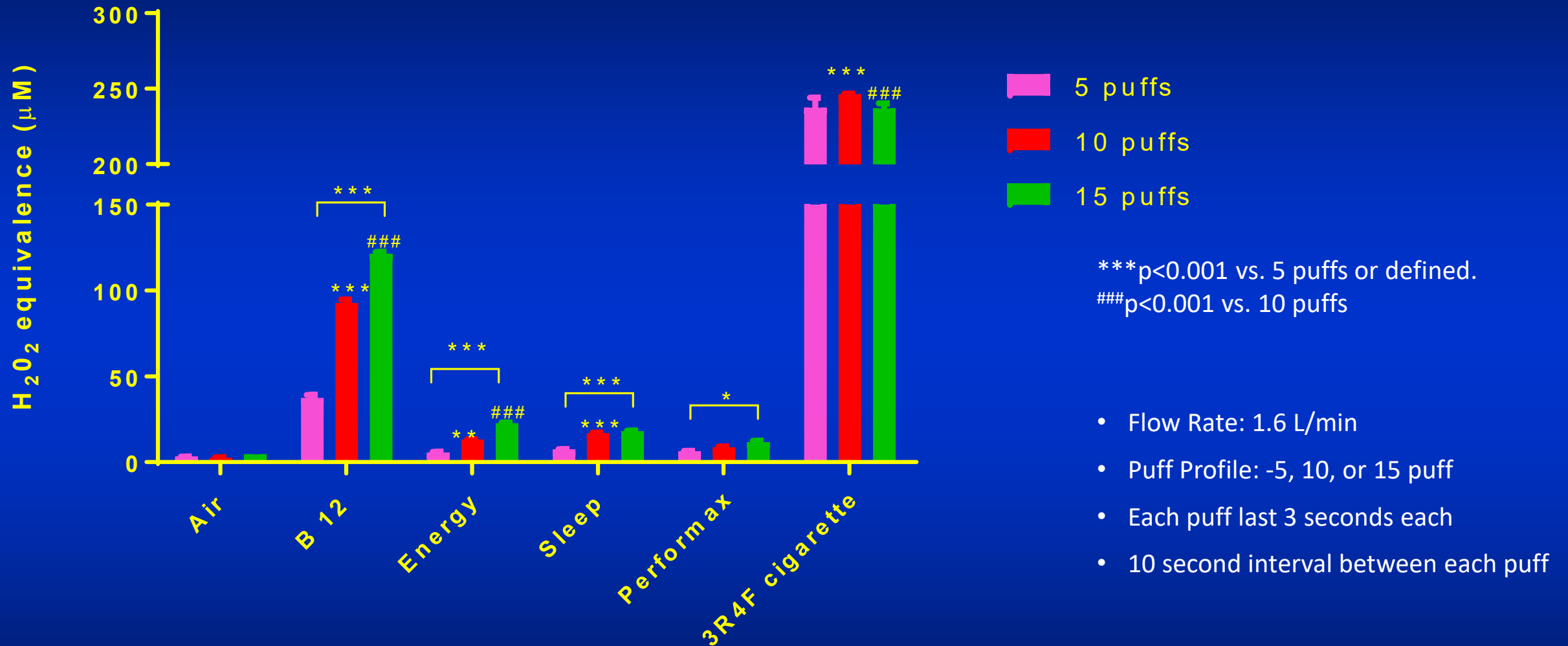
[Vitamin vaping clip](#)

Vaping pen ingredients

- VitaminVape B-12
 - Composed of vitamin B-12
- Nutrovape Energy
 - Vegetable Glycerin, Propylene Glycol, Caffeine, L-Theanine, Vitamin B-12, Natural and Artificial Flavorings
- Nutrovape Sleep
 - Vegetable Glycerin, Propylene Glycol, Melatonin, Chamomile Extract, Passion Flower Extract, L-Theanine, Natural and Artificial Flavorings
- Nutrovape Performax
 - Vegetable Glycerin, Propylene Glycol, Horny Goat Weed Extract, Passion Flower Extract, Kava Kava Root Extract, Longjack Extract (Tongkat Alie), Tribulus Terrestris Extract, Guarana Extract, Ginseng Extract, Maca Extract, L-Theanine, Natural and Artificial Flavorings

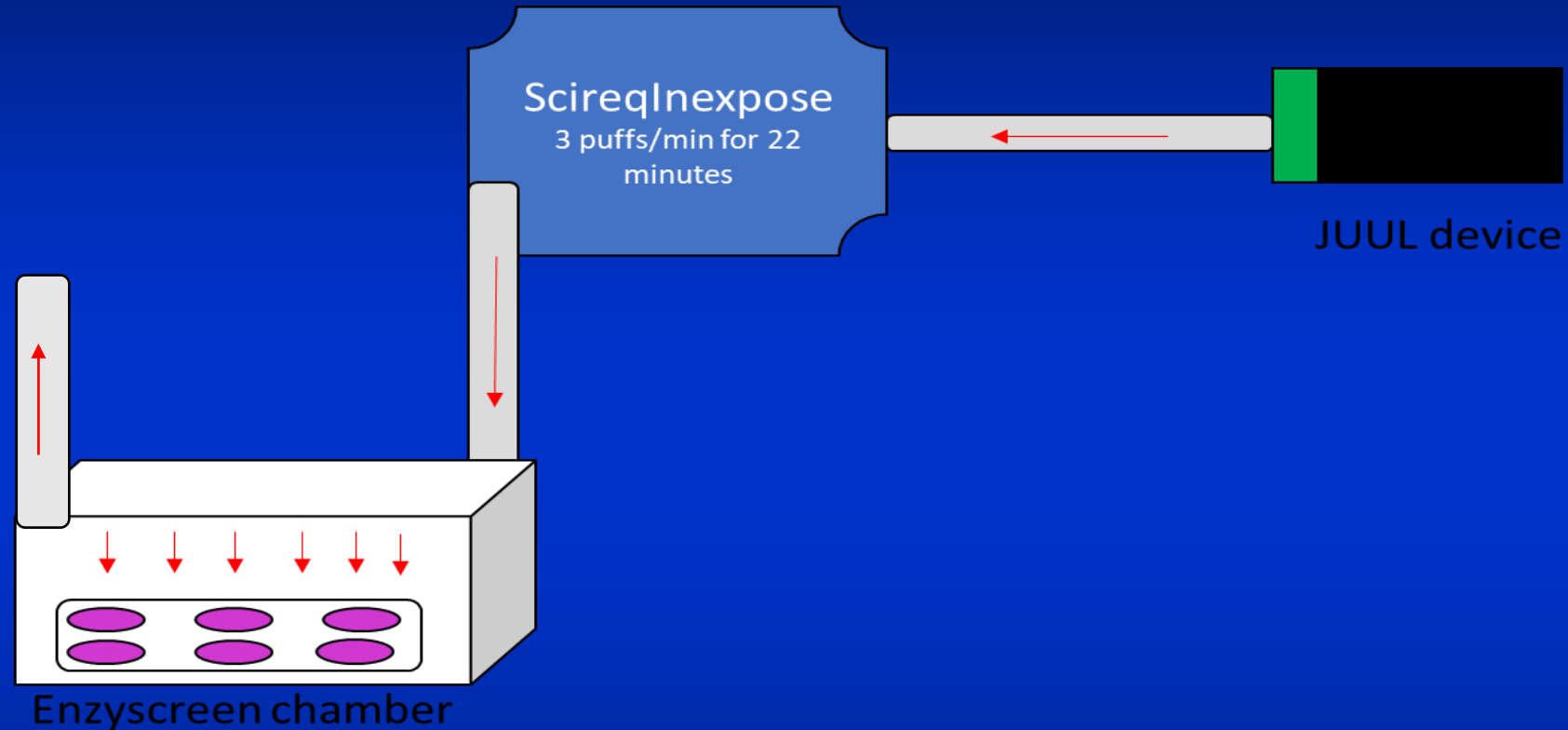


Acellular ROS generated by vape pens



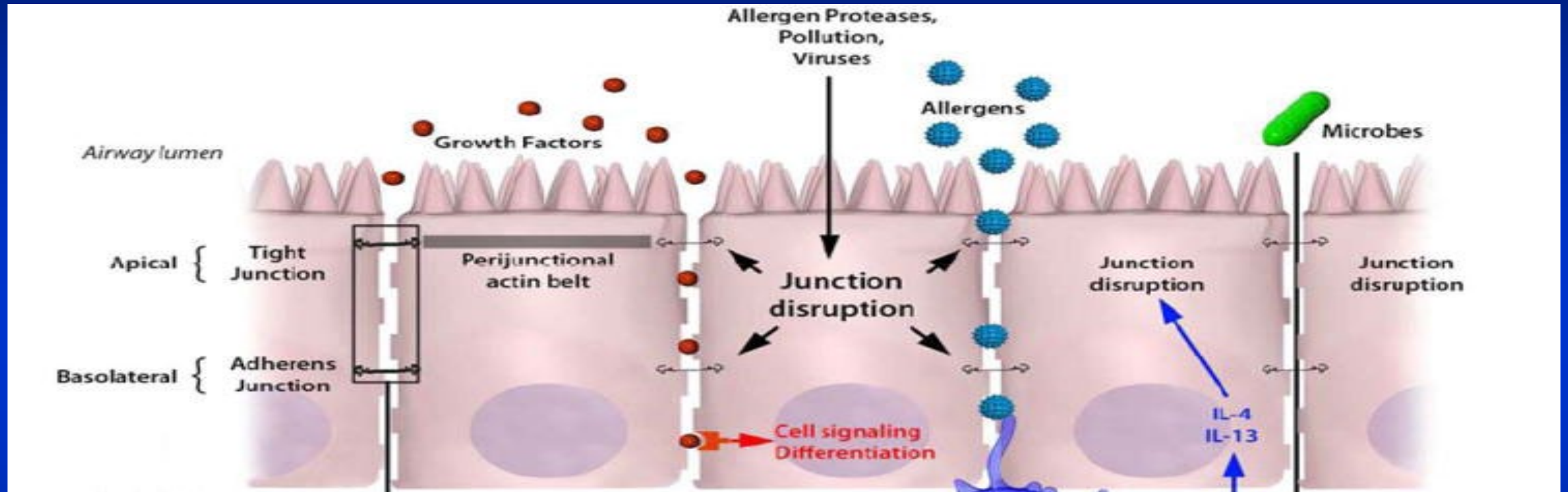
- Dose-dependent increase in acellular ROS generation by vaping pens.
- B12 showed highest approaching ROS levels generated by conventional cigarettes
- Energy, sleep, and performax showed significantly less ROS levels generated compared to cigarettes.

In vitro JUUL aerosol exposure setup



A session of exposure was defined as 3 puffs per minute for 22 minutes. Let the cells be exposed for 8 more minutes in the chamber (total 30 minutes of JUUL exposure per session).

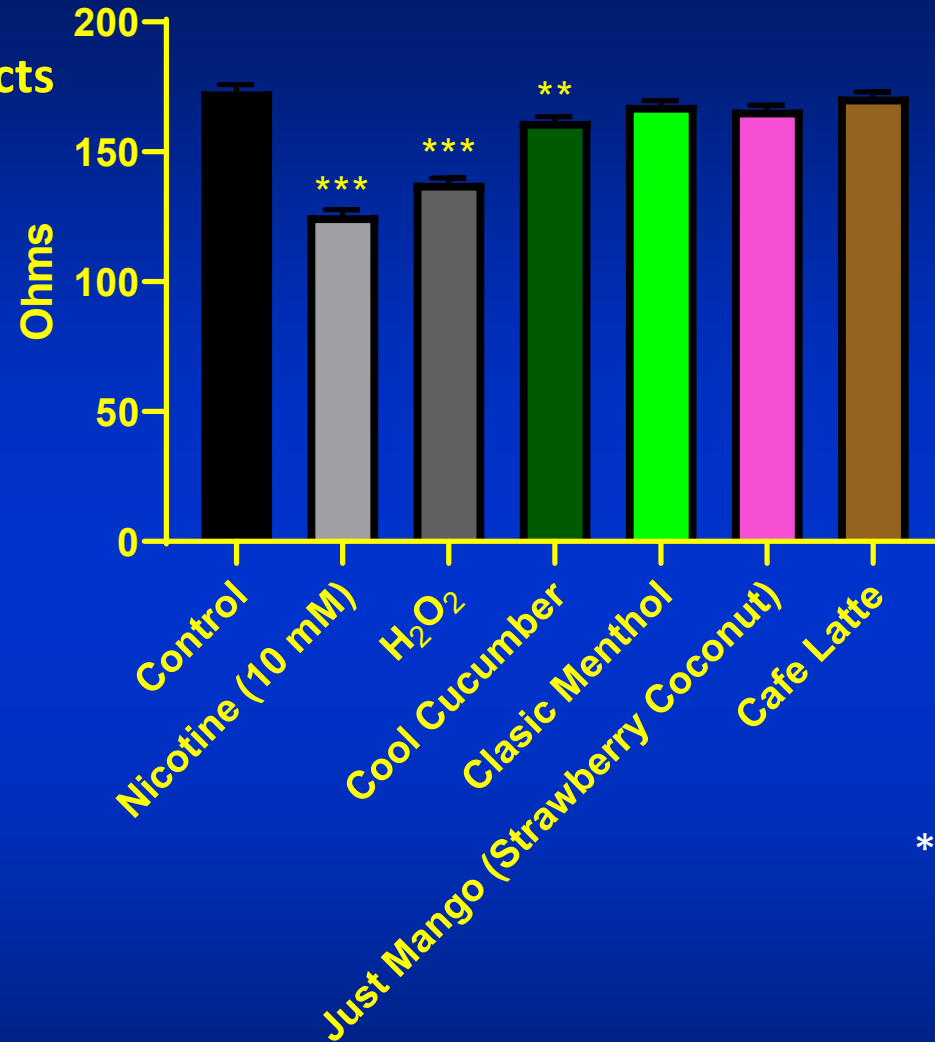
Exposure and epithelial junction disruption



Adapted from *J Allergy Clin Immunol.* *J Allergy Clin Immunol.* 2014; 134(3): 509–520.

Acute exposure to Cool Cucumber JUUL flavor impairs barrier function

Crème Brûlée flavored aerosol affects barrier function

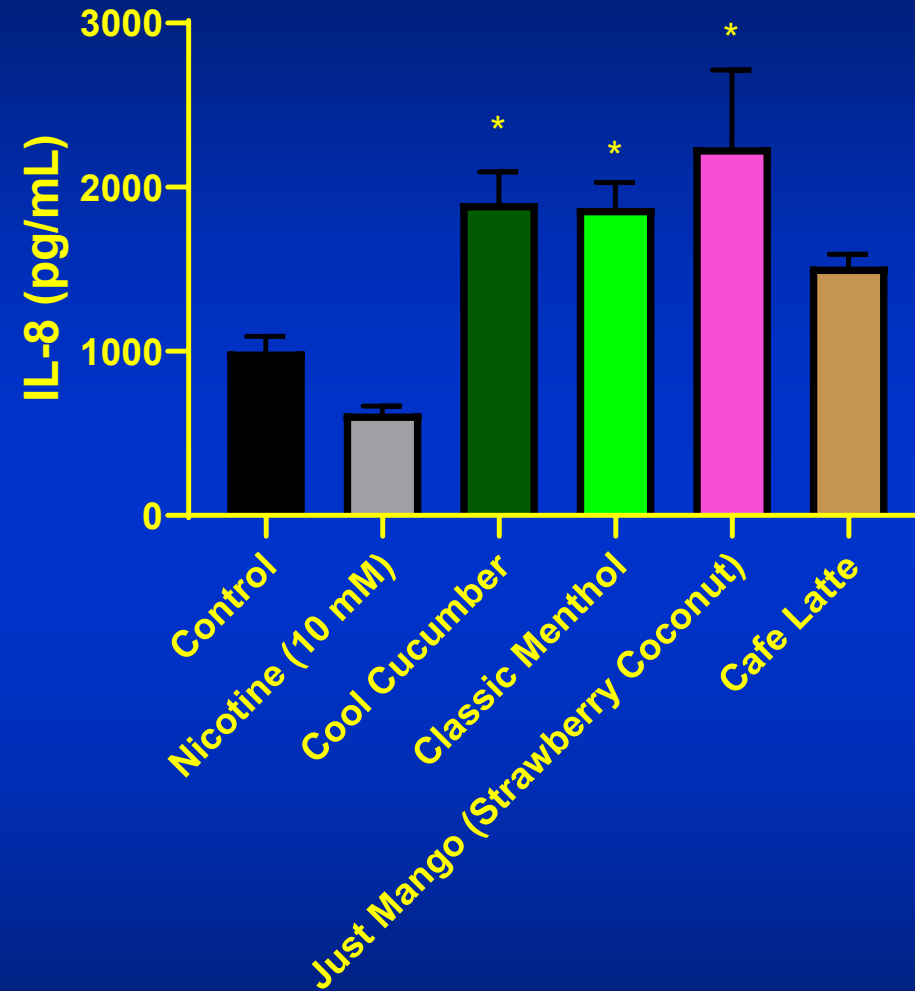


N=3-12 per group

p<0.01, *p<0.001 vs. Control

16-HBE cells grown in 24-well transwell plates exposed to **three-sessions (each 66 puffs)** of JUUL with **equal intervals of 12 hours between sessions**. After the final exposure, the resistance was measured by EVOM2 device.

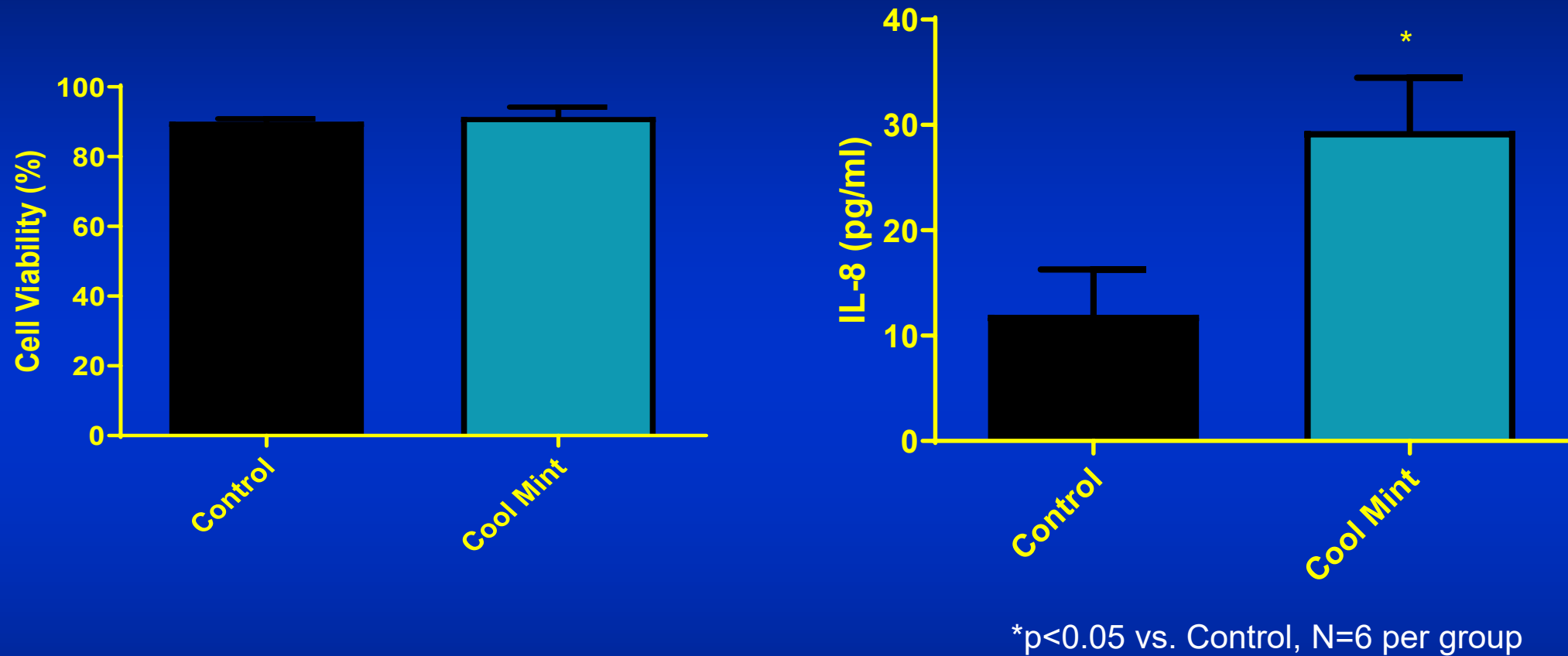
Acute exposure to JUUL aerosols elicits an inflammatory response in 16-HBE bronchial epithelial cells



*p<0.05 vs. Control (N=3)

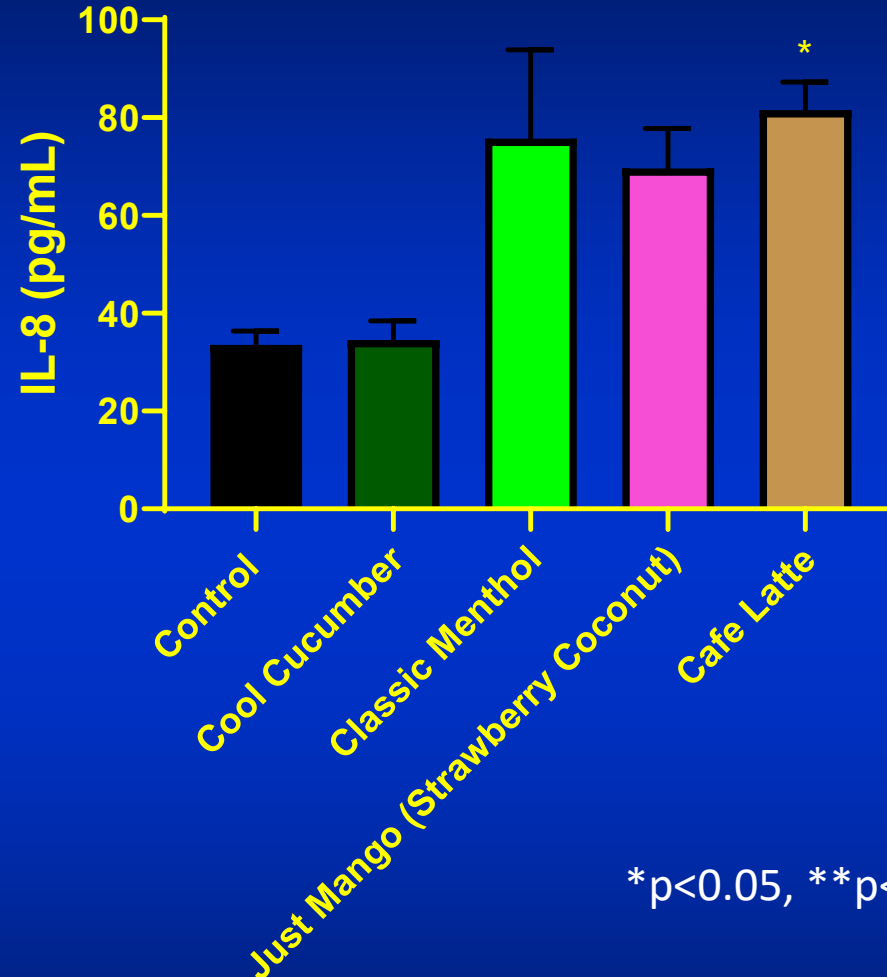
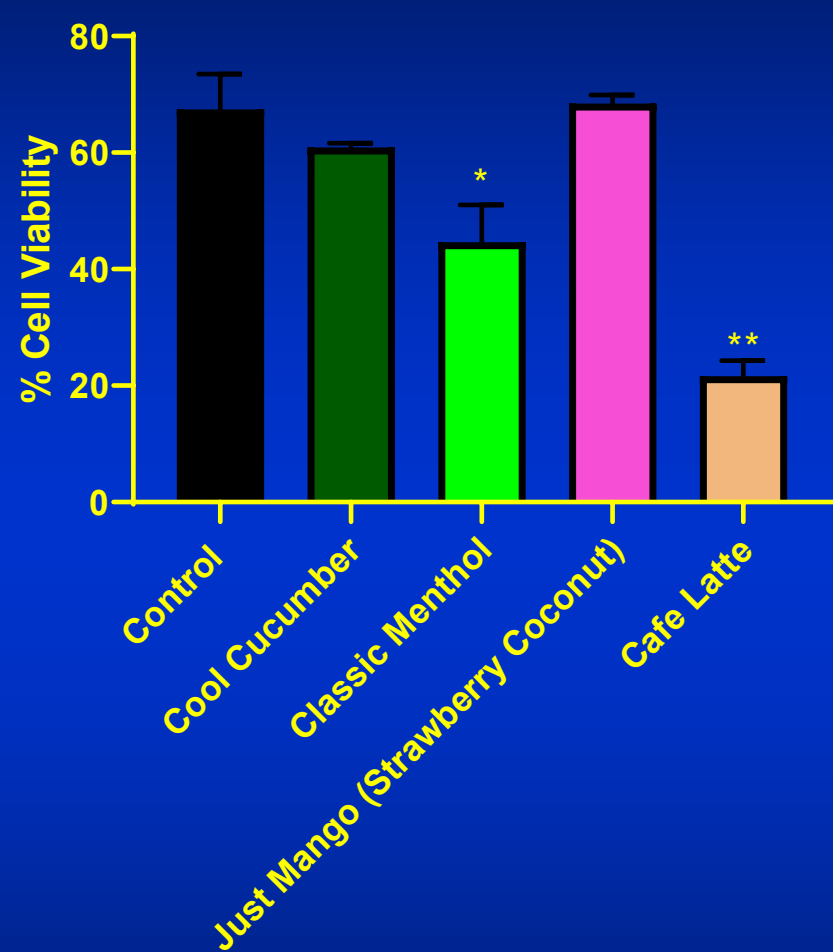
16-HBE cells grown in 24-well transwell plates exposed to three-sessions of JUUL aerosol with equal intervals between sessions. After the last exposure, IL-8 was measured in conditioned media

Inflammatory response by a single Cool Mint flavored JUUL session in bronchial epithelial Beas2b cells



BEAS 2B cells were grown in 6 well plates and exposed to a **single session (22 puffs)** of JUUL aerosol with minimum media during exposure. 24-hours after incubation the conditioned media was used for IL-8 analysis and cell viability was measured by AO/PI staining.

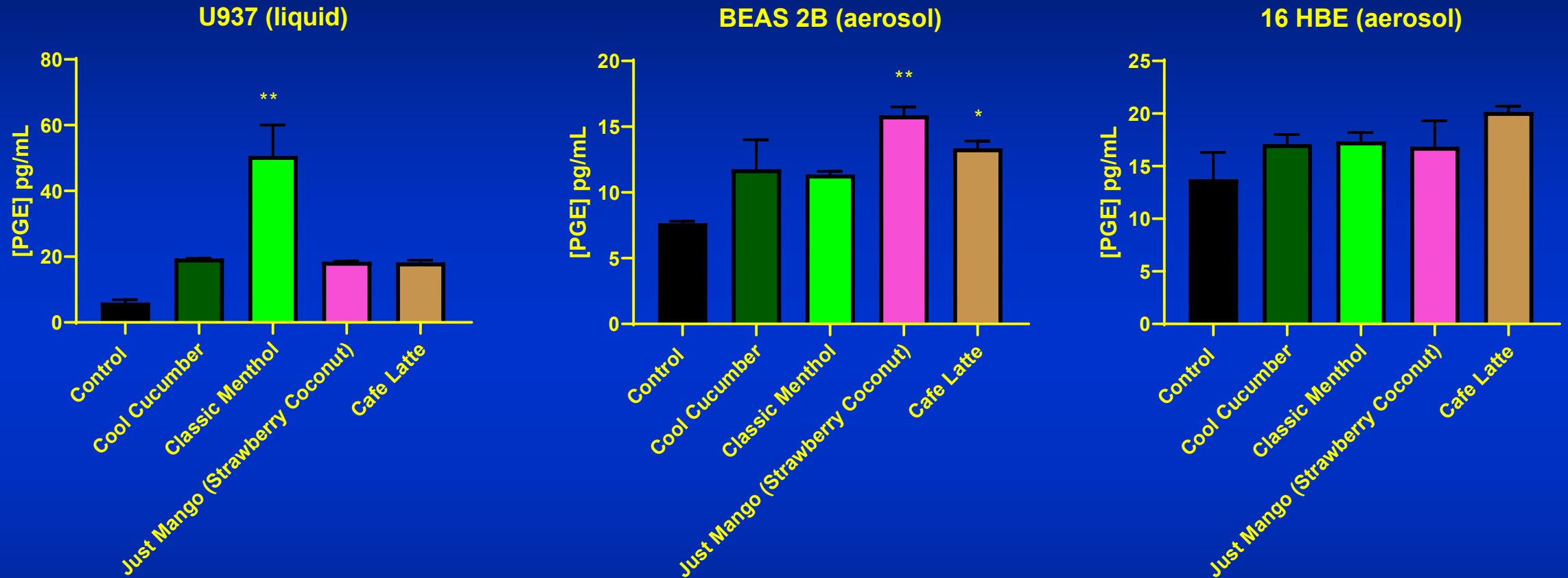
JUUL flavors induce cytotoxicity and inflammation in monocytes



*p<0.05, **p<0.01 vs. Control
N=2/group

U937 cells were cultured in a 12-well plate in complete medium. Serum deprived for 24 hours and treated with 0.5% JUUL flavors by well-volume. 24 hours later the cell viability was assessed by AO/PI staining and the conditioned media was used for cytokine assessment.

JUUL flavors differentially induce PGE2 secretion in different cells



*p<0.05, **p<0.01 vs. respective control. (N=2/per group)

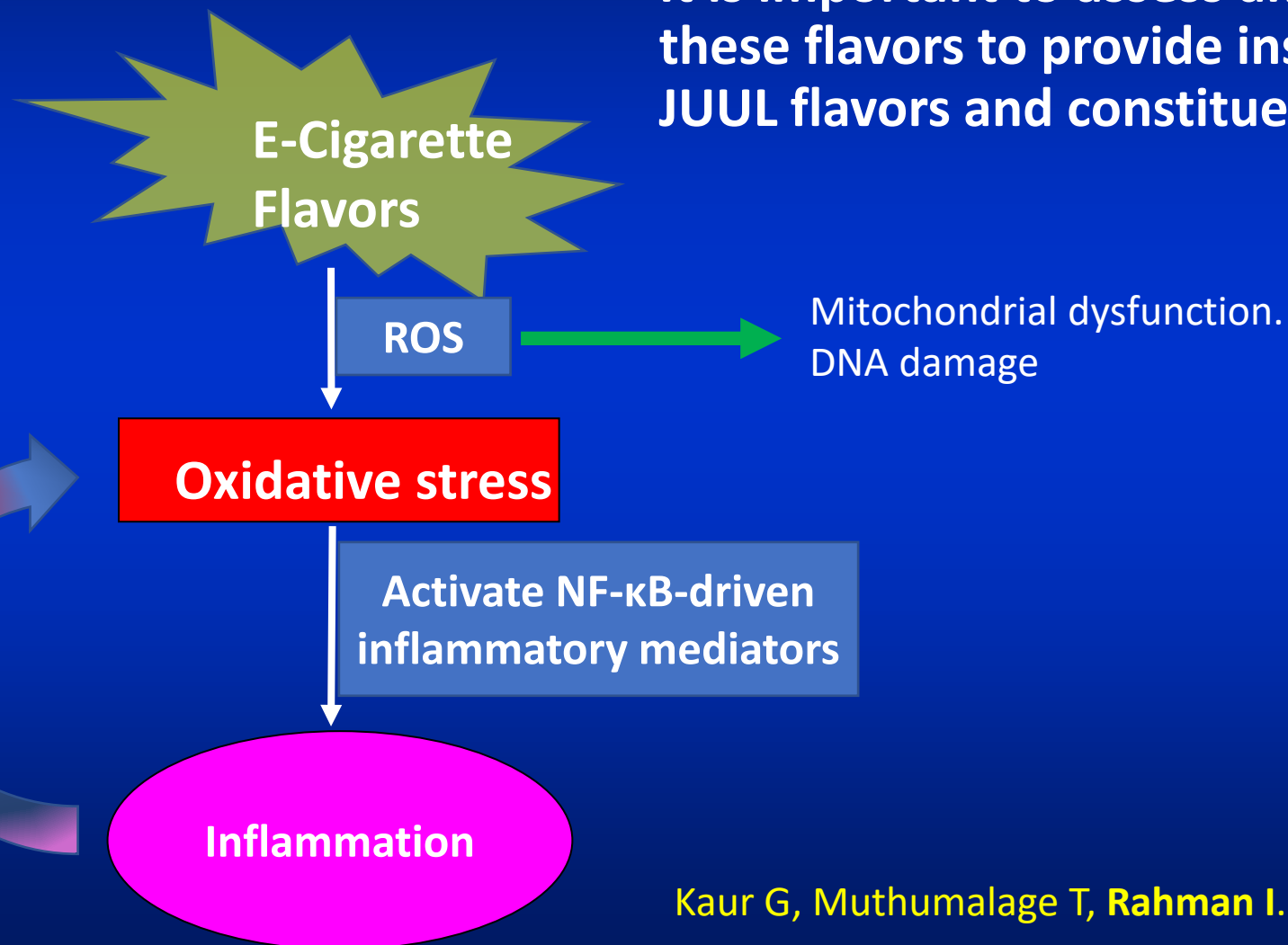
U937, BEAS-2B, and 16-HBE cells were exposed to JUUL flavors (liquid or aerosols) in serum deprived medium. Prostaglandin E2 was measured in conditioned media.

Summary

- JUUL flavors generate ROS (less than CS)
- JUUL Vitamin B12 e-vape pen generate more ROS vs energy and sleep pen
- JUUL flavors (crème brulee, cool cucumber) caused epithelial barrier dysfunction.
- JUUL flavors induced cell type dependent differential inflammatory response.
 - IL-8 levels were elevated by cool mint, cool cucumber, classic menthol, just mango (strawberry coconut), and café latte.
 - PGE2 levels were elevated by classic menthol and just mango (strawberry coconut)
 - IL-6 levels were elevated by classic menthol.

Conclusions

- JUUL flavors induce an inflammatory response mediated by reactive oxygen species.
- It is important to assess and quantify the chemicals imparting these flavors to provide insights into appropriate regulation of JUUL flavors and constituents.



Acknowledgements

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