Evaluating the Bioenergetic Effects of Flavored Electronic Cigarettes on Respiratory Immune Cells

E-cigarette liquids or "vaping" liquids are complex chemical mixtures; therefore, users are exposed to inhaled aerosols of varying chemical composition. These ingredients and mixtures are the focus of research now underway to evaluate the health effects of e-cigarettes that extend beyond those of nicotine, with a focus on the respiratory system. This data interpretation activity enables students to analyze experimental data from studies assessing the impact of e-cigarette flavorings on the structure and function of the respiratory innate immune system and features the research of toxicologists at the University of North Carolina at Chapel Hill. **Students learn about the experimental models and technologies being used to investigate the health effects of flavored e-cigarettes by interacting with published scientific data showing how flavored e-liquids and inhaled aerosols derived from these liquids impair cellular functions, such as <u>ATP generation that drives ciliary beating in respiratory epithelial cells</u>.**

This activity features data from the following peer-reviewed articles:

Clapp P.W., Lavrich K.S., van Heusden C.A., Lazarowski E.R., Carson J.L., & Jaspers I. (2019). Cinnamaldehyde in flavored e-cigarette liquids temporarily suppresses bronchial epithelial cell ciliary motility by dysregulation of mitochondrial function. *American Journal of Physiology-Lung Cellular and Molecular Physiology.* 316(3), L470-L486. https://doi.org/10.1152/ajplung.00304.2018

Hickman E., Herrera C.A., Jaspers, I. (2019). Common E-Cigarette Flavoring Chemicals Impair Neutrophil Phagocytosis and Oxidative Burst. *Chem Res Toxicol*. 2019 Jun 17;32(6):982-985. https://doi.org/10.1021/acs.chemrestox.9b00171

Learning Objectives

Upon completion of this lesson, students will be able to:

- Describe the components of vaping liquids and inhaled e-cigarette aerosols
- Describe the components of the innate immune system (respiratory epithelial cells + white blood cells) in the lungs and explain how some flavoring agents impair this system
- Explain how scientists are studying how flavoring agents in inhaled aerosols disrupt homeostasis through impairment of cellular metabolism
- Interpret published scientific data (graphs and images) to assess how some flavorings impair cellular functions, such as ATP generation that drives ciliary beating
- Construct explanations of experimental results using evidence from published scientific data (graphs and images)

Materials

- Companion Slide Set (Google slides); **click here to access the slide set**
  *This is a google set that you can **customize to support your instructional goals**.*
- Student worksheet (included at end of this guide), either digitally or in print (double sided), one per student
- Guiding question slides (one data set + data interpretation prompts) either digitally or in print (Note: color copies are critical for guiding question 3), at least one set per group

Background

Few consumer products have evolved as an environmental health concern as rapidly as electronic cigarettes or e-cigarettes. Officially known as “electronic nicotine delivery systems” or “ENDS”, these devices deliver nicotine, flavorings, and other additives to users through an inhaled aerosol, in a process known as vaping. Among youth, e-cigarettes have been the most commonly used tobacco product since 2014, and the use of these devices skyrocketed in 2017 with the advent of sleek and discreet ENDS devices like JUUL (Gentzke, et al., 2019; Cullen et al., 2018). JUUL’s rise in popularity among youth led the US Food and Drug Administration and the US Surgeon General to declare youth e-cigarette use an epidemic in 2018 (US DHHS, 2018). According to the 2020 National Youth Tobacco Survey, more than 3.5 million youth in the United States were e-cigarette users, including 19.6% of high schoolers (Wang et al., 2020). Results also revealed that among high school students who exclusively used e-cigarettes, 82.9% used flavored e-cigarettes, with fruit, menthol or mint, and other sweet flavors being most commonly reported flavors (Wang et al., 2020). Results also revealed that among high school students who exclusively used e-cigarettes, 82.9% used flavored e-cigarettes, with fruit, menthol or mint, and other sweet flavors being most commonly reported flavors (Wang et al., 2020). With most flavored e-cigarettes also containing nicotine, and with nicotine exposure during adolescence known to be harmful to the developing brain, there is a concern not only about nicotine addiction among youth but also about research findings that indicate these youth are more likely to smoke combustible cigarettes in the future (NASEM, 2018). Thus, **e-cigarettes provide a timely and relevant context in which students can explore and refine their knowledge of fundamental biology concepts while communicating risk to a vulnerable population**.

This rise in e-cigarette use and in the frequency of use among youth is not only concerning because of exposure to nicotine but also because **e-liquids and aerosols derived from e-liquids contain flavoring agents and a variety of chemicals (such as formaldehyde and benzene) that can harm respiratory health**. For example, scientists have learned that the activity of leukocytes (white blood cells) commonly found in the respiratory mucosa, such as natural killer cells, neutrophils, and macrophages can be modified by exposure to e-liquids (Clapp et al. 2017). Furthermore, research is revealing that some flavoring agents, such as cinnamaldehyde, impair mitochondrial function in respiratory epithelial cells, which results in decreased ATP production, decreased cilia motility and reduced ability to keep the lungs clear of mucus (Clapp et al. 2019). Research in animals has also shown that e-cigarette exposure can impair bacterial and viral responses to common pathogens (Madison et al. 2019, Miyashita et al. 2018, Hwang et al. 2016). These research findings are especially concerning in light of SARS-CoV-2, which targets the respiratory system and causes the disease known as COVID-19.

Recognizing that flavored e-cigarettes were contributing to the teen vaping epidemic, the US FDA banned the sale of all flavored “cartridge-based ENDS”, except for those containing menthol and tobacco flavoring agents effective January 2, 2020. However, the ban excluded disposable e-cigarettes as well as flavored e-liquids that can be used in refillable devices, also referred to as “tank systems” or “box mods.” **Disposable e-cigarette devices are now the most commonly reported e-cigarette device among youth**; popular brands among youth include

Puff Bar, Vuse, JUUL, SMOK, NJOY, Hyde, and blu (Park-Lee et al., 2022). Disposables, such as Puff Bar, can include e-liquids that contain more nicotine than JUUL and may have additional chemical components that augment their toxicity (Kaplan, 2020; Omaiye et al., 2021).

Regulatory Update: In August 2016, the FDA finalized a rule known as “the deeming rule” to regulate all tobacco products, including e-cigarettes. Companies manufacturing e-liquids had to submit product applications for review by Sept. 9, 2020. Some e-liquid manufacturers responded by switching to the use of synthetic nicotine or “tobacco-free nicotine” to bypass FDA regulation (Jordt, 2021). However, on March 15, 2022, the FDA extended its jurisdiction to include products “containing nicotine from any source,” not just nicotine derived from tobacco. As of March 2023, the FDA has received applications for more than 26 million deemed products and authorized “23 tobacco-flavored e-cigarette products and devices, which are the only e-cigarettes that currently may be lawfully sold or distributed in the U.S.” (FDA, 2023). The FDA is next expected to finalize its ban on menthol flavored tobacco products, which are of concern because of their appeal to youth given that menthol masks the flavor of tobacco.

The evolution of e-cigarette regulation and the tensions that arise when regulating these products for smoking cessation in adults while preventing nicotine addiction among youth is ripe for class discussion. You and your students can keep up with current and future FDA regulations by visiting https://www.fda.gov/news-events/fda-newsroom/press-announcements or https://www.fda.gov/tobacco-products.

While the long-term health effects of flavored e-cigarettes are unknown due to the relatively recent entry of these devices into society, **research emerging from UNC-Chapel Hill and other institutions suggests that flavored e-liquids convey health risks beyond those associated with nicotine use.** Recent research from the Jaspers lab has shown that cinnamon-flavored e-liquids and aerosols, and even just the flavoring chemical cinnamaldehyde alone, can impair ciliary beating in airway epithelial cells. Cinnamaldehyde decreases mitochondrial function in these cells, therefore decreasing ATP production, and ATP is needed for the cilia to beat. This research was conducted using cells that grow outside of the body. If this same effect occurs in people who vape cinnamaldehyde-containing e-liquids, their lungs might not be able to clear mucus and anything trapped in the mucus very well, which could potentially increase the risk for respiratory infections.

In this activity, students learn about the experimental models and technologies being used to investigate the health effects of flavored e-cigarettes by interacting with published scientific data showing how flavored e-liquids and inhaled aerosols derived from these liquids impair cellular functions, such as ATP generation that drives ciliary beating in respiratory epithelial cells.

Overview of the innate immune system in lungs

The innate immune system in the lungs is responsible for protecting the body from inhaled pathogens (e.g., bacteria and viruses) and chemical pollutants. The innate immune system in your lungs is made up of **two primary components**:

1) **The mucociliary escalator.** The epithelial cells that line your airways produce mucus and have cilia along the airway side of the cell. The cilia beat this mucus along with anything trapped in it up and out of the airway. This mucus is then cleared through either swallowing or coughing.

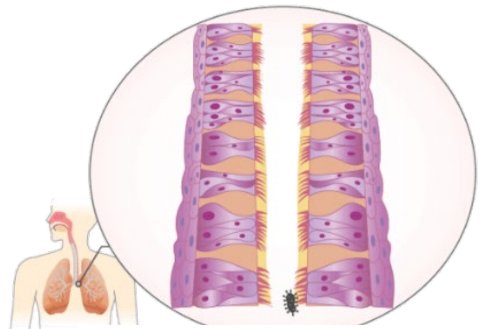

Image Credit: National Institutes of Health

2) **White blood cells (macrophages, neutrophils, natural killer cells).** Macrophages are found in the lungs, and neutrophils can be attracted to the lungs from the blood in response to inflammation. These cells can kill pathogens and clear debris through a process called **phagocytosis.** They can also signal other white blood cells to the site of infection or inflammation. In addition, neutrophils can release DNA and other molecules to the outside of the cell to bind and trap pathogens like bacteria. This release of extracellular DNA is known as a **neutrophil extracellular trap (NET)**. Natural killer (NK) cells are another type of white blood cell that patrol the body and identify and kill abnormal cells, including diseased cells.

Overview of e-liquids

Flavored e-liquids are chemical mixtures that, in addition to flavorings, include the base ingredients **propylene glycol (PG)** and/or **vegetable glycerin (VG)** and either nicotine or nicotine salts at concentrations ranging from 0% to 7% (which is very high).

Flavorings are chemicals that give flavored e-liquids their predominant taste and smell. Examples include cinnamaldehyde (spicy or cinnamon), benzaldehyde (cherry or almond), vanillin (vanilla), and ethyl vanillin (vanilla). However, many e-liquids include combinations of two or more flavors. PubChem (https://pubchem.ncbi.nlm.nih.gov/) is a great resource for looking up chemical formulas, molecular weights, structures, etc. for specific flavorings (or really any other chemical) you might be interested in having your class study. *Note: "Sini-cide" is a cinnamon flavored e-liquid referenced in this activity.*

E-liquids become aerosolized during vaping. The "vapor" generated by e-cigarettes is a complex mixture of particles and gases derived from aerosolizing e-liquid. An **aerosol** is a suspension of fine solid particles or liquid droplets in air or another gas. Due to their small size, these particles can be inhaled deep into lungs. Furthermore, when e-liquids are heated and aerosolized by e-cigarettes, the chemicals can be broken down or changed during the heating and aerosolizing process. How much the chemicals change depends on the type of e-cigarette and the device settings.

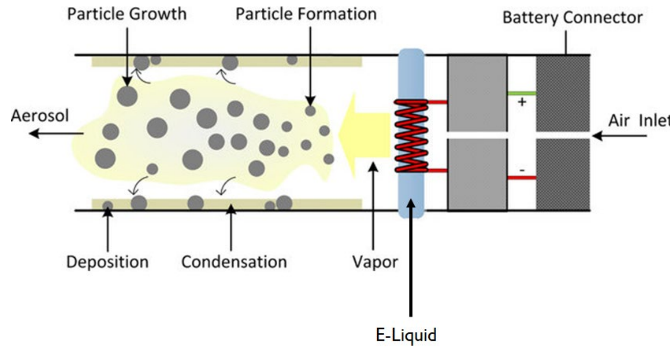

Image credit: Talih et al. November 2016 Aerosol Science and Technology

Experimental design and key toxicology concepts

Dose refers to how much of a chemical enters the body either through the nose, mouth or skin.

Dose response is the concept in toxicology that with increasing dose, or amount, of a chemical, you will see an increasing effect of that chemical on whatever function you are measuring.

In this activity, two **control groups** are used:

1. **Media control** – this refers to cell cultures grown under normal growth conditions, with nothing extra added to the cells. Cells are taken from human subjects through blood draws or airway sampling and then placed in plastic culture dishes. In these dishes, they are covered by a liquid mixture called media that provides nutrients and a suitable growth environment for the cells. Treatments, like cinnamaldehyde, can also be added to the media. When a treatment is not added, that is called the media control.
2. **PG/VG vehicle control** - this refers to cell cultures being treated with PG/VG only to determine if there were any effects of the base liquid without the flavoring components and to determine whether effects were caused by the base liquid alone or just the flavoring components.

Statistical significance is indicated by asterisks (*) above specific bars on a bar graph. The number of asterisks indicates the extent of statistical significance. Statistical significance is important because it tells researchers that their results are likely real effects of a treatment or experiment versus being random effects due to variability in the experiment. P values indicate whether a comparison is statistically significantly different; a $P < 0.05$ is generally considered statistically significant.

Activity Procedure
This activity utilizes the 5E instructional model and can be conducted as a jigsaw activity, with a small group of students evaluating one or more guiding question(s), or it can be adapted into worksheets, lecture-based discussion, or stations.

Prior to conducting this activity:

1) Introduce students to e-cigarettes, e-liquids, components of e-liquids and the basic concepts of toxicology covered in the background section *(See companion slide set).*
2) Review the function of cellular respiration and the role of glycolysis, mitochondria and aerobic respiration in ATP production.

1. **Engage:** Show the time series of short videos provided in the Companion slide set that provide visual evidence that Sini-cide (cinnamaldehyde) alters ciliary beating on respiratory epithelial cells. Use this to prompt students to think about what might be causing this biological response (reduced cilia movement). Tells students they will be exploring experimental results to learn what might be responsible for this observation.

2. **Explore | Step I:** Introduce (or review) the structure and function of the human respiratory system and the innate immune system, including the ciliated respiratory epithelial cells. Describe the experimental approach (source of cells and use of cell cultures) used by the Jaspers lab to prepare students for the data interpretation activity. *See Companion slide set for accompanying visuals.*

3. **Explore | Step 2:** Begin jigsaw activity by distributing the set of figures for each guiding question to be covered. Ask students to read their assigned question prompts and examine the accompanying figures in order to formulate the answer to their guiding question.

4. **Explain:** Students should use available evidence to answer the questions on their worksheet; you may choose to have students answer their guiding question by writing a claim-evidence-reason (CER) paragraph. Students also could make a poster or google slide presentation to summarize their findings in preparation for sharing with the class. One at a time, ask groups to report their findings to the class, summarizing the answer to their guiding question. <u>A key is provided at the end of this activity.</u> To keep the class on task, ask students to complete the summary table on their worksheet while they hear from other groups.

5**. Elaborate:** Once all groups have reported out, summarize the activity as a class and ask one or more of these culminating discussion questions:

- What broad conclusions can you make based on these data?
  - Cinnamaldehyde and cinnamaldehyde-containing e-liquids can impair cellular respiration and glycolysis, resulting in decreased epithelial cell ciliary beating and decreased neutrophil oxidative burst.

- Why did the authors use nicotine-free e-liquids in these experiments?
    - The authors were interested in the effects of the flavorings specifically and the presence of nicotine could impact the data.
    - Nicotine concentration is not always accurately reported in e-liquids, so if nicotine-containing e-liquids were used, it could still be hard to control for the concentration of nicotine.
- Based on these data, would you consider cinnamaldehyde-containing e-liquids safe to inhale?
    - Hot Cinnamon Candies, Kola, Sini-cide
- Why is it important that our respiratory cells function properly?
    - In the case of respiratory epithelial cells and cells of the innate immune system, proper function means the lungs can clear/remove pathogens (bacteria and viruses) and debris to maintain health.
- What ideas do you have for follow-up experiments to expand on the data provided here?
    - Study other flavoring chemicals and other flavors of e-liquids.
    - Figure out what the most accurate dose is to use on these cells (what dose is most like a vaper's exposure?).
    - Determine if there are certain types of people are more susceptible to these effects (age, sex, ethnicity, previous or current cigarette smoker).
    - Study other cell types or other cell functions.
    - Study how these flavored e-liquids affect immune cell function (mechanism).

6. **Evaluate**: Assess student learning by doing one or more of the following:
- Collect completed worksheets
- Ask students to summarize their responses to their assigned guiding question in writing or by creating a short presentation
- Ask students to identify a new research question based on what they have learned, develop a hypothesis and, on paper, design an experiment to test their hypothesis. Students can submit their ideas to the Jaspers lab at jasperslabunc@gmail.com

7. **Extend:** There are opportunities to **extend student learning by inviting them to read, evaluate, and communicate information** about this topic in the context of the E-cigarette or Vaping Use-Associated Lung Injury (EVALI) outbreak 2019 and of emerging evidence that vaping could impair the respiratory immune response to influenza or SARS-CoV-2.

E-cigarette or Vaping Use-Associated Lung Injury (EVALI)
According to the CDC, from August 2019 to February 2020, over 2,800 people were hospitalized and 68 died as a result of e-cigarette or vaping use-associated lung Injury (EVALI). While scientists don't know the cause of EVALI, there is evidence that vitamin E acetate used to dilute and/or thicken marijuana containing vaping liquid may have contributed to the outbreak (CDC). While this additive is safe for use in skin creams and nutritional supplements it is not safe to inhale but scientists do not know the mechanism by which it could have caused EVALI. This

finding reinforces one of the key messages of this lesson, that chemicals (such as flavorings, or vitamin E acetate) may be safe for ingestion but not safe for inhalation.

Vaping and respiratory immune response to influenza or SARS-CoV-2 (Novel Coronavirus)
Scientists do know that e-cigarette exposure can impair the body's immune response to viruses; in one recent experiment mice exposed to unflavored e-cigarette aerosol with or without nicotine for 4 months were not able to clear flu infection as well (Madison et al., 2019). In another study from the Jaspers lab, e-cigarette users, smokers, and nonsmokers were administered the nasal spray flu vaccine (a safe way to study viral responses) and their bodies' responses to the flu vaccine were monitored to determine if there were differences in antiviral response between groups. Data revealed that e-cigarette users had significantly lower levels of a flu-specific antibody (proteins that help the body remember a past viral exposure) than nonsmokers or e-cigarette users (Rebuli et al., 2021). Some scientists and doctors have speculated that e-cigarette use may make people more susceptible to COVID-19, the infection caused by SARS-CoV-2 (Novel Coronavirus), though it is too soon to know if this connection exists (Lewis, 2020).

Curriculum Alignment

Advanced Placement Biology	
Science Practices	**Big Ideas**
This activity utilizes all six science practices: • Concept Explanation • Visual Representations • Questions and Methods • Representing and Describing Data • Statistical Tests and Data Analysis • Argumentation	• **BIG IDEA 2: ENERGETICS (ENE)** *Enduring understanding 2.D, 2.E* • **BIG IDEA 3: INFORMATION STORAGE AND TRANSMISSION (IST)** *Enduring understanding 3.B* • **BIG IDEA 4: SYSTEMS INTERACTIONS (SYI)** *Enduring understanding 4.C*
Relevant Units: **Unit 1: Chemistry of Life** (Biological Macromolecules (Proteins)) **Unit 2: Cell Structure and Function** **Unit 3: Cellular Energetics** (Enzymes)	

Next Generation Science Standards		
Disciplinary Core Ideas	**Relevant Science & Engineering Practices**	**Relevant Cross Cutting Concepts**
LS1: From Molecules to Organisms: Structures and Processes **LS3:** Heredity: Inheritance and Variation of Traits	• Asking questions and defining problems • Analyzing and interpreting data • Developing and using models • Constructing explanations • Obtaining, evaluating, and communicating information	• Patterns • Cause and effect: mechanism and explanation • Scale, proportion, and quantity • Systems and system models • Structure and Function • Stability and change

Authors

Dana Brown Haine, MS, K-12 Science Education Manager, Center for Public Engagement with Science, UNC-Chapel Hill Institute for the Environment
Elise Hickman, PhD, Curriculum in Toxicology & Environmental Medicine and Department of Environmental Sciences & Engineering, UNC-Chapel Hill

For inquiries about this lesson please contact Dana Haine at dhaine@unc.edu

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Resources and other papers that might have interesting data for your class to consider:

Other Recent Jaspers Lab Research:

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Chemical Characterization of E-Cigarettes:

Behar, R. Z., Luo, W., McWhirter, K. J., Pankow, J. F., and Talbot, P. (2018). Analytical and toxicological evaluation of flavor chemicals in electronic cigarette refill fluids. *Sci. Rep. 8*, 8288. https://doi.org/10.1038/s41598-018-25575-6

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Review of Respiratory Effects of E-Cigarettes:

Gotts J.E., Jordt S.E., McConnell R., Tarran R. (2019). What are the respiratory effects of e-cigarettes? *BMJ* 366:l5275. https://doi.org/10.1136/bmj.l5275

Activity Key

Guiding Question 1: Do cinnamon-flavored e-liquids and aerosols impair ciliary beating in respiratory epithelial cells?

Group 1 will evaluate the effects of cinnamon-flavored e-liquids and aerosols on ciliary function. Figure A shows the effects of 1% dilutions of flavored e-liquids (left side) on ciliary beat frequency and the percent of the imaged area that had cilia in motion (active area). Figure B shows the effects of vaped Sini-cide on ciliary beat frequency and the percent of the imaged area that had cilia in motion (active area).

Review Questions:

1. What is the mucociliary escalator?

The cells that line your airways have cilia on the airway side of the cell. There is a layer of mucous on top of the cilia, and the cilia beat this mucous and anything trapped in it up and out of the airway. This is called the mucociliary escalator.

2. Why do you think cilia beat frequency and percent active area are endpoints the researchers were interested in studying?

These endpoints are a good representation of how well the mucociliary escalator would be able to function since cilia beating is what moves the mucus up and out of the lungs.

3. Describe one consequence of impaired ciliary beat frequency in the lungs.

Impaired ciliary beat frequency in the lung could cause mucus, pathogens, and or toxicants to get stuck in the lungs.

Data Interpretation Questions:

1. Which e-liquid had the greatest effect on ciliary function? Why might this be?

Sini-cide had the greatest effect on ciliary function. This could be because it has the highest concentration of the chemical(s) that are affecting the cilia.

2. Compare and contrast the effects of Sini-cide e-liquid and aerosol. Are they the same or different? Why might this be?

Both e-liquid Sini-cide and vaped Sini-cide significantly reduced ciliary function. This could be because whatever chemical(s) in the e-liquid affect the cilia are also found in the aerosol.

Guiding Question 2: Does cinnamaldehyde impair ciliary beating in the respiratory epithelial cells?

Group 2 will evaluate the effects of cinnamaldehyde on ciliary function. These figures show the effects of different concentrations of cinnamaldehyde on ciliary beat frequency and the percent of the imaged area that had active cilia.

Review Questions:
1. What is the mucociliary escalator?
The cells that line your airways have cilia on the airway side of the cell. There is a layer of mucous on top of the cilia, and the cilia beat this mucous and anything trapped in it up and out of the airway. This is called the mucociliary escalator.

2. Why do you think cilia beat frequency and percent active area are endpoints the researchers were interested in studying?
These endpoints are a good representation of how well the mucociliary escalator would be able to function since cilia beating is what moves the mucous up and out of the lungs.

3. Describe one consequence of impaired ciliary beat frequency in the lungs.
Impaired ciliary beat frequency in the lung could cause mucous, pathogens, and or toxicants to get stuck in the lungs.

Data Interpretation Questions:
1. Which doses impaired ciliary function?
10mM and 15 mM cinnamaldehyde significantly impaired ciliary function.

2. What do you think would happen if cinnamaldehyde was inhaled as part of an e-cigarette aerosol? Why?
If cinnamaldehyde was inhaled, the mucociliary escalator could be slowed or stopped because cinnamaldehyde can decrease ciliary beating.

Guiding Question 3: Does cinnamaldehyde change how energetic mitochondria are in respiratory epithelial cells?

Group 3 will evaluate the effects of cinnamaldehyde on the activity of the mitochondria. The set of images shows effects of cinnamaldehyde on the energetic state of the mitochondria (red = highly energetic, green = not energetic) with different doses of cinnamaldehyde. The graph quantifies the differences seen in the image. The y-axis is the red/green ratio.

Review Questions:
1. What is the primary way that epithelial cells lining our airway get energy to power the beating of cilia?
ATP

Group 3, continued

2. What is mitochondrial membrane potential?
Mitochondrial membrane potential is the proton gradient created by the electron transport chain during aerobic respiration. The movement of protons from high concentration in the intermembrane space to a low concentration in the mitochondrial matrix provides the energy needed for the cell to make ATP.

3. Describe one consequence of reduced mitochondrial function in airway cells.
Reduced ATP levels, so reduced function for any process that requires ATP, such as ciliary beating.

Data Interpretation Questions:

1. Describe what changes you see in the images as the dose of cinnamaldehyde increases.
As the dose of cinnamaldehyde increases, the amount of red in the photos decreases and the amount of green increases which indicates that mitochondria are losing their membrane potential and therefore their ability to make ATP.

2. What does the decreased red/green ratio at higher doses of cinnamaldehyde mean for cellular function?
It means that the mitochondria have been impaired and therefore ATP levels will be reduced in the cell. Lower levels of ATP will reduce the ability of the cell to perform any functions that require ATP, such as ciliary beating.

Guiding Question 4: Does cinnamaldehyde impair mitochondrial function?

Group 4 will evaluate the effects of cinnamaldehyde on mitochondrial function. These graphs show the effects of increasing doses of cinnamaldehyde on basal respiration (oxygen consumption) and ATP production.

Review Questions:

1. Why is mitochondrial function an important endpoint for this study?
The mitochondria are the main cellular source of ATP, and ATP is what provides energy for ciliary beating.

2. Describe the relationship between basal respiration and ATP production.
Basal respiration is the amount of oxygen that the mitochondria are consuming to move electrons through the electron transport chain and create the proton gradient necessary for ATP Production. However, this process isn't 100% efficient -- some of the energy (protons) is lost in the process. Measuring ATP production tells us how much of the oxygen and electrons were actually turned into ATP versus lost.

Group 4, continued
3. Describe one consequence of reduced mitochondrial function in airway cells.
Reduced ATP levels, so reduced function for any process that requires ATP, such as ciliary beating.

Data Interpretation Questions:
1. What was the effect of cinnamaldehyde on mitochondrial function?
Cinnamaldehyde decreased mitochondrial function at 0.25, 0.5, and 5 mM.

2. What dose of cinnamaldehyde would you say is "non-toxic" and why?
There was no effect of 0.05 mM cinnamaldehyde, suggesting that that dose is non-toxic.

3. Do these results agree with the results that group 3 discussed and why?
Yes. Group 3's results showed that mitochondrial membrane potential is decreased, which correlates with decreased basal respiration and ATP production in this set of graphs.

Guiding Question 5: Does cinnamaldehyde impair glycolysis?

Group 5 will evaluate the effects of cinnamaldehyde on glycolysis. These graphs show the effects of increasing doses of cinnamaldehyde on glycolysis.

Review Questions:
1. Why is glycolysis an important endpoint for this study?
Glycolysis is also a source of ATP in the cell and does not require oxygen. If mitochondria aren't functioning properly, the cell can acquire ATP from glycolysis.

2. Describe the relationship between basal glycolysis and glycolytic capacity.
Basal glycolysis is glycolysis that is occurring normally in the cells. Glycolytic capacity is the maximum glycolysis that the cell can perform, typically to compensate for decreases in mitochondrial ATP production.

3. Describe one consequence of reduced glycolytic function of airway cells.
Reduced ATP levels, so reduced function for any process that requires ATP, such as ciliary beating.

Data Interpretation Questions:
1. What was the effect of cinnamaldehyde on glycolysis?
Cinnamaldehyde decreased basal glycolysis at 0.25, 0.5, and 5 mM and decreased glycolytic capacity at 0.5 and 5 mM.

2. What dose of cinnamaldehyde would you say is "non-toxic" and why?
There was no effect of 0.05 mM cinnamaldehyde, suggesting that that dose is non-toxic.

Group 5, continued
3. What is the significance of these results in the context of the results from groups 3 and 4?
These results show that glycolysis is also affected by cinnamaldehyde, meaning that glycolysis will not be able to compensate for decreases in mitochondrial function. This partially explains why cilia function was decreased.

Guiding Question 6: Does cinnamaldehyde impair neutrophil oxidative burst?

Group 6 will evaluate the effects of cinnamaldehyde on neutrophil oxidative burst. One way that neutrophils kill bacteria is through turning oxygen into a more reactive form of oxygen that can act as an antimicrobial agent. This is called an oxidative burst. Neutrophils rely on glycolysis to generate the energy (ATP) needed for oxidative burst. These graphs show the effects of increasing doses of cinnamaldehyde on neutrophil oxidative burst and glycolysis (which is the source of ATP for oxidative burst).

Review Questions:
1. Why are neutrophils an important cell type to study in the lung?
Neutrophils are part of the innate immune system that protects your body from inhaled pathogens.

2. What is oxidative burst? Where do neutrophils get the energy for oxidative burst?
Oxidative burst is when neutrophils turn oxygen into more reactive/toxic types of oxygen that can then kill bacteria (acting as an antimicrobial agent). Neutrophils get the energy (ATP) to do this from glycolysis.

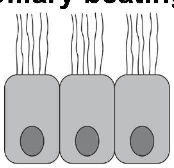
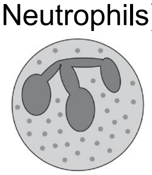
3. Describe one consequence of decreased oxidative burst.
Neutrophils would not be able to fight invading pathogens as well, so the body would not be as well protected from disease.

Data Interpretation Questions:
1. What was the effect of cinnamaldehyde on oxidative burst (antimicrobial activity)?
Cinnamaldehyde decreased oxidative burst at 0.25-2mM.

2. What was the effect of cinnamaldehyde on glycolysis?
Cinnamaldehyde decreased glycolysis at 0.25-2mM.

Summary of Data: ***Complete this section as your classmates share their findings.***
Use the following symbols to summarize findings from each group:

X = no effect ↑ = stimulation ↓ = suppression

Cell Function	Summary of Findings	
	Impact of Liquids	Impact of aerosol
Ciliary beating 	• Sini-cide ↓ cilia beating (Q1) • Cinnamaldehyde ↓ cilia beating (Q2)	Sini-cide ↓ cilia beating (Q1)
Glycolysis	Cinnamaldehyde ↓ basal glycolysis and glycolytic capacity (Q5)	
Mitochondria	• Cinnamaldehyde ↓ mitochondrial energy (membrane potential) (Q3) • Cinnamaldehyde ↓ basal respiration and ATP production (Q4)	
Oxidative Burst (Neutrophils) 	Cinnamaldehyde ↓ neutrophil antimicrobial function and glycolysis (Q6)	

In one or two sentences, summarize the major conclusion(s) from these studies and reference specific data points if possible.
Cinnamaldehyde-containing e-liquids and cinnamaldehyde have the ability to impair cilia beating and bioenergetic functions such as glycolysis and respiration in airway cells. Cinnamaldehyde can also impair bioenergetics in neutrophils, decreasing their antimicrobial function. Together these data suggest that vaping cinnamaldehyde-containing e-liquids could cause increased chance of respiratory infection.

What is a follow up question or experiment the researchers could use to find out more about the effects of e-cigarettes on the respiratory immune system?
Many options for this, including doing bioenergetics in different cell types, figuring out exactly which enzymes are affected, and performing similar experiments with different flavors/flavoring chemicals.

Updated September 2023

Beyond Nicotine: Evaluating the Bioenergetic Effects of Flavored Electronic Cigarettes on Respiratory Immune Cells

Name ________________________________ Group Number: ____

Write down your assigned guiding question:

Data Review Questions: ***Record your answers to your group's questions below.***

1.

2.

3.

Data Interpretation Questions:

Be sure to ***cite specific data points*** *to support your answers with evidence.*

1.

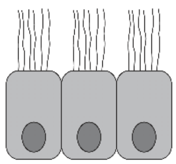
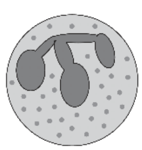
2.

3.

What additional questions do you have?

Summary of Data: ***Complete this section as your classmates share their findings.***
Use the following symbols to summarize findings from each group:

X = no effect ↑ = stimulation ↓ = suppression

Cell Function	Summary of Findings	
	Impact of e-liquids	Impact of aerosols
Ciliary beating 		
Glycolysis		
Mitochondria		
Oxidative Burst (Neutrophils) 		

In one or two sentences, summarize the major conclusion(s) from these studies and reference specific data points if possible.

What is a follow up question or experiment researchers could use to find out more about the effects of e-cigarettes on the respiratory immune system?