E-Cig DNA Day Module Slide Guide

Timeline:

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| **Time (minutes)** | **Lesson component** | **Slides** |
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1. **Introduction**

* Greet the class and introduce yourself.

1. **What is North Carolina DNA Day?**

* DNA Day was created to commemorate the completion of the Human Genome Project in 2003 and the discovery of DNA’s double helix in 1953.
* On DNA Day, scientists from universities visit high school and middle school classrooms to talk about science and do fun activities.

1. **Why do we call it DNA Day?**

* The story of how scientists discovered DNA is an example of how science progresses.
* In April of 1953, Drs. James Watson and Francis Crick determined the structure of DNA   
  (double helix), with contributions by Drs. Rosalind Franklin and Maurice Wilkins.
* 50 years later, in April of 2003, the Human Genome Project determined the entire DNA sequence of a human (3 billion letters)!
* In 50 years, we went from learning the structure of DNA to determining the entire DNA sequence of a human. This is amazing! But how does the scientific process work?

1. **How are discoveries made?**

* Scientists come up with ideas, and then they test these ideas using experiments. Each idea is like one piece of a big puzzle, except we don’t know what the completed puzzle will look like.
* Experiments must be repeated many times to ensure the results are accurate.
* Scientists then present their research and eventually write a paper about it.
* The paper is then submitted to a journal. If it is rejected, more experiments might be needed. If it is accepted, the paper is published, and it contributes to scientific knowledge!

1. **Today’s Topic: The Science of E-Cigarettes**

* Let’s move on to talk about the science of e-cigarettes and some of the research going on at UNC on the health effects of e-cigarettes!
* How many of you know someone who vapes?
* Who has seen someone using e-cigarettes at school?

1. **What is the connection between e-cigarettes and DNA/DNA Day?**
   * Some scientists have studied how e-cigarette use changes gene expression. Gene expression comes from DNA. You can see here that there were some shared genes between cigarette smokers and e-cigarette users, but there were an additional 305 genes that were decreased by vaping alone!
   * Another connection is that e-cigarette research is in its infancy now, just like DNA research was over 50 years ago. This is a really exciting time to learn about e-cigarettes!
2. **E-Cigarette Venn Diagram Game – see separate instructions.** 
   * Instruct Venn Diagram game. Read separate instructions
3. **What is an e-cigarette?**

* First, let’s talk about what e-cigarettes are.
* E-cigarettes allow users to inhale nicotine and flavorings.
* Even though there are a lot of types of e-cigarettes, all of them have a power source (battery) and compartment for e-liquid, which is heated up and aerosolized by the e-cigarette. The user then inhales the aerosol.
* Before advancing to next slide, ask students to predict how many young people in middle school versus high school use e-cigarettes. They can give a number or a percentage.

1. **How many young people use e-cigarettes?** 
   * Read off slide.
2. ***Class Brainstorm:* Why do people use e-cigarettes?**

* Let’s brainstorm about why people use e-cigarettes!
* **Activity:** Have one student come up to the board to write down the ideas, and have the other students propose ideas. Discuss the ideas they come up with, which might include:
  + To stop smoking (yes, this is true, and does work for some people, but there isn’t a lot of evidence that this works, and e-cigarettes are not approved by the FDA as a smoking cessation device)
  + Because they are addicted to nicotine
  + It tastes/smells good or better than cigarettes
  + Because it makes them feel cool
* Let’s talk more about why e-cigarettes are so appealing to young people.

1. **Advertising: Smoking v. Vaping**

* Many perceptions about e-cigarettes come from targeted marketing.
* The tobacco industry used targeted marketing to make cigarettes appealing to young people.
* **Questions:** Let’s look at these ads – do you think the e-cigarette ads on the left look similar to cigarette ads on the right? What is similar about them? How do you feel about this?
* (1958 v. 2013) Some e-cigarette ads are tapping into the cool, rugged masculinity that became famously linked with cigarettes.

1. **Advertising: Smoking v. Vaping (continued, use above prompts)**
   * Other e-cigarette ads use the exact same artwork as was used in cigarette ads. These ads were meant to convey the message that doctors approve of using these products.
2. **Advertising: Smoking v. Vaping (continued, use above prompts)**

* Many e-cigarette and cigarette advertisements use bright, youthful colors and models that look “cool” to appeal to younger audiences.
* So, now that we understand what e-cigarettes are and why people might use them, let’s get more into science behind e-cigarettes.

1. **Smoking versus vaping?**

* A lot of people want to know how vaping compares to smoking.
* [Go through venn diagram, talk about in context of intro activity].
* Smoking and vaping are actually pretty different, making them hard to compare. Because of this, it’s likely that the effects of vaping are different from smoking, rather than on the same continuum. It is kind of like comparing apples to oranges.

1. **Smoking versus vaping?**

* Smoking and vaping also affect the body differently.
* The difference between the effects of smoking and vaping is also shown in these photographs of human airways. This picture is taken at the first bronchial branching.
* You can see that the airways from the vaper almost look like they have been sunburned, and they look different from the smoker’s airways, which are very mucousy.
* Did you know that the estimated temperature of vaped aerosol is 158F (70C)?
* So, keep all of this in mind the next time you or someone else thinks about vaping as just a “lesser evil” in comparison with smoking.

1. **Diversity of E-Cigarette Devices**

* As you can see on this slide, there are a lot of different types of e-cigarettes! Some of the older device types include disposable e-cigarettes and tank or box-mod e-cigarettes.
* Vape pens are also an older type of e-cigarette that are resurging in popularity for vaping marijuana.
* Current e-cigarettes that are very popular include JUUL, Sourin Drop, and STIG disposable e-cigarettes. Some e-cigarettes even look like asthma inhalers or USB thumb drives!

1. **E-Liquids are a Complex Mixtures**

* But what is in the e-liquid that all of these devices use? E-liquids are complex mixtures that include base liquids propylene glycol (PG) and vegetable glycerin (VG), flavoring chemicals, nicotine OR nicotine salts, and potentially other chemicals.
* PG and VG are humectants, or chemicals that preserve moisture. PG provides the feeling of a “throat hit” (like you would have with smoking), while VG is easier on the throat, sweeter, and makes a larger vapor cloud. These two chemicals are mixed at varying concentrations to create the desired effect.
* Flavoring chemicals give the e-liquid its smell/taste.
* E-liquids can be nicotine free or have nicotine in varying concentrations.
* Next, let’s talk about each of these e-liquid components in more detail.

1. **Nicotine is Addictive!**
   * One of the major components in e-cigarettes is nicotine.

* [Read through this slide, placing special emphasis on the fact that nicotine is as addictive as heroin and cocaine – REALLY addictive].
  + Ask students if they were aware of the addictiveness of nicotine
* This is why e-cigarettes are so addictive!
* Nicotine addiction can be costly and can affect your quality of life.

1. **Nicotine: So What?**
   * Let’s discuss why you should care about nicotine exposure.
   * In addition to being addictive, nicotine can also cause various health effects.
   * [Read through slide]
2. **Two Forms of Nicotine in E-Cigarettes**

* Traditional e-cigarettes use freebase nicotine (also found in cigarettes) while newer, low output mods, use nicotine salts.
* Nicotine salts give a smoother hit because they are a neutral pH, which is not as irritating to the throat as freebase nicotine, which has a basic pH. This means that you can vape more nicotine in the salt form before it irritates your airways, allowing increased nicotine consumption.

1. **Activity slide: Rank the Nicotine Content**
   * Now we are going to do an activity to see if you can rank e-cigarettes in terms of their nicotine content.
   * [Read from slide]
2. **Debrief slide 1: Rank the Nicotine Content**
   * [Read from slide]
3. **Debrief slide 2: Rank the Nicotine Content**

* [Read through this slide, reporting how much nicotine is in each type of e-cigarette/pod]
  + Is anyone surprised by the order?
  + How often do you think people go through a pod?
* We are seeing an increase in the concentration of nicotine in newer e-liquids/pods.
* It is important to keep in mind that the concentration of nicotine in the pod or e-liquid may not translate to the amount that your body actually absorbs.
* Different devices, different e-liquids, different user behaviors, means there is a lot of variation how much aerosol and what chemicals are inhaled with each puff.
* How do you think these higher nicotine concentrations will affect addiction?

1. **Addiction & E-Cigarettes**

* As you can see in this graph, high school use of e-cigarettes has increased dramatically in the past few years. This graph is based on survey data from North Carolina students.
* This means that an increasing number of youth are becoming addicted to e-cigarettes and are more likely to start smoking cigarettes.
* **Question:** In the cartoon, Nicotine Addiction says “It’s good to be back in school.” What is the “back” referring to?

1. **Addiction & E-Cigarettes**
   * There are many resources available for people who want to quit using e-cigarettes and even for people supporting someone in recovery.
2. **Dose & Route of Exposure Matter**

* Part of the reason that we want to study e-cigarettes is that dose and route of exposure matter.
* One of the first toxicologists, Paracelsus, said [read quote off slide], meaning that the dose makes the poison, or the amount of a chemical you are exposed to determines how severe the effects are.
* For e-cigarettes, we also know that the route of exposure matters! There are three primary routes of exposure (ingestion, inhalation, and skin contact), and for e-cigarettes, we are interested in inhalation.

1. **Activity slide: Toxic or Not?**
   * Now we are going to do an activity to think critically about the importance of route of exposure.
   * [Read instructions from slide]
2. **Debrief slide 1: Toxic or Not?**
   * [Read off slide]
3. **Debrief slide 2: Toxic or Not?**
   * This is one way to sort the items.
   * What may be safe to eat, may not be safe to inhale, and vice versa.
4. **Food-safe flavoring chemicals that can be toxic when inhaled**

* Some of the common flavoring chemicals in e-liquids include cinnamaldehyde (cinnamon flavor), benzaldehyde (almond or cherry flavor), and vanillin & ethyl vanillin (vanilla flavor).
* These flavoring chemicals are present in the food we eat, and they are safe for us to consume as part of food.
* However, they are also found in e-liquids, and we don’t know if it is safe to inhale them. Research is emerging that suggests that these flavoring chemicals can be toxic when inhaled.

1. **Activity slide: Scratch and Sniff**
   * [Read off slide]
2. **Debrief slide: Scratch and Sniff**
   * [Read off slide- emphasizing that what they are smelling is a chemical]
3. **Okay to eat but not inhale?**

* We already have an example of a flavoring chemical that is okay to eat but not inhale.
* Diacetyl is a butter flavoring chemical found in microwave popcorn factories.
* When workers in the factory inhaled too much diacetyl, they developed popcorn lung, which damages the lungs’ smallest airways, causing their lungs to become stiff and not work very well. There is no treatment for this disease.
* The reason that diacetyl is safe to eat but not inhale is that your liver, which detoxifies food you eat, has a lot of the enzyme that detoxifies diacetyl, but your lungs have very little.
* Interestingly, diacetyl has been detected in some e-liquids (though it is not one of the more common flavoring chemicals).

1. **E-Cigarettes Produce an Aerosol**

* The “vapor” generated by e-cigarettes is a complex mixture of particles derived from aerosolizing e-liquid [emphasize that it is not water vapor].
* The aerosol is generated by heating the e-liquid.
* An aerosol is a suspension of fine solid particles or liquid droplets, in air or another gas. These particles, due to their small size, can be inhaled deep into lungs.
* These particles are very small! We think they are around or below 1 micron in size.
* So, when you see someone vaping, think about how complex the cloud is and visualize something like this.

1. **What is in e-cigarette “vapor”?**

* PG and VG are humectants, or chemicals that preserve moisture. PG provides the feeling of a “throat hit” (like you would have with smoking), while VG is easier on the throat, sweeter, and makes a larger vapor cloud. These two chemicals are mixed at varying concentrations to create the desired effect.
* When PG and VG are aerosolized through heating, they can break down into different chemicals, such as acrolein and formaldehyde, which are known carcinogens.
* This means that e-cigarettes even without nicotine or flavoring chemicals could have health impacts, as shown in this paper.
* Other than thermal decomposition products, what else is in e-cigarette “vapor” (which we now know is actually an aerosol)?
* [Discuss contents of slide]
  + Note: metal nanoparticles can also be present and arise from the heating element in presence of e-liquid/heat.
  + Note: e-cigarette aerosol generally contains fewer harmful chemicals than smoke from burned tobacco products.
* Different devices, different e-liquids, different user behaviors, means there is a lot of variation how much aerosol and what chemicals are inhaled with each puff.

1. **Why study e-cigarettes?**

* Now that we know all about how e-cigarettes work, let’s talk about why people are interested in studying them.
* This is because we don’t know the complete toxicology (health effects) of e-cigarettes, and we are still learning about them.

1. **What are the effects of e-liquids and aerosols on the respiratory immune response?**

* Scientists at UNC, including Dr. Ilona Jaspers, are studying the effects of e-liquids and aerosols on the respiratory immune response.
* Dr. Jaspers runs a large research team, pictured on the left! Dr. Phillip Clapp, in the photo on the right, performed the experiments we will talk about today.
* Let’s talk more about the respiratory immune system and why it matters.

1. **The Respiratory Immune System**

* The immune system in your lungs protects you from harmful substances you inhale.
* Let’s think – what do you think is the function of these ciliated cells that line the airways?
* They beat mucous and anything trapped in it up and out of the airways as shown in these images.

1. **What are the effects of e-liquids and aerosols on respiratory immune response?**

* When we think about the immune response, we think about it on a spectrum. Normally, we have healthy immune responses and proper host defense. But if we go in either direction from normal, that can have health consequences.
* If the immune system is too active, that can result in inflammation and autoimmune disease. If the immune system is not active enough, there is immune suppression and insufficient host defense.
* For this research, we want to know what the effects of e-liquids and aerosols are on cilia beating, which is part of the respiratory immune response.

1. **Where do respiratory epithelial cells come from?**

* You might wonder how we can study this when cilia are so small and are in the human body!
* To study cilia beating, we take respiratory epithelial cells from human subjects or donors and then grow them in a special type of cell culture well. In this well, they can grow with air on one side, and media (liquid food for the cells) on the other side, replicating how they would be in the body. You can see a real picture of these cells (as viewed under a microscope) on the left.

1. **Experimental Design: Treatments and Controls**

* When you expose cells to something, you also have to have unexposed cells so that you can compare the results.
* Here we see a cell culture plate. There are cells in each well.
* Some cells are exposed only to media, some cells to media and a flavored e-liquid, and other cells to just PG/VG.
* The reason that some cells are exposed to only PG/VG is that the e-liquids contain PG/VG *and* flavorings. So if we only exposed the cells to the e-liquid, we wouldn’t know whether the effect was from the PG/VG or from the flavorings.

1. **Movement of Cilia on Lung Epithelial Cells**

* In this video, taken using a microscope, you can see the cilia beating rapidly. This is how they look when they are normal.

1. **Sini-cide alters movement of cilia on lung epithelial cells**

* When Sini-cide, a cinnamon-flavored e-liquid, is put on top of the cells, you can see that the cilia start to slow down.

1. **Sini-cide alters movement of cilia on lung epithelial cells.**

* 40 minutes after the exposure to Sini-cide, the cilia have almost completely stopped.

1. **Sini-cide alters movement of cilia on lung epithelial cells.**

* After two hours, the cells have completely recovered. This could be for a few reasons:
  + The Sini-cide evaporated off of the cells.
  + The cells turned the Sini-cide into something less toxic.
  + The cells adapted to counteract the effects of Sini-cide.

1. **Which e-liquid had the greatest effect on ciliary beat frequency?**
   * Have students answer. Answer: Sini-cide
2. **Let’s brainstorm!**

* Lead the class through the slide to get them thinking about cellular respiration and what might be happening to the cells when they are expose to cinnamon flavored e-liquids and cinnamaldehyde.

1. **Now it’s your turn to be a scientist!** 
   * We will break up into four groups of students and you will each get to interpret one or two graphs. Then, you will share what you found back to the class.
   * [Give students ~5 minutes to complete activity]
2. **Overall Research Question: How does the flavoring chemical cinnamaldehyde impair ciliary beating in lung epithelial cells?**

* Each group’s graphs will help answer this question.

1. **Team 1: What are the effects of e-liquids and aerosols on respiratory immune response?**

* Team 1 answered this question. If we expose ciliated cells to cinnamon-flavored e-liquids and cinnamon-flavored aerosols, what happens? Let’s find out.

1. **Team 1: What are the effects of Sini-cide e-liquid and aerosol on cilia beat frequency? (Compare & Contrast)**

* [Have students from that group walk the other students through the graph]
  + Note the different scales on the Y-axes of the graphs.
* Now, we have one graph showing what happens to cilia beating when they are exposed to Sinicide aerosol and the other shows what happens when they are exposed to Sinicide e-liquid.
* [have students answer the question] **Answer:** It looks like for the aerosol, CBF is about 200 with PG/VG and 30 with Sinicide, and with the e-liqud, 600 (PG/VG) and 200 (Sinicide). So, both were greatly decreased but the aerosol looks like it decreased the CBF more relative to PG/VG.
* What is a possible explanation for this?
  + **Answer:** A reason for this could be that more toxic chemicals were generated when the e-liquid was vaped.

1. **Team 2: How does the concentration of cinnamaldehyde impact cilia beat frequency?**

* [have students answer] **Answer:** there is a dose-dependent decrease in CBF, with the highest dose decreasing CBF the most.

1. **Team 3: How does the concentration of cinnamaldehyde impact mitochondrial function?**

* You can see pictures of the ciliated cells here. They have been exposed to a special dye that makes energetic mitochondria red and non-energetic mitochondria green.
* What do you notice about the cells at the highest dose? **Answer:** There is a dose dependent decrease in mitochondrial function, with the highest dose decreasing mitochondrial function the most.

1. **Team 3: How does the concentration of cinnamaldehyde impact mitochondrial function?**

* Here we see the mitochondrial function quantified using the pictures we saw on the last slide.
* Does this match up to what you thought based on the images?
* [if students ask, the mitochondrial function is calculated by dividing the intensity value for red fluorescence by the intensity value for green fluorescence].

1. **Team 4: How does the concentration of cinnamaldehyde impact ATP production?**

* [have students interpret the graph] **Answer:** again, we see a dose-dependent decrease in ATP production by the cells with increasing dose of cinnamaldehyde.

1. **Interpretation and Discussion**
   * Wrap up data activity and see if students have any additional questions.
2. **Predict: How might decreased mitochondrial function in lung cells impact human health?**

* There isn’t any right answer, but some possible answers include: increase risk for infection, mucous buildup in lungs and coughing, increased debris in lungs from cilia not clearing particles, etc.
* Also ask the class what they think potential health effects would be if ciliated cells cannot function properly.

1. **E-Cigarette and Vaping Associated Pulmonary Injury (EVALI)**

* You may have also heard about e-cigarette and vaping associated pulmonary injury (EVALI), which has been in the news a lot lately.
* [Read symptoms]
* Patients with EVALI had lungs that looked like they were filled with ground glass, very different from the healthy lung pictured on the left.
* **Question:** Does anyone know what chemical is thought to have caused these injuries? *Answer:* Vitamin E Acetate

1. **Vitamin E Acetate**

* [Read through slide about Vitamin E Acetate]
* Point out that we don’t know exactly how Vitamin E acetate affects cells, and studies haven’t been done like have been done for cinnamaldehyde

1. **In the News**

* What has been the government’s response to the EVALI outbreak?
* There have been responses at the city, state, and national levels [talk about each one].
* Most recently, a bill was signed into law to raise the age for buying any tobacco products (including e-cigarettes) to 21 and to ban flavored e-cigarette pods.
* Note that the e-cigarette flavored pod ban does not apply to tobacco and menthol flavors or to refill (non-pod) e-liquids.

1. **Let’s Listen** 
   * Here is a short NPR report about disposable vape pens.
   * Has anyone heard of these products?
   * What do you think it will take to deter youth from using e-cigarettes?
   * What was effective about anti-cigarette campaigns that can be used to discourage e-cigarette use?
2. **What have we learned?**

* Ask students to summarize what they have learned.
* Reveal bullets on slide.

1. **Discussion questions**
2. **Wrap up**
   * Answer any remaining questions about e-cigarettes, what it is like to be a scientist, etc.