Facilitator Guide

Beyond Nicotine Lesson 3

Biomedical Research on E-Cigarettes & Vaping | Investigating the Impact of E-Cigarettes on Airway Inflammation and Asthma

A Digital Interactive Notebook

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. In this activity, students work through a Google slide set also known as a **digital interactive notebook (DIAN)**, to learn about and interpret toxicological data from human subjects research to examine one approach researchers are taking to evaluate the respiratory effects of e-cigarettes use. **By comparing levels of biomarkers (e.g., neutrophil activation, mucus production and detoxification pathways) among nonsmokers, cigarette smokers and e-cigarette users, researchers can determine the extent to which e-cigarette use might contribute to airway inflammation and ultimately lead to airway injury or disease.** Given that this is an emerging area of study, students will learn about and analyze biomarker data to get a glimpse into how researchers are assessing the extent to which e-cigarettes and the chemicals they contain can influence respiratory health. This data interpretation activity culminates with a critical thinking assignment that invites students to use what they have learned to design a clinical study to answer the following research question: are asthmatics more susceptible to the respiratory health effects of e-cigarettes than non-asthmatics?

*This activity features data from the following peer-reviewed article:*

**Learning objectives**
Upon completion of this activity, students will be able to:

- Describe the respiratory tract and the cells involved in respiratory immune function.
- Describe processes in the body (e.g., mucus production) associated with airway inflammation and dysregulated airway homeostasis.
- Explain what a biomarker is and describe the role of biomarkers in the biomedical field.
- Distinguish between different kinds of biomarkers (e.g., proteins (e.g., enzymes) and cells).
- Interpret toxicological data from sputum samples from non-smokers, smokers, e-cig users to assess trends in the inflammatory response and determine whether people who vape exhibit biomarkers of inflammation and dysregulated airway homeostasis (neutrophil activation, mucus production and detoxification pathways).
- Describe the features of asthma, including symptoms and cell types involved.
- Formulate a hypothesis and design a clinical study to answer the question: are asthmatics more susceptible to the respiratory health effects of e-cigarettes than non-asthmatics?
- Explain how researchers are studying the effects of e-cigarettes on asthmatics and asthma-like symptoms.
Part II. Does e-cigarette use cause inflammation of the airway?
In this part, students learn about toxicologists and how they are using biomarkers to determine if e-cigarette users have increased markers of inflammation (e.g., neutrophil activation) in addition to increased mucus production and detoxification pathways compared to non-vapers and smokers because these findings could indicate that some sort of disease process is occurring. Students examine biomarker data from human subjects to determine the extent to which e-cigarette use appears to promote inflammation and result in aggravated airways. Students evaluate biomarker data from sputum samples from non-smokers, smokers, e-cig users to assess trends in the inflammatory response and airway homeostasis (via the following protein biomarkers):

Biomarkers associated with neutrophil activation:
- Neutrophil elastase
- Myeloperoxidase
- Calprotectins

Biomarkers associated with detoxification pathways:
- Alcohol dehydrogenase
- Glutathione S-transferase

Biomarkers associated with mucus production:
- MUC5AC
- MUC5B

Note: These proteins are described in the DIAN; direct students to https://www.genecards.org/ to learn more details about these proteins and their corresponding genes.

[Optional] Hands-on Biomarker Simulation Activity (Hands-On Activity)
For teachers who do not want to have their students do the data analysis in Part II OR who want to supplement the data analysis with a hands-on activity, this hands-on wet-lab simulation is suggested prior to data analysis. Developed in collaboration with the University of Rochester, this qualitative data analysis activity has students test simulated “sputum” samples (buffers of varying pH) from smokers, vapers and non-smokers/non-vapers to look for the protein biomarkers above using assorted pH test strips.

Link to Biomarker simulation activity

Part III. Emerging research question: Are asthmatics more susceptible to the respiratory health effects of e-cigarettes?
In this part, students are guided to develop a hypothesis based on what they have learned and design a human subjects research study that would use biomarkers to test their hypothesis. Students select subjects from the following populations: Non-asthmatics: non-vapers and vapers and Asthmatics: non-vapers and vapers and then identify relevant biological samples and biomarkers to investigate in addition to describing expected results if their hypothesis is true. See the Teacher edition of the DIAN (slides 54-57) for details and possible experimental design scenarios.

Additional activities for advanced learners
- Research an additional biomarker that could be used in their study based on peer-reviewed literature.
- Research other biological specimens could be used to measure biomarkers of respiratory tract processes (nasal swabs, bronchial brushings, bronchoalveolar lavage). What are the pros and cons of each? What types of studies are they typically used for?
● Choose one of the biomarkers from the lesson and research its detailed functions and importance in diseases. If it is an enzyme, describe the reaction it catalyzes. One website that could be helpful for this is: https://www.genecards.org/

● Read one of the papers provided in the references for this activity and summarize the main findings in one paragraph. Have students highlight and define terms that are new to them.

Assessment
Assess student learning by doing one or more of the following:
● Collect completed experimental design visual (interactive notebook slide 40, student edition)
● Ask students to summarize their experimental design visual in writing, by narrating their slide, or by creating a short presentation

Extensions
There are opportunities to extend student learning by inviting students 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 (Novel Coronavirus):

E-cigarette or Vaping Use-Associated Lung Injury (EVALI)
According to the Centers for Disease Control and Prevention (CDC, 2020), 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, 2020). 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).
Background Information

Few consumer products have evolved as an environmental health concern as rapidly as electronic cigarettes, or e-cigarettes. E-cigarettes 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 e-cigarettes such as JUUL (Gentzke et al., 2019). 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, over 3.5 million youth reported using e-cigarettes in 2020, with 19.6% of high school students and 4.7% of middle school students reporting current e-cigarette use (Wang et al., 2020). Results also revealed that among high school students, 84.7% 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 acrolein) 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 flavored 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 the recent outbreak 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.

Overview of E-Liquids & Aerosols

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.

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.

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. Researchers at UNC are also interested in how e-cigarettes could uniquely affect people with underlying respiratory disease, such as asthma.

Overview of the respiratory tract
Air enters the body through the nose and mouth and travels down the respiratory tract to the lungs. Within the lungs, the respiratory tract branches into smaller and smaller airways until it reaches the alveoli, small sacs where gas exchange with the blood occurs. Gas exchange is not the only activity happening in the respiratory tract, though! Because the respiratory tract is exposed to the outside world through contact with the air we breathe in, it also must defend the body from inhaled pathogens and pollutants.

Each region of the airways contains many different cell types that act together to perform the necessary functions of the respiratory tract. The lining of the airways is made up mostly of epithelial cells, which separate the rest of the body from the airways. Higher up in the respiratory tract, ciliated epithelial cells beat mucus and anything trapped in it up and out of the airways. The mucus is produced by goblet cells, which can be found in the lining of the respiratory tract along with other cell types such as club cells (which make proteins that help defend the respiratory tract) and neuroendocrine cells (which can interact with nerves and make signaling molecules). In the alveoli, type I and type II epithelial cells, which are non-ciliated, make up the barrier between the alveoli and the body and allow for gas exchange. Basal cells are the stem cells of the respiratory tract and are able to become ciliated epithelial cells, club cells, or goblet cells as needed to replace cells that have died. The airways are also patrolled by immune cells, which can sense and attack allergens and pathogens. Common immune cells found in the respiratory tract include neutrophils and macrophages. When people are healthy, all of these cell types work together smoothly, and the respiratory tract is in homeostasis. However, when someone has a respiratory disease, this homeostasis is altered, leading to imbalances that can result in inflammation (too much cellular activity) or inadequate protection from environmental insults (too little cellular activity).

Cells of the respiratory epithelium (in alphabetical order)

- Basal cells are the stem cells of the respiratory tract and are able to become ciliated cells, club cells, or goblet cells as needed to replace cells that have died.
- Ciliated epithelial cells make up the majority of cells that line the upper and middle airways. These cells have cilia that beat mucus and anything trapped in it up and out of the airways.
- Club cells are found more prevalently lower in the airways, and they protect the airways by secreting a variety of defense proteins. They are also important because they can detoxify chemicals and become ciliated cells if needed.
- Goblet cells produce mucin proteins that make up the mucus layer in the airways.
- Pulmonary neuroendocrine cells are the interface between the airways, nervous system, and endocrine system. They produce signaling molecules and can respond to changes in the airway environment. Note: This cell type is not included in the activity but could be added by the teacher if desired.
Common immune cells found in the respiratory tract

- **Macrophages** are the most prevalent immune cell type in the airways. They perform a wide variety of functions, including phagocytosis of pathogens and debris, sending signals to epithelial cells and other immune cells, and helping with tissue repair after damage.

- **Neutrophils** are an innate immune cell that are found in the airways in high numbers during acute inflammation and disease. They can be found in low numbers in healthy airways.

- **Eosinophils (asthmatics)** are another innate immune cell type that play a role in allergic responses, and they are often found in the airways in people who have asthma.

Other important features & terms:

- **Lumen**: generally, a lumen is the inside space of a tubular structure. For the airways, the lumen is the space where air goes in and out of the respiratory tract.

- **Mucus** is a thick, slippery liquid that lines the airways and is important for helping trap pathogens and debris and for forming a barrier between the lumen and the respiratory epithelium. The two major proteins found in mucus are MUC5AC and MUC5B.

- **Induced sputum** is a sample that can be taken from the central airways. Sputum is induced by having the patient or research participant inhale nebulized saline (a salty solution), which liquefies airway secretions and allows them to be coughed up. The sputum is then cleaned up and separated into cellular and non-cellular components in a laboratory so that it can be used to measure biomarkers.

- **Mucociliary escalator** refers to cilia beating mucus up and out of the airways, as if the mucus is rising on an escalator.

- **Chemical detoxification** is performed in multiple cell types found in the respiratory tract and involves both enzymatic and non-enzymatic processes to change toxic chemicals into less toxic chemicals that can then be removed or repurposed in the body.
Experimental design and key clinical toxicology concepts
The data included in this lesson are derived from human subjects research at UNC Chapel Hill. **Human subjects research** must follow extremely strict guidelines and must be approved by an Institutional Review Board to certify that the research is designed appropriately and minimizes risk to participants. For example, it is much more feasible and less risky to the subjects to do research where you collect samples from people who are currently e-cigarette users (already exposing themselves) or have them use an e-cigarette for a certain amount of time as part of the study, rather than exposing non-vapers to e-cigarettes.

As part of clinical research, many different biological samples can be collected for measurement of **biomarkers** that could indicate chemical exposures and/or disease processes in the body. According to the National Institutes of Health, a biomarker refers to a “quantifiable biological parameter that is measured and evaluated as an indicator of normal biological, pathogenic, or pharmacologic responses to a therapeutic intervention (FDA-NIH Biomarker Working Group, 2016). Biomarkers (short for “biological markers”) are measurable indicators of specific biological processes that can be associated with health and disease. There are many different types of biomarkers that have different purposes and applications. Biomarkers are used by doctors and researchers to predict disease risk, assess presence of diseases, determine severity of diseases, and choose best courses of treatment. Biomarkers can be measured in a wide variety of bodily fluids and tissues depending on what disease or biological process is of interest. Not all biomarkers are used in the same way. In this lesson, we will be focused on biomarkers that are markers of inflammation, chemical exposure, and changes in cellular homeostasis.

**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.

**Overview of asthma**
Asthma is a chronic disease of the airways that occurs when the airways are inflamed and sensitive to environmental triggers. This often results in coughing, wheezing, difficulty breathing, and chest tightness. There are many different types of asthma that can be classified by cause, severity, immune cell type present in the airways, environmental trigger, and age of disease onset. Because asthma is such a varied disease, there is no one test that a doctor can give to see if someone has asthma. Rather, they use a wide range of diagnostic tests and procedures to determine if someone has asthma, and if so, what type of asthma they have. There is no cure for asthma, but there are treatments that can help manage symptoms. This webpage is a great resource to learn more about asthma symptoms, diagnosis, and treatment: [https://www.aaaai.org/conditions-and-treatments/asthma](https://www.aaaai.org/conditions-and-treatments/asthma).

**Are asthmatics more susceptible to e-cigarette-related health effects than non-asthmatics?**
Research into the health effects of e-cigarettes has only been going on for about 10 years. In that time, scientists have found that e-cigarettes affect the respiratory system and the way that the
immune system in the respiratory tract functions. These studies have been focused on how e-cigarettes affect healthy people who do not have any pre-existing conditions. However, how e-cigarettes affect susceptible subpopulations, such as individuals with asthma, has not yet been thoroughly studied. This is particularly concerning because there is evidence that young asthmatics may be more likely to use e-cigarettes and may perceive them as less harmful than their non-asthmatic counterparts (reviewed in Hickman and Jaspers, 2020). Because the respiratory immune system is already disrupted in asthma, further changes in cellular function by e-cigarettes could impact asthma development, severity, and/or exacerbations. Currently, there aren’t any controlled studies that address direct cause-and-effect relationships between e-cigarettes and asthma, but there are epidemiological (population based) studies that show associations between e-cigarette use and asthma and asthma-like symptoms in adults and adolescents. Future research is needed to investigate this relationship using controlled human exposure studies and exposure of cell culture or animal models of asthma to e-cigarettes.

How do scientists investigate research questions like this?

There are many different ways that scientists can pursue research to help them answer questions about the toxicity of e-cigarettes and whether certain people may be more susceptible to their effects. While this activity features toxicological research, you may also want to discuss epidemiological research studies, which have revealed an association between e-cigarette use and asthmatic symptoms (Bhatta and Glantz, 2020, Schweitzer et al., 2017, McConnell et al., 2017).

- **Toxicologists** study how chemicals impact human health. Because it is not typically ethical or feasible to expose humans to chemicals to assess toxicity, toxicologists often expose animals (typically mice or rats) or cells to the chemical(s) they are interested in. Then, they can collect samples from the animals or cells and measure specific endpoints or effects they are interested in. This type of experiment is called a **controlled exposure**, and it is the best way to understand cause-and-effect relationships between chemicals and health effects because the researchers can control every parameter of the experiment.
  - Toxicologists can also use **clinical research**, in which human subjects who are already exposed to a chemical (e.g., someone who vapes) provide researchers with samples, which can then be compared with samples from human subjects who are not exposed. Sometimes researchers can also get permission to do a controlled exposure with human subjects, but these types of experiments are subject to strict regulations to protect the subjects.

- **Epidemiologists** study the causes and effects of diseases in human populations using large-scale **observational studies**, with the goals of identifying people who are at high risk of disease and preventing future disease. Unlike with controlled exposure studies, observational studies collect information from people who are already exposed to a chemical in daily life. Because the chemical exposures in epidemiological studies are not strictly controlled, and because the study population is often heterogeneous and has many confounding factors, epidemiological studies can only show association between a chemical and health effect, not that a chemical causes a health effect.
## Curriculum Alignment

### Advanced Placement Biology

<table>
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<th>Science Practices</th>
<th>Big Ideas</th>
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| This activity invites students to use all six science practices: | • BIG IDEA 2: ENERGETICS (ENE)  
Enduring understanding 2.D, 2.E |
| ● Concept Explanation | • BIG IDEA 3: INFORMATION STORAGE AND TRANSMISSION (IST)  
Enduring understanding 3.B |
| ● Visual Representations | • BIG IDEA 4: SYSTEMS INTERACTIONS (SYI)  
Enduring understanding 4.C |
| ● Questions and Methods | |
| ● Representing and Describing Data | |
| ● Statistical Tests and Data Analysis | |
| ● Argumentation | |

### Relevant Units:

- **Unit 1: Chemistry of Life** (Biological Macromolecules (Proteins))
- **Unit 2: Cell Structure and Function**
- **Unit 3: Cellular Energetics** (Enzymes)

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### Next Generation Science Standards

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<th>Disciplinary Core Ideas</th>
<th>Relevant Science &amp; Engineering Practices</th>
<th>Relevant Cross Cutting Concepts</th>
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| **LS1**: From Molecules to Organisms: Structures and Processes | ● 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 |
| **LS3**: Heredity: Inheritance and Variation of Traits | |

**Relevant Performance Expectation**: HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
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References


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

**Other Recent Jaspers Lab Research:**


**Chemical Characterization of E-Cigarettes:**


**Review of Respiratory Effects of E-Cigarettes:**