UNIVERSITY OF CALIFORNIA SAN DIEGO

Effects of Prolonged E-cigarette Exposures on Lung Physiology and Asthmatic Response in a Murine Model.

A thesis submitted in partial satisfaction of the requirements
for the degree of Master of Science

in

Biology

by

Jarod Maxwell Olay

Committee in charge:

Laura Crotty Alexander, Chair
Alisa Huffaker, Co-Chair
Stanley Lo

2020

Signature Page

The Thesis of Jarod Maxwell Olay is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

_____
(Co-chair)

_____
(Chair)

University of California San Diego

2020

DEDICATIONS

To my family for always supporting and pushing me to achieve more as well as to never give up.

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

E-cig	Electronic Cigarette
Rrs	Total Airway Resistance
Crs	Total Airway Compliance
Ers	Total Airway Elastance

ACKNOWLEDGMENTS

I would like to acknowledge Dr. Laura Crotty Alexander for the opportunity and support to conduct this research project in her lab. Through her guidance I have found a passion for research.

I would like to also acknowledge our lab manager and lab technician, Alex Moshensky and John Shin for assisting during harvests and data computation.

Abstract of the Thesis

Effects of Prolonged E-cigarette Exposures on Lung Physiology and Asthmatic Response in a Murine Model.

by

Jarod Maxwell Olay

Master of Science in Biology

University of California San Diego, 2020

Professor Laura Crotty Alexander, Chair
Professor Alisa Huffaker, Co-Chair

In the last decade, e-cigarettes (e-cigs) have grown in popularity among the youth leading to increased cases of E-cigarette Vapor Associate Lung Injury, EVALI. At the time of this study, according to the CDC, there have been 68 deaths and 2,800 hospitalizations due to e-cig use. The e-cig market is constantly changing and relevant research on the effects of the devices are absent, therefore creating a niche for a study that analyzes the popular devices at a dosage similar to general population usage. We exposed mice to either mango or mint JUUL vapor for one hour three times a day for a month and three months. The exposed mice illustrated trends that demonstrated changes in the lung physiology hinting that prolonged use leads to increase in airway resistance and compliance. Our findings illustrate that at a dosage similar to human usage JUUL can negatively impact the lung physiology of mice. Future experiments would be to test the effects of e-cigs in an animal that is more closely related to humans, potentially rhesus macaque.

INTRODUCTION

The lungs are a necessary organ of the human body and allows us to function as a multicellular organism. Because the lungs play an integral role in our bodies, it is important that we know what processes could potentially damage it. One such process is the inhalation of smoke or vapors other than air.

At the beginning of the century, electronic cigarettes, also known as e-cigs, were created to resemble the traditional cigarette in order to wean addicted users off of them [1]. Around this time, the harmful side effects of cigarettes were starting to be discovered. One of the many effects include lung diseases such as chronic obstructive pulmonary disease, COPD, which makes it harder for a person to breathe [2]. Unfortunately, if a patient smokes and is diagnosed with COPD, they are 12 times more likely to die from the disease than compared to nonsmokers [3]. Adding to the list of negative symptoms, if a patient were to have asthma, smoking would predispose them to an increased risk of triggering an attack [4]. Smoking can also lead to an increased risk of coronary heart disease by 2 to 4 times more than nonsmokers [5].

What started as a way to get traditional smokers to stop smoking has turned into a $19.3 billion industry with roughly 460 different brands of e-cigs currently on the market [6][7]. Constant evolution of these e-cigs has led to devices becoming smaller, discrete and flavored. Through social media, the devices have reached a younger generation of users and has become a “gateway” into smoking for others[8]. This has led to a surge in hospitalizations linked to e-cig use, with physicians labeling the cases as E-cigarette Vaping Associated Lung Injury (EVALI)[9]. As of now, there have been

2,700 documented cases of EVALI[10]. Out of these cases, 52% of the users are under the age of 24 [10].

A feature that popularized e-cigs is the ability to create “clouds” of e-cigarette vapor, which allows the user to perform tricks with the vapor. This created a niche for devices that could produce larger vapor “clouds”, which in turn gave rise to a device called “box mods” [11]. The box mod consists of a battery in a box, about the size of a fist, with a glass tank and a mouthpiece mounted above it [12]. These devices use a piece of cotton to absorb different types of e-cig liquid, also known as “e-juice”, and this allows for the vaporization of the liquid. Around 99% of e-juice on the market contains nicotine as well as exposes the user to toxic chemicals such as acetaldehyde and formaldehyde that are known to be carcinogenic. [13][14]. Some of the other chemicals include diethylene glycol and diacetyl that are linked to lung diseases such as bronchiolitis obliterans more commonly known as popcorn lung [13]. The main ingredients of e-juice are propylene glycol (PG) and vegetable glycerin (VG) with varying concentrations of liquid nicotine [11]. The e-juice can be modified by the user to change the ratio of the PG:VG to create bigger clouds [11]. This variability and simplicity of ingredients allows avid users to make their own cocktail and change the percentages to their own liking. In recent years, recipes for e-juice containing illegal drugs have become easily accessible online [15]. Similar to how users changed the nicotine concentrations and PG:VG in their e-cig juice, they are able to change the flavors. The large number of flavors on the market is another one of the factors that has promoted the use of e-cigs in younger generations. While the customizability of the box mod allows the user to tailor the device to their liking, it was also one of its pitfalls.

One of the drawbacks of the box mod is that refilling it requires unscrewing the tank and carrying around extra e-juice. The e-juice is thick and sticky, which makes the process of refilling the glass tank an ordeal. Therefore, a replacement with a sleek design, about the size of usb called the JUUL, took over the market swiftly. The lightweight design was referred to as the “iphone” of e-cigs when it was first released. JUULs do not have refillable tanks but rather a disposable pod. The newly designed pods deliver nicotine from nicotine salts. The amount of nicotine in one JUUL pod is equivalent to the amount of nicotine found in a whole pack of cigarettes, approximately 200 puffs [16]. The ease of smoking from a JUUL makes finishing a single pod a simple task compared to smoking a pack of cigarettes. With the devices delivering a nicotine dosage higher than that of a single cigarette, many wonder if e-cigs are truly achieving the intended goal of helping users ween off cigarettes[1].

Within the last year there has been a spike in reported EVALI cases in the United States [18]. Lung biopsies conducted on patients diagnosed with EVALI show a buildup of fluid in the lungs and scarring of the lung tissue [19]. Physicians reported these disease states as organizing pneumonia and fibrinous pneumonitis, respectively [19]. The EVALI patients not only showed injury to the lung tissue itself but also saw histological changes to systems associated with the lungs. Among these changes were activated immune cells lining the lungs as well as inflammation induced from these cells [19].

It is now known that traditional cigarettes affect the physiology of the lungs; however, the effects of e-cigs on the lungs has not been fully explored. Initial testing had promising data supporting e-cig's effectiveness, however these studies were

sponsored by tobacco companies and had severe design flaws [17]. This skewed information about the devices has misinformed the general public about the risks of the devices and their detrimental effects. Therefore, it is of utmost importance to understand the effects to fully inform users of all the potential risks when using these devices.

In response to the discovery of negative side effects being presented in humans from the use of traditional cigarettes, scientists created mouse models to try to quantify the changes happening to the body. Understandably, when the usage of e-cigs started to become more popular, scientists shifted their experiments to include the new devices. Early reports exposed c57bl/6 mice, a strain of black mice, to either cigarette smoke, e-cig vapor or air. The mice exposed to traditional cigarette smoke for four weeks showed decreased lung compliance and increased lung elastance[23]. Similar changes in lung physiology were witnessed in the e-cig vapor exposed mice, however these effects arose sooner at three day. The mice exposed to e-cig vapor had an increase in airway resistance and elastance as well as a decrease in airway compliance compared to non-exposed air group [23]. However, while the vapor from the devices elicited changes in lung physiology, the dosages from the devices used were not comparable to human usage. Therefore, we strive to see how these devices, primarily JUULs, affect a mouse model in regards to changes in lung physiology at doses comparable to human use.

In order to expose our mice to a schedule similar to human usage of e-cigs, we surveyed e-cig users on usage and preference for devices. From this data, we decided to use JUULs. We exposed our mice to a chamber full of the e-cig vapor on a regular schedule that represents acute human usage/dosage. The goal of our study was to

analyze the short-term effects of JUUL vapor when exposed at relativity short exposure timelines of one month and three months. Once the mice were exposed to the e-cig vapor, we analyzed the effects by measuring the airway resistance and compliance to quantify changes in lung physiology. Another aspect we were looking at was the lung resistance and elastance response to a hyperresponsive state, such as an asthma attack. From these data sets, we hope to document the direct effects of e-cig vapor on the lung physiology. We predict that the mechanism of how e-cigs affect our mouse model will be similar to that of the past data from traditional cigarettes. We also hope to document the changes on the lung physiology from the popular devices at dosages that the general population are using.

RESULTS

Extended exposure to JUUL vapor doesn't alter lung physiology significantly compared to air group.

After a one month exposure to either mint or mango JUUL vapor, the airway resistance did not show statistical changes over the air group at a confidence interval of 95%. With a prolonged exposure of three months, the airway resistance still did not show statistical change between the exposure groups. However there is a slight trend of the mango and mint having increased airway resistance compared to the air group. The total lung compliance after a one month exposure did not show change across all three exposure groups at a confidence interval of 95%. All three exposure groups had a close grouping close to each other. After an increased exposure time point of three months there was no statistical change between the three groups, however there was less tight grouping.

In response to an asthmatic attack, JUUL vapor exposed mice were trending with increased total airway resistance and elastance.

To evaluate how prolonged exposure to JUUL vapor affects the lungs response to airway hyperresponsiveness such as asthmatic conditions, a methacholine challenge was performed on the mice at the one month and three month time points. Both total airway resistance and elastance were measured to quantify any changes. After a month of exposure the three groups of mint, mango and air did not show any statistical difference in total airway resistance at a confidence interval of 95%. Similar results were seen at the one month time point for the three groups when measuring total airway compliance with no statistical differences. However it should be noted that all three

groups had a close grouping at each increasing dose of methacholine. After a three month exposure the air group was trending lower in both total airway resistance and elastance compared to the two JUUL vapor groups, however there was no statistical significance at a confidence interval of 95%. This might suggest with prolonged use of JUULs that users might be at risk for altered lung response when exposed to asthmatic conditions.

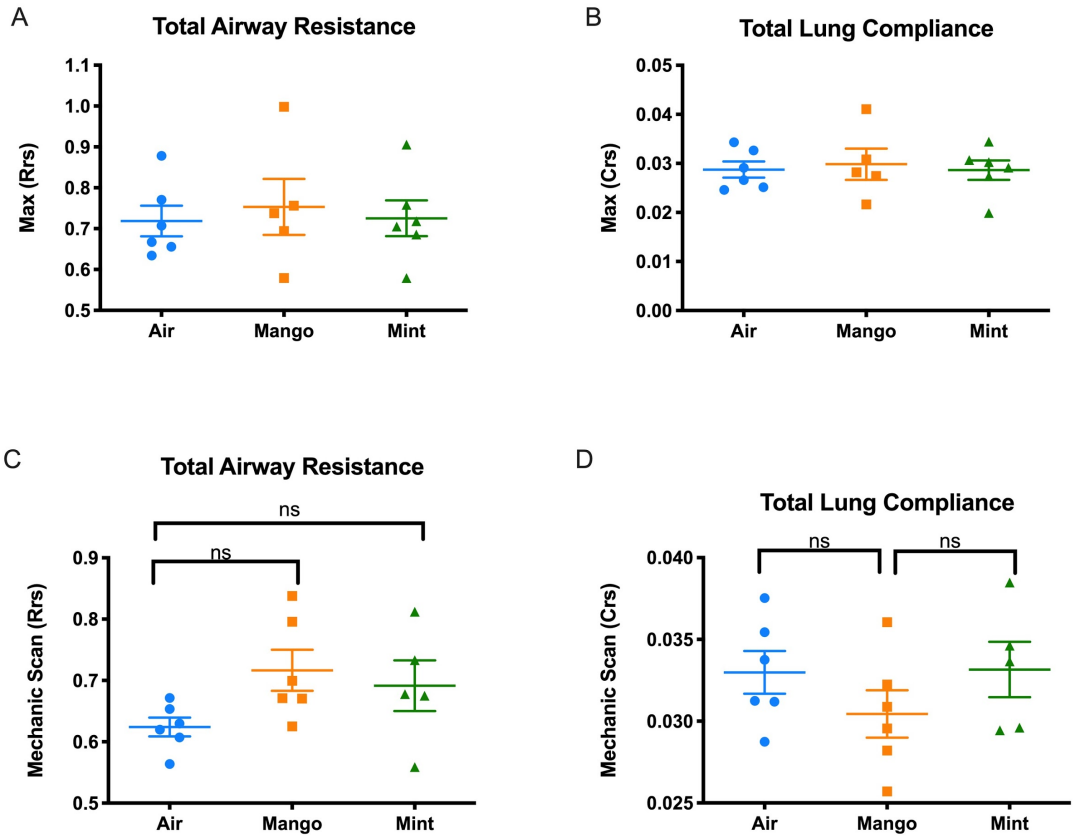

Figure 1. **Lung physiology is not significantly altered after three month exposure to JUUL Vapor compared to air group.** Resistance (Rrs) and Compliance (Crs) are arbitrary units. **(A and B)** Mechanic scan for resistance and compliance measured by ventilator for total airway resistance done on c57BL/6 mice after 1 month exposure to JUUL vapor. Air group was not exposed to any JUUL Vapor. 1 month air and mint groups (n=6), 1 month mango (n=5) **(C and D)** Mice exposed for 3 months to either air or JUUL vapor(either mango or mint). 3 month mango and air groups (n=6), 3 month mint group (n=5). Data graphed on an individual scatter plot. All data shown as mean ± SEM and analyzed by Sidak Multiple comparisons test.

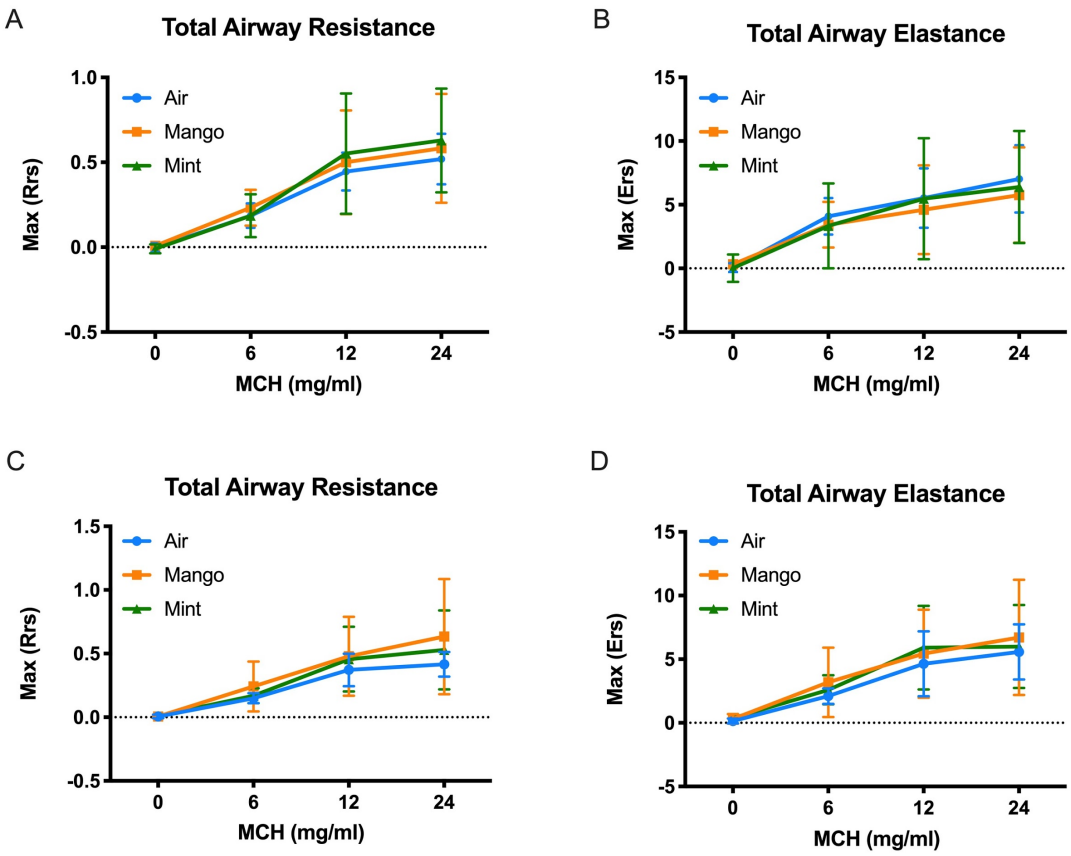

Figure 2. **Prolonged exposure to JUUL vapor is possibly linked to altered lung physiology response to an astmatic attack.** Methacholine challenge performed on exposure groups of either one month or three months. Increasing dosage of methacholine 0,6,12 and 14 mg/ml. Resistance (Rrs) and Elastance (Ers) are arbitrary units. **(A and B)** Mechanic scan for resistance and elastance measured by ventilator for total airway resistance done on c57BL/6 mice after 1 month exposure to JUUL vapor. **(C and D)** Mice exposed for 3 months to either air or JUUL vapor(either mango or mint). Data graphed on a connecting line chart. All three groups were tested with six mice (n=6). All data shown as mean ± SEM and analyzed by Sidak Multiple comparisons test.

DISCUSSION

In order to quantify how JUUL vapor directly affects the lung tissue, the resistance and compliance of the airways are measured in three groups of mice, which were either exposed to JUUL vapor or air. The two JUUL vapor groups were composed of mice being either exposed to mango or mint JUUL flavor pods. Another aspect of this study analyzes prolonged exposure to JUUL vapor using two time points either one month or three months.

Here we show that after a month exposure to JUUL vapor, there was no statistical change in the measured total airway resistance or compliance between all three exposure groups. These results are similar to past studies comparing exposure to cigarette smoke and e-cig vapor at time points of three days to one month. However, in their study the e-cig vapor was being delivered through a different device, a box mod. After a month's exposure their results indicate no statistical change in airway resistance between cigarette smoke, e-cig vapor and air, it should be noted that this study was funded by a tobacco company. Our results could indicate that like cigarette smoke and e-cig vapor from a box mod, that JUUL vapor effects on lung physiology, if any, are not seen at such a short time point of one month. Therefore, the next step in the study was to extend our exposure to a three month time point, to look for changes that could arise after prolonged use of the devices. Another e-cig exposure study in the field, exposed their mice to a time point of 2 months and was able to indicate no statistical changes in airway resistance, but saw trends with groups exposed to nicotine having lower resistance. However, this study was aimed to discover which components that make up the e-cig liquid are causing change. This study was comparing e-cig vapor from a box

mod to cigarette smoke[23].Therefore with a combination of looking at other studies and their exposure model and we decided to expose our mice to a 3 month exposure. Our results were similar after a three month exposure illustrating that total airway resistance and compliance did not show statistical significance between the three exposure groups. A possible explanation for the result could potentially be the nicotine component in the JUUL vapor. The other study saw lower trends of total airway resistance, only in the group that was exposed to e-cig liquid that lacked nicotine. Therefore a future study would be to conduct an experiment with JUUL pods that lack nicotine, which would analyze if JUUL's mechanism of delivering its vapor can elicit similar trends.

Interestingly, both studies referenced earlier, the cigarette smoke groups did not elicit a change in lung physiology. It is known that in humans, prolonged use of cigarette smoke alters lung function, therefore it might be that our exposure model, being considered an acute model is too short to elicit changes.

When comparing our time points of one month exposed to three months exposed the total airway resistance in the JUUL vapor exposed groups trended with an increase over the air group. This trend could become significant at an increased exposure duration. An increase in airway resistance is a negative elicited response, since there is an increase in opposition to airflow through the pathway towards the lungs [25]. This would lead to the lungs not being able to properly conduct gas exchange, in turn leading to respiratory failure [26]. Airway resistance can be determined by the density of the gas, size/number of the airways and the type of flow of airflow, either laminar or turbulent[25]. Our findings also mirror the results of a nicotine electronic cigarette

aerosol inhalation study completed with human volunteers. [27] The study saw an increase in airway flow resistance 30 mins after exposure to 30 puffs of an e-cig device.

To further quantity the effects that JUUL vapor might have on lung function a methacholine challenge was conducted on the mice during the harvest. The goal of a methacholine challenge is to determine the lungs response when being stimulated to a hyperresponsive state, such as an asthmatic attack. After one month or three months of exposure to either JUUL vapor or air the total airway resistance and elastance were measured. The one month exposed mice showed no statistical difference, however the three groups were grouped close together. At an extended exposure of three months, the air group was trending with lower resistance and elastance compared to the JUUL exposed mice. This result might suggest with prolonged use of JUULs that users might be at risk for altered lung response when exposed to asthmatic conditions.

Shown in the other studies, when exposed to cigarette smoke, both measurements of resistance and elastance saw an increase when exposed to a hyperresponsive state at either a three day exposure or two month exposure. However when it came to exposure to e-cig vapor the elicited effects depended on the components in the e-cig liquid. The first study at the three day exposure was indicating that it might be the flavoring mixed with vg and pg that might be causing a significant change. However when comparing these findings to the study that was looking at the two month time point it looked like it is the vegetable glycerin, regardless of nicotine that was causing the changes. Combining these two data sets it might point to vegetable glycerin being the driving force for an increase in resistance during an asthma attack. With our study we would look to see if the JUUL vapor is causing similar changes to

lung response in a hyperresponsive state like the cigarette smoke and the vegetable glycerin component of e cig liquid. Analyzing the results of our data indicate that JUULs might not elicit the same changes as cigarettes and box mod e-cigs in regards to the lungs being exposed to a hyperresponsive state.

Our study did not show statistical significance in our measurements at either the one month or three month one hour daily exposure. When comparing our exposure schedule to others in the field, our model could be considered to be an acute exposure to JUUL vapor. Other models in the field that were looking at long term exposure to cigarette smoke exposed their mice to four hours daily for almost a year[28]. Therefore a future direction for this study would be to look at a long term exposure to JUUL vapor by increasing exposure daily as well as long term.

One of the potential pitfalls in our animal model is that mice have a high nasal airflow and filter rate compared to humans [29], which could alter the amount of e-cig vapor particles deposited in the lungs. To determine if nicotine e cig vapor is being inhaled by the mouse, we conducted an ELISA for the presence of cotinine, a metabolite of nicotine. However, this method does not offer insight on how or where the nicotine enters the body, rather it just proves nicotine was absorbed by the mouse. While our ELISA showed significant levels of cotinine in the blood, we are not able to confidently state whether the nicotine e cig vapor was absorbed through the lungs compared to a different route such as the nasal capillaries. A potential solution would be to analyze the lungs of the exposed mice for the chemical products of e-cig vapor through mass spectrometry.

In the future we look to determine which of the various factors known to calculate airway resistance are altered by exposure to e cig vapor. Possible factors to focus on would be the change in the dimension of the airways. Another avenue of interest is that in our study all the e-cigs contain nicotine, a possible future study would be to test e-cig liquid that does not have nicotine to determine whether the nicotine or other ingredients in the e-cigs are causing the changes. Further in the future we plan to change our model organism to one that is more similar to humans, primates.

MATERIALS AND METHODS

Female C57BL/6 mice were exposed daily for 1 month or 3 months to either JUUL (Mint and Mango with 59 mg/mL nicotine) e-cigarette vapor. Mice were exposed for 20 minutes 3 times daily. All exposures were conducted in the Scireq whole body Expose System. At the time of harvest, mice were given ketamine/xylazine for sedation. Tracheostomy was performed on mouse. The mouse then underwent lung physiology testing via the Scireq Flexivent mouse ventilator. Methacholine challenge was also conducted with increasing dosages of 0,6,12,24 mg/ml. Lungs were then removed from chest cavity under 25cm of water pressure. Lungs were sent off for histology. All graphs and statistical analyses were made and run through Graphpad Prism.

REFERENCES

[1] Who Invented the Electronic Cigarette? - Who Invented It. (n.d.). Retrieved February 9, 2020, from https://www.whoinventedit.net/who-invented-the-electronic-cigarette.html

[2] U.S. Department of Health and Human Services. How Tobacco Smoke Causes Disease: What It Means to You. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010 [accessed 2020 Jan 14].

[3] U.S. Department of Health and Human Services.The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2014 [accessed 2020 Jan 14].

[4] U.S. Department of Health and Human Services. How Tobacco Smoke Causes Disease: What It Means to You. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010 [accessed 2020 Jan 14].

[5]U.S. Department of Health and Human Services. Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General External. Rockville (MD): U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 1989 [accessed 2020 Jan 14].

[6] Craver R. Analyst projection: E-cigs will overtake traditional tobacco revenue at Reynolds in 2021. Winston-Salem Journal, 2013.

[7]Zhu S-H, Sun JY, Bonnevie E, Cummins S, Gamst A, Yin L, & Lee M Four hundred and sixty brands of e-cigarettes and counting: Implications for product regulation. Tob Control. 2014;23 Suppl 3:iii3-iii9. doi:10.1136/tobaccocontrol-2014-051670

[8] Luo, C., Zheng, X., Zeng, D. D., & Leischow, S. (2014). Portrayal of electronic cigarettes on YouTube. BMC Public Health, 14(1), 1028. https://doi.org/10.1186/1471-2458-14-1028

[9] For Healthcare Providers, Outbreak of Lung Injury Associated with E-Cigarette Use, or Vaping. Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 25 Feb. 2020, www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease/healthcare-providers/index.html.

[10] Outbreak of Lung Injury Associated with the Use of E-Cigarette, or Vaping, Products | Electronic Cigarettes | Smoking & Tobacco Use | CDC. (n.d.). Retrieved February 9, 2020, from https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html

[11] Salzman, G. A., Alqawasma, M., & Asad, H. (2019). Vaping Associated Lung Injury (EVALI): An Explosive United States Epidemic. Missouri medicine, 116(6), 492–496.

[12] NIDA. (2020, January 8). Vaping Devices (Electronic Cigarettes). Retrieved from https://www.drugabuse.gov/publications/drugfacts/vaping-devices-electronic-cigarettes on 2020, February 8

[13] American Lung Association. What's in an E-Cigarette. Smoking Facts, 19 Aug. 2019. Retrieved from https://www.lung.org/stop-smoking/smoking-facts/whats-in-an-e-cigarette.html on 2020, February 8

[14] National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. The Health Consequences of Smoking—50 Years of Progress. A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US); 2014.

[15] Drugs-Forum. Dissolving Heroin in E-Cigarette Liquid Then Smoking it in a E-Cig? Available online: https://drugs-forum.com/forum/showthread.php?t=173636

[16] What You Should Know About JUUL. (n.d.). Retrieved February 8, 2020, from https://www.martinhealth.org/what-you-should-know-about-JUUL

[17] Polosa R, Caponnetto P, Morjaria JB, Papale G, Campagna D, & Russo C. Effect of an electronic nicotine delivery device (e-Cigarette) on smoking reduction and cessation: a prospective 6-month pilot study. BMC Public Health 2011;11:786

[16] Kalininskiy A., Bach C. T., Nacca N. E., Ginsberg G., Marraffa J., Navarette K. A., McGraw M, &Croft D. (2019). E-cigarette, or vaping, product use associated lung injury (EVALI): case series and diagnostic approach. Lancet Respir. Med. 7 (12), 1017–1026. 10.1016/S2213-2600(19)30415-1

[19] Kligerman, S., Raptis, C., Larsen, B., Henry, T. S., Caporale, A., Tazelaar, H., Schiebler, M.L., Wehrli, F. W., Klein, J. S., & Kanne, J. (2020). Radiologic, Pathologic, Clinical, and Physiologic Findings of Electronic Cigarette or Vaping Product Use–associated Lung Injury (EVALI): Evolving Knowledge and Remaining Questions. *Radiology*, 192585. https://doi.org/10.1148/radiol.2020192585

[20] Chaffee, Benjamin W. “Electronic Cigarettes: Trends, Health Effects and Advising Patients Amid Uncertainty.” Journal of the California Dental Association vol. 47,2 (2019): 85-92.

[21]E-cigarette use increases susceptibility to bacterial infection by impairment of human neutrophil chemotaxis, phagocytosis, and NET formation Ross Corriden, Alexander Moshensky, Christine M. Bojanowski, Angela Meier, Jason Chien, Ryan K. Nelson, and Laura E. Crotty Alexander American Journal of Physiology-Cell Physiology 2020 318:1, C205-C214

[22]Pahwa R, Goyal A, Bansal P, & Jialal I. Chronic Inflammation. [Updated 2020 Feb 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan

[23] Glynos, C., Bibli, S.-I., Katsaounou, P., Pavlidou, A., Magkou, C., Karavana, V., Topouzis, S., Kalomenidis, I., Zakynthinos, S., & Papapetropoulos, A. (2018). Comparison of the effects of e-cigarette vapor with cigarette smoke on lung function and inflammation in mice. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *315*(5), L662–L672. https://doi.org/10.1152/ajplung.00389.2017

[24] Omaiye, E. E., McWhirter, K. J., Luo, W., Pankow, J. F., & Talbot, P. (2019). High-Nicotine Electronic Cigarette Products: Toxicity of JUUL Fluids and Aerosols Correlates Strongly with Nicotine and Some Flavor Chemical Concentrations. *Chemical Research in Toxicology*, *32*(6), 1058–1069. https://doi.org/10.1021/acs.chemrestox.8b00381

[25] Airway Resistance. Interactive Respiratory Physiology , Johns Hopkins School of Medicine , 1995, oac.med.jhmi.edu/res_phys/Encyclopedia/AirwayResistance/AirwayResistance.HTML.

[26] Respiratory Failure | Lung Disease | Lung Problems. MedlinePlus, NIH U.S. National Library of Medicine, 18 Oct. 2019, medlineplus.gov/respiratoryfailure.html.

[27] Antoniewicz L, Brynedal A, Hedman L, Lundback M & Bosson J. “Acute Effects of Electronic Cigarette Inhalation on the Vasculature and the Conducting Airways.” *Cardiovascular toxicology* vol. 19,5 (2019): 441-450. doi:10.1007/s12012-019-09516-x

[28] Tang MS, Wu XR, Lee HW, Xia Y, Deng FM, Moreira A, Chen LC, Huang W, Lepor H. Electronic-cigarette smoke induces lung adenocarcinoma and bladder urothelial hyperplasia in mice [published correction appears in Proc Natl Acad Sci U S A. 2019 Nov 5;116(45):22884]. Proc Natl Acad Sci U S A. 2019;116(43):21727-21731. doi:10.1073/pnas.1911321116

[29]Oka Y, Takai Y & Touhara K. “Nasal airflow rate affects the sensitivity and pattern of glomerular odorant responses in the mouse olfactory bulb.” *The Journal of neuroscience : the official journal of the Society for Neuroscience* vol. 29,39 (2009): 12070-8. doi:10.1523/JNEUROSCI.1415-09.2009