4-2020

It's Not Cuul to JUUL: Nicotine Addiction, the Rise of e-Cigarette Usage, and How Zebrafish Can Help

Anna Pearson
DePauw University

It's Not Cuul to JUUL:

Nicotine Addiction, the Rise of e-Cigarette Usage, and How Zebrafish Can Help

Author

Anna Pearson

Sponsor

Dr. Henning Schneider - Department of Biology

First Reader

Dr. Kevin Moore - Department of Psychology & Neuroscience

Second Reader

Dr. Scott Ross - Department of Psychology & Neuroscience

Class of 2020

DePauw University

Honor Scholar Program

Acknowledgments

I want to express the deepest and most sincere gratitude to Dr. Henning Schneider, not only for his willingness to sponsor this project but also for the multitude of research and growth opportunities he has provided me during my four years at DePauw University. Dr. Schneider taught me everything I know about both zebrafish and nicotine, giving me all of the tools necessary to carry out this project. Thanks to Dr. Schneider, I am a better writer, a better thinker, and a better scientist. Researching under his guidance for the past two years has been a great privilege and becoming a member of the NicoTeam has been one of my most rewarding college experiences.

Further, I am grateful for Dr. Maria Bondesson and Beas Bhattacharya at Indiana University - Bloomington's Luddy School of Informatics, Computing, and Engineering. Their interest in my project and their willingness to collaborate were instrumental to this thesis.

Next, I would like to thank Dr. Kevin Moore and Dr. Scott Ross for offering their advice, expertise, and time to the success of this project. I am especially appreciative of their feedback, direction, and enthusiasm in encouraging my project from the very beginning.

I would also like to acknowledge the Douglas and Mary Hallward-Driemeier Fund for the Honor Scholar Program for their support of my thesis. I am incredibly grateful for the resources this award provided to assist me in carrying out the empirical aspect of my thesis.

Finally, and most remarkably, I am incredibly grateful for the support of my parents as I worked through my ideas. Mom, thank you for the hugs, laughs, and for your irreplaceable friendship. Dad, thank you for your encouragement and words of support. I owe a great deal of my success to the persistent love and motivation I receive from you both.

This thesis is dedicated to my brother and sister, Sam and Eva. Real recognizes real.

Table of Contents

Introduction

The recreational use of tobacco products materialized centuries ago, with almost every part of the world taking part in this behavior in some capacity (Hammond, 2009). Throughout history, a global slew of shifting cultural norms and ideals has contributed to dynamic perceptions of tobacco product usage and, therefore, nicotine consumption (Hammond, 2009). As the popularity of these products has fluctuated, so have their impacts on the world's medical and economic sectors, forming fascinating cycles of clinical and legislative responses. More specifically, within the United States, a lack of ongoing research focused on types of nicotine consumption has constructed patterns of significant inaction followed by rapid changes in order to counter the consequences of various nicotine-containing products. Organizing consistent research efforts, however, could help to establish more efficient preventative methods for this global health issue. Most recently, the introduction of electronic cigarettes (also known as e-cigarettes or vapes) has again demonstrated the need for more thorough and persistent research in light of an expanding health crisis (Centers for Disease Control and Prevention, 2019).

Many tobacco and nicotine products have a complicated history regarding their health perception. At the height of cigarette popularity in the United States, it was common for tobacco companies to pair with physicians to make advertising claims such as "More doctors smoke Camels than any other cigarette" and "20,679 physicians say Luckies are *less irritating*" (Little, 2018). Unfortunately, it was not until decades later2 that scientists linked smoking to lung cancer (Proctor, 2012). Since this discovery, blunt warnings concerning cigarettes and related products have gained prevalence. For example, in 2006, the World Health Organization (WHO) stated, “cigarettes are among the most deadly and addictive products ever produced by mankind. When used as intended…they kill approximately one-half of their users” (WHO, 2006). Although that particular statement was issued over ten years ago, today, according to the Centers for Disease Control and Prevention (CDC), “smoking is the single largest preventable cause of death and disability in the United States” (2017a). Smoking kills more people than HIV/AIDS, TB, and malaria do combined, although smoking is the most choice-directed and preventable (WHO, 2018). Further, 14% of adults in the United States still report using cigarettes, with 75% of them smoking every day (CDC, 2017b).

The continued use of cigarettes despite such clear cautioning about their effects can be largely attributed to the addictive nature of nicotine (Stolerman & Jarvis, 1995). Only about 19% of smokers are successful in quitting (Viswesvaran & Schmidt, 1992). To further complicate the situation, most smokers have to make multiple attempts before successfully quitting (United States Department of Health and Human Services, 2011), implying that smokers must be highly motivated and tenacious to succeed in cessation. In 2006, electronic cigarettes were introduced to the world as a potential cessation tool (Hajek et al. 2014). The products were designed to simulate the experience of smoking while significantly reducing a person’s exposure to nicotine, tobacco, and other additives in traditional cigarettes (Kaisar et al., 2016). Although the invention was initially slow to gain traction, e-cigarette use drastically increased in popularity in 2015 with the founding of JUUL Labs, Inc., a company specializing in the creation of sleek, modern-looking e-cigarette devices (JUUL Labs, Inc., 2019). Within two years of the company’s founding, JUUL products alone made up 40% of the entire e-cigarette market (Huang et al., 2018).

Despite their stated mission “to improve the lives of the world's one billion adult smokers by eliminating cigarettes,” (JUUL Labs, Inc., 2020), JUUL managed to bring a whole new dilemma to the table: the recreational use of their devices by youth and young adults who had never smoked before. According to the 2019 Monitoring the Future (MTF) Survey conducted by the United States’ National Institute of Drug Abuse, “One in 4 twelfth-graders say they vaped nicotine in the past month, along with 1 in 5 tenth-graders, and nearly 1 in 10 eighth-graders.” Youth gained interest in JUUL devices for several reasons, none of them connecting to cessation purposes. The MTF survey states, “Many teens say they vape for the flavor, to experiment, for social reasons, or to feel good.” In 2019, 20.7% of eighth-graders, 36.4% of tenth-graders, and 40.5% of twelfth-graders reported having ever tried a nicotine vaping product (Miech et al, 2019). To further compound the issue, some research indicates that JUUL devices may serve as a gateway for the use of other tobacco products by children and young adults (Kaisar et al., 2016), completely reversing the intended purpose of JUUL devices.

In addition to influencing an incredibly vulnerable audience, the effectiveness of e-cigarettes as a cessation tool for adults is questionable. Existing literature pertaining to their short- and long-term effects are largely contradictory or inconclusive (Kaisar et al., 2016). This deficit in knowledge proved to be especially problematic in 2019, as e-cigarette use was linked to several thousand hospitalizations, cases of lung damage, and deaths in the United States (CDC, 2019a). As the problem increased in severity, the government quickly began to roll out legislative solutions. For example, in September of 2019, the Trump administration announced plans to remove all flavored e-cigarette products from the market as a response to the outbreak (Peltz, 2019). In early November, however, Trump refused to sign his own decision memo, stating that his proposed ban would lead to significant job losses within the country (Kelly, 2019). Instead, the minimum sales age for all tobacco purchases was increased from 18- to 21-years-old throughout the country in order to make it more difficult for young adults to obtain the products (Howard, 2019). Because most youth e-cigarette users were already under the age of 18, however, the ban may not make as dramatic a difference as one would hope. At the beginning of 2020, the Trump administration did impose a partial flavor ban, calling for the removal of mint- and fruit-flavored products, the flavors most often used by youth, from market shelves and preserving the sale of menthol products (Alltucker, 2020). Due to how recently these policies were established, the effectiveness of these changes in curbing underage use of e-cigarettes remains unclear.

Considering how closely related e-cigarettes are to traditional nicotine-containing products, it is interesting to consider how little attention has been directed towards researching the potential consequences and impacts of e-cigarettes. One reason as to why this type of research was neglected may be tied to the importance of e-cigarettes and other tobacco products within the national and global economies. The economic impact of nicotine products goes beyond the sales between companies and users. Instead, it also encompasses the money that companies pour into the economy on marketing, testing, and employment. For example, the CDC notes that "e-cigarette companies have rapidly increased advertising spending, from $6.4 million in 2011 to $115 million in 2014." (CDC, 2017b). This number has only continued to increase, as the United States Federal Trade Commission (FTC) reports that in 2017, "tobacco companies spent $9.36 billion marketing cigarettes and smokeless tobacco in the United States. This amount translates to more than $25 million each day, or more than $1 million every hour"

(2019). This amount is nearly five times more than the United States spends on advertising and marketing alcoholic beverages (Strasburger, 1999). Therefore, completely eliminating tobacco companies from financial systems could influence other private and public sectors. Further, this influence extends to the global economy. One account states that the sale of tobacco products "produces huge tax revenues for most governments, especially in high-income countries, as well as employment in the tobacco industry" (Ekpu & Brown, 2015). The widespread effect of tobacco and nicotine on many economies likely contributes to the hesitance government officials have to fund research investigating health concerns that may indirectly call for a negative impact on the economy.

As Dr. Neal Benowitz, a prominent physician who has focused much of his career on the effects of tobacco dependence, stated in a 2010 review article, "Tobacco addiction involves the interplay of pharmacology, learned or conditioned factors, genetics, and social and environmental factors (including tobacco product design and marketing)." The current thesis aims to further investigate this interdisciplinary nature of the current vaping crisis in the United States via several objectives. More specifically, this project seeks to better understand the recent spike in e-cigarette popularity by analyzing trends of tobacco usage and nicotine consumption in a historical context. Additionally, this project will investigate the medical and economic influence that e-cigarettes have in the United States, arguing that the United States has largely ignored the importance of proactive, continued, and medically-based research on nicotine-containing products due to the notable influence these items have on the economy.

Moreover, in an effort to emphasize the need for consistent research on e-cigarettes and their constituents while also contributing to the current pool of available studies, the current thesis includes a research project which investigates the impacts of e-cigarette liquids on the behavior of larval zebrafish (*Danio rerio*). Although rodent models have previously proven to be useful in pharmacological studies, they are more expensive and take longer to develop than zebrafish (Kimmel et al., 1995). Zebrafish can be tested much more quickly and at a lower cost than rodent or non-human primate models, making them a useful screening tool for pharmacology before a potential medication advances to rodent, non-human primate, or human trials (Grunwald & Eisen, 2002; Kimmel et al., 1995; Stewart et al., 2014). Previous research has found that about 20% of larval zebrafish seek nicotine (Schneider et al., 2018). The current study is designed to better understand how e-cigarette liquids and their components influence previously established levels of seeking-behavior.

Finally, this thesis aims to better understand how current and potential users of e-cigarettes should approach these devices. Despite the many illnesses and deaths linked to e-cigarettes, many researchers believe that vaping is safer than smoking traditional cigarettes. Research further states that the secondhand smoke from e-cigarettes is likely less harmful than the byproducts of combustible tobacco products (Hajek et al., 2014). With respect to current legislative actions and clinical implications surrounding e-cigarettes, the final objective of this project is to determine whether there are any benefits to e-cigarettes, and how those benefits can be reaped considering the many complications associated with vaping.

A Brief History of Tobacco and Nicotine

Tobacco has a history spanning thousands of years in North America, with its first cultivation taking place around 6000 BCE (Brandt, 2007). Beginning at approximately 1 BCE, the crop was used in Native American religious ceremonies and medical practices (Brandt, 2007). When Europeans reached America in 1492, the indigenous people offered dried tobacco as a gift to the colonists. At first, the settlers did not immediately understand the plant's value, accepting the gift but not fully comprehending its use and potential (Hammond, 2009). They eventually realized, however, that tobacco possessed qualities that made it especially desirable and useful for trade. In 1612, John Rolfe was the first settler to grow tobacco in North America at his plantation in Jamestown, Virginia, launching the commercialization of the plant and marking the beginning of its economic influence (U.S. National Park Service, 1998). Within the decade, tobacco became the leading export in the United States (U.S. National Park Service, 1998).

During this early phase of tobacco's history in North America, many people embraced the Native American's belief in the medicinal powers of the plant, especially for colds and fevers (Charlton, 2004). At the onset of its popularity, the tobacco grown by settlers was used for pipe-smoking, snuffing, and chewing (Brandt, 2007). Over time, the use of tobacco became more leisurely and reliance on its curative uses was minimized. In the early 1800s, cigars gained notable popularity in the United States as a more recreational means of consuming tobacco and nicotine (Altman, 2009). While the introduction of cigars certainly increased tobacco consumption in the United States, the automation of cigarette production completely revolutionized the tobacco industry (Burns et al., 1997).

James Albert Bonsack developed the first cigarette-making machine in 1881, an invention that significantly increased cigarette production and allowed for a significant rise in distribution (Witschi, 2001). Prior to Bonsack's invention, the habit of smoking cigarettes was considered especially glamorous as cigarettes were almost exclusively used by the wealthy elite (Rodrigues, 2009). This was largely due to the fact that cigarettes had to be hand-rolled, a slow process that yields little output, with one person typically rolling about 200 cigarettes in a day (Burns et al., 1997). Bonsack's machine, on the other hand, could produce 120,000 cigarettes in approximately 10 hours (Burns et al., 1997). The ability to mass-produce cigarettes made them more accessible to the general public, and soon the means of tobacco consumption began to shift. In 1900, cigarettes went from composing 2% of tobacco consumed in the United States, whereas chewing tobacco made up 50% of consumption (Hammond, 2009). Fifty years later, cigarettes constituted 80% of the country's tobacco consumption (Hammond, 2009).

A 1999 study by the CDC analyzing smoking trends in the United States throughout the 20th century found several factors, in addition to the rise of mass production, that contributed to the increase in smoking during this period. These factors include: "the introduction of blends and curing processes that allowed the inhalation of tobacco, the invention of the safety match…transportation that permitted widespread distribution of cigarettes, and use of mass media advertising to promote cigarettes" (CDC, 1999). All of these factors significantly increased the ease by which people could obtain cigarettes. Throughout the 20th century, more people were drawn to cigarettes than ever before, but with little knowledge of exactly why their appeal was so strong or of the impacts that these products could possibly have on the user's health.

While tobacco was increasing in popularity throughout North America, other parts of the world were trying to better understand the addictive qualities of the crop. Two German physicians were the first people to extract nicotine from tobacco in 1828. Wilhelm Heinrich Posselt and Karl Ludwig Reimann concluded that the chemical compound they discovered was responsible for the addictive qualities of tobacco products (Goodman, 2005). Additionally, they demonstrated that the compound possessed poisonous characteristics, causing hypertension and various types of organ malfunctions in dogs and rabbits (Mishra et al., 2015). They ended their report by declaring strong suspicions that the chemical would also have harmful effects on humans (Goodman, 2005). Despite the alarming conclusions Posselt and Reimann drew from their studies, the scientific community expressed minimal interest concerning further research efforts on nicotine and its effects. Consequently, tobacco remained a staple crop around the world and its use continued to serve as a favored pastime in the United States (Goodman, 2005).

It was not until nearly a century later that the first connection between smoking and lung cancer was reported, although the study only presented correlational data. In 1912, Isaac Adler, another German physician, reported that smoking was likely to blame for an increase in lung tumors found during the autopsies of soldiers from German tobacco workers (Proctor, 2012). Due to the lack of causal information, society expressed minimal concern and little change came about in response to the findings. Several more decades passed before the American Cancer Society finally released its first warning to smokers in 1944 suggesting that cigarettes may influence a user’s susceptibility to developing lung cancer. (U.S. Department of Health and Human Services, 2014). This initial announcement, however, did little to affect the general public's loyalty to their tobacco products and cigarettes remained America's choice of tobacco product (Hammond, 2009), likely due to the ambiguous nature of the available data.

From there, the American Cancer Society sought to conduct its own research on the link between cigarettes and cancer. In 1952, Dr. E. Cuyler Hammond and Dr. Daniel Horn were hired to conduct a study following approximately 188,000 American men aged 50 to 69 (Mendes, 2014). The men were interviewed extensively about their cigarette use. For about 20 months, researchers checked in on the men to determine whether or not they were still living. If the participants had died since the last check-in, the researchers obtained their death certificate to determine their cause of death. Hammond and Horn found that men who had smoked cigarettes had a significantly higher death rate than men who consumed tobacco through other means and men who did not use tobacco at all. They also noted that the cause of death for men who smoked cigarettes was typically cancer or heart disease (Hammond & Horn, 1958). When this data was first presented in 1954, it was the largest and most convincing study on the subject, even influencing Hammond and Horn to give up cigarettes themselves (Mendes, 2014).

After obtaining this preliminary data, Hammond continued to work with the American Cancer Society on collecting evidence of the harmful effects of cigarettes. His work influenced a 1961 letter to President John F. Kennedy urging him to create a coalition tasked to tackle this public health issue (Mendes, 2014). President Kennedy assigned the job to Luther Terry, the Surgeon General at the time. In 1964, with help from Hammond's latest data, Terry made a landmark report on smoking in the United States, calling for more extensive research. This report stated that cigarettes were a cause of both lung and laryngeal cancers in men, a probable cause of lung cancer in women, and the single most important cause of chronic bronchitis (CDC, 2006).

As a result of this report, many scientists and doctors began to focus on the relationship between smoking and cancer at this time (Mendes, 2014). As a result of Terry's report and the resulting studies, the United States began to respond to warnings regarding nicotine with unprecedented urgency.

Eleven days after Terry's announcement, the FTC required that cigarette manufacturers place warning labels on all of their products (Cummings, 2002). As a result, cigarette sales began to decline, but manufacturers began to advertise their "filtered" cigarettes, marketing them as a safer alternative that would not expose the user to as many harmful toxins (Cummings, 2002). Research has since established that negative health outcomes of filtered and unfiltered cigarettes are the same, indicating that the filtered versions do not provide a health advantage, with some studies even claiming that the filtered versions pose an even greater health risk (Hall, 2017).

Throughout the 50 years that have passed since Terry's 1964 report, many new control mechanisms have been established to hinder smoking. For example, tobacco advertising decreased, the circulation of public warnings increased, and cigarette taxes were implemented (CDC, 2006). Since the establishment of these early policies, smoking in the United States smoking rate has dropped by approximately 50% (Mendes, 2014). While this drop is certainly significant and a step in the right direction, the CDC estimates that smoking still kills more than 480,000 Americans every year (CDC, 2017b). In other words, although the problem of cigarette and tobacco use has slowly diminished, a severe public health issue still exists.

The sustained prevalence of smoking likely exists due to the physical and psychological hardships that frequently accompany cessation attempts. In a 2018 report, the American Heart Association stated that "Nicotine is addictive – reportedly as addictive as cocaine or heroin."

With this statement, the American Heart Association communicated two main points: 1) Nicotine addiction is just as serious and concerning as other kinds of drug addictions, and 2) Quitting is hard. With this kind of severe addiction comes the emergence of withdrawal symptoms when people try to give up smoking. These symptoms include experiences such as: "irritability, frustration or anger, anxiety, dysphoric or depressed mood, restlessness, difficulty concentrating, insomnia… cough, dizziness, increased dreaming, and mouth sores" (Hatsukami, Stead, & Gupta, 2008). In an effort to avoid these symptoms, people often relapse as they lean into the temptation of immediate gratification. In other words, users may conceptualize the immediate experience of quitting as more unpleasant than the potential of developing the various long-term consequences of smoking. As a response to the tension between the short-term effects of cessation and the long-term effects of prolonged use, a continuously-growing market of cessation programs and devices has emerged to help mitigate the experience of withdrawal symptoms and assist patients in kicking their habit of smoking.

Some of the most common cessation programs include combinations of pharmacological interventions, psychotherapeutic methods, and nicotine-replacement therapies (Hatsukami, Stead, & Gupta, 2008). The effectiveness of each of these programs is highly variable. Just as the likelihood of an individual developing a nicotine addiction depends on a combination of genetic and environmental factors, the effectiveness of treatments also depends on many of the same elements (Hatsukami, Stead, & Gupta, 2008). Because of the inconsistency of success amongst these treatments, an ongoing search exists for a more reliable device.

In response to this important search, the e-cigarette was invented by Chinese pharmacist Hon Lik in Hong Kong in 2003 (Hajek et al., 2014). The devices generally consist of a mouthpiece where the user inhales vapors, a cartridge that contains a nicotine solution dissolved in propylene glycol and/or glycerin, a heating element that turns the nicotine solution into vapor, a microprocessor that activates the heating element when the mouthpiece is used, and a battery (Goniewicz et al., 2013). The e-cigarette was a popular choice for people interested in quitting because the original design looked just like a cigarette, giving the user the experiential component of smoking while allegedly eliminating many of the concerning contents of traditional cigarettes. Further, at this time the world began to widely accept the fact that cigarette smoke is toxic to both the user and those who were just in close proximity to the smoke. E-cigarettes were designed to be less toxic to all parties, therefore enhancing their overall appeal as users began to believe that they would be able to use the product anywhere, regardless of preexisting smoking laws banning cigarette use in many public areas (Grana, Benowitz, & Glantz, 2014).

E-cigarettes were first introduced to the United States in 2006 (Hajek et al. 2014). Although manufacturers enthusiastically advertised these products as a cessation tool, little empirical evidence backed up their claims. In 2008, WHO asserted that it did not consider e-cigarettes a valid smoking cessation device, demanding that companies remove any advertisements implying efficacy (World Health Organization, 2008) for the time being. Regardless of this uncertainty, Americans still took faith in e-cigarettes, showing an increased interest in these products. This behavior undoubtedly warrants continued research on the ability of e-cigarettes to curb smoking habits. The inflexibility of American smokers in 2008 reflected smokers' behaviors in 1944 when large-scale public announcements began to circulate regarding the dangers of traditional cigarettes. Researchers began to respond again with studies attempting to confirm the underlying processes of this unconventional means of smoking and its influence on the human body.

The United States Food and Drug Administration (FDA) published its first studies in 2009, attempting to answer basic questions regarding e-cigarettes. They first tested two brands of e-cigarettes, NJOY and Smoking Everywhere, to determine the amount of nicotine in each product. Although both brands were advertised as nicotine-free products, the FDA found that both products contained low-levels of nicotine (FDA, 2009). Two months after this conclusion, the FDA released a warning to consumers about the risks of using e-cigarettes. In this warning, the FDA stated concerns that "consumers currently have no way of knowing whether e-cigarettes are safe for their intended use" (2009). Additionally, for the first time, the FDA warned that "e-cigarettes can increase nicotine addiction among young people and may lead kids to try other tobacco products, including conventional cigarettes" (2009). Although research skeptical of e-cigarettes was gaining traction, the e-cigarette market was quickly expanding. One study suggests that "In 2014, there were an estimated 466 brands and 7764 unique flavors of e-cigarette products" (Dinakar & O'Connor, 2016). Although the use of e-cigarettes at this time was obviously common, it would soon skyrocket. In fact, the devices would eventually begin to influence the lives of millions of Americans beginning in 2015, the year that JUUL, Inc entered the equation (JUUL, Inc, n.d.).

JUUL Inc. was founded by Stanford graduates Adam Bowen and James Monsees. Their device, simply known as a "JUUL," was designed as a fresh take on e-cigarettes. Like most e-cigarette and vape products at the time, JUUL, Inc. was co-founded as an attempt to find an effective alternative for smoking. Both co-founders had been smokers themselves and attributed their previous vice as a chief reason as to why their product had such an appeal amongst smokers (JUUL, Inc, n.d.). They used their insight on the various struggles smokers face in quitting to develop their idea, and their knowledge of industrial design to create a sleek product that appealed to many people. According to the JUUL website, Bowen and Monsees were so wildly successful in creating their product because “As smokers, they knew a true alternative to cigarettes would have to offer a nicotine level found in no other alternative on the market. It would also have to invite its own ritual. The result was JUUL” (JUUL, Inc, n.d.). Smooth, black, and discreetly resembling a flash drive, the devices have been referred to as “the iPhone of e-cigarettes” by several sources (Huang et al., 2014), and they have certainly become ritualistic for many groups, but not in the same manner that Bowen and Monsees proposed.

Instead of adults using JUUL products for smoking cessation, youth and young adults have taken hold of the trend and are using these devices for recreation. Youth usage is alarming because adolescents and young adults are often unaware of the effects of e-cigarettes or carefree to the potential risks (Fadus et al., 2019). To further compound the issue, e-cigarettes often serve as a type of gateway device for other means of tobacco and drug use (Fadus et al., 2019). A meta-analysis by Fadus et al. (2019) found that e-cigarette use is positively associated with traditional cigarette and cannabis use. The study also found that adolescents and young adults using JUULs are three times more likely to be using these other products compared to age-matched controls who do not JUUL (Fadus et al., 2019).

Although JUUL claims that they did not purposefully advertise their products to adolescents, not everyone believed that JUUL’s actions were unintentional. JUUL’s advertising techniques have been blamed a number of times for targeting this particular demographic group

(Mitchell & Young, 2019). In October 2019, Sheila Kaplan, an investigative reporter for the New York Times, criticizes JUUL's advertising tactics on a podcast by *The Daily*:

> Publically, JUUL is saying that they want to be an alternative for smokers. But the advertising and social media are really all about how cool JUUL is… JUUL takes on a very creative, silicon valley-like ad campaign. Ads that have attractive young people vaping in glamorous settings like the beach and concerts. They have influencers on Instagram and Facebook who are beautiful and in love and vaping together. (Mitchell & Young)

Furthermore, the New York Times article claims that JUUL "began targeting consumers in their 20s and early 30s… in a furious effort to reward investors and capture market share before the government tightened regulations on vaping" (Creswell & Kaplan, 2019). In other words, some groups believe that JUUL had always plotted to target these specific groups, while others believe that their advertising was a result of legal pressures. The FDA made it necessary for e-cigarettes to include health warnings on their products (Fadus et al., 2019), but by this point, the damage was already done. As a result, JUUL is facing hundreds of ongoing lawsuits. Some of these lawsuits address the aforementioned advertising concerns, while others pose claims that the company's products were linked to the development of various health problems in users, ranging from nicotine addiction to lung cancer (Ducharme, 2019).

On November 13th, 2018, because of FDA regulations, JUUL removed all posts from their Instagram account, aside from a message stating that the account would become inactive following the current post (Fadus et al., 2019). Although their Twitter account once displayed celebrity testimonials and colorful photos displaying their range of flavors, the account now features research efforts, and many of the tweets are focused around preventing underage vaping. Today, JUUL's official YouTube channel only features testimonials of adults who stopped using combustible tobacco products in favor of JUULs as opposed to the upbeat commercials showcasing the JUUL's modern design (Fadus et al., 2019).

Unfortunately, these changes came too late for many users. In June of 2019, patients began to enter hospitals around the United States complaining of pneumonia-like symptoms, such as weight loss, shallow breath, and increased fatigue (Abbott, 2019). Considering that pneumonia cases are far more prevalent during the colder months of Winter, doctors were especially stumped by the timing of these complaints (Abbott, 2019; Butala et al., 2019). Health care professors quickly discovered a striking commonality between all of these unseasonal cases: all patients used vaping devices or e-cigarettes (Abbott, 2019). Not long after this discovery, the United States reported its first death related to an e-cigarette, or vaping, product use-associated lung injury (EVALI). As of February 18th, 2020, the CDC reported 2,807 hospitalized EVALI cases from all 50 states, the District of Columbia, and two U.S. territories (Puerto Rico and the U.S. Virgin Islands). Further, 68 deaths had been confirmed in 29 states and the District of Columbia.

These alarming statistics pushed the world to the quickest, most dramatic actions it had ever taken in response to a nicotine product. China banned the devices from online markets, and they are completely banned in India (Diaz, 2019). Massachusetts placed a four-month ban on the sale of vaping products and Walmart completely stopped selling e-cigarettes. The United States government was in the spotlight as the world awaited its response.

As opposed to acknowledge a dire need for increased research on e-cigarettes and exactly how they were producing these symptoms, the government reacted to the illnesses and deaths by quickly imposing several legislative actions. In early September of 2019, the Trump administration proposed a ban on all flavored tobacco products in order to minimize youth use of e-cigarettes. Trump later feared that a strict ban would have a significant negative influence on unemployment, causing hundreds of tobacco workers to lose their jobs. Instead, the administration chose to raise the legal age to buy tobacco products to 21 in December of 2019 (Howard, 2019). The legal age increase was catalyzed by a statistic from the Surgeon General claiming that 95% of smokers begin using tobacco before 21 (Preston, 2019). Research has supported this claim; according to Jordan & Andersen (2017), "As individuals continue to mature between 13 and 21 years, the likelihood of lifetime substance abuse and dependence drops 4-5% for each year that initiation of substance use is delayed." The thought process behind the legal age increase is, if people are not smoking before age 21, they may never start.

Many health and law officials did speculate that the age increase would be strong enough to curb current smoking and vaping habits among youth, so at the beginning of 2020, the FDA passed a ban on mint- and fruit-flavored e-cigarette products. This ban was much more narrow than the one that the Trump administration proposed in early September of 2019, as the current restrictions still allow for menthol- and tobacco-flavored products to be sold with minimal. Further, the ban temporarily allows vape and smoke shops to continue selling flavors from open-tank systems where customers can mix their own nicotine with various flavoring agents. While the ban is not perfectly restrictive, in combination with the raised tobacco age, it does have the potential to combat a large portion of youth use (Alltucker, 2020).

While the government was rapidly issuing these back-to-back policy changes, the CDC pushed out an important announcement regarding the Summer's medical cases, stating, "National and state data from patient reports and product sample testing show tetrahydrocannabinol (THC)-containing e-cigarette, or vaping, products… are linked to most EVALI cases and play a major role in the outbreak" (CDC, 2019a). In other words, physicians found that many of the people experiencing effects vaped a combination of THC, the active chemical in marijuana, and nicotine, implying that companies like JUUL were not responsible for the majority of the hospitalizations. Because THC is still illegal in the United States and therefore is not federally regulated, users often resort to the black-market to get their devices (Sawrey, 2019). These illicit devices are sometimes called "dank vapes," and almost all of them contain Vitamin E acetate, the substance that may be the culprit behind this particular lung disease (Sawrey, 2019). Dank vapes use Vitamin E acetate to increase the size of the smoke cloud a user blows after using their e-cigarette or vaping device, and it was found in the lungs of the majority of EVALI patients (CDC, 2019a). Conversely, this compound is not found in solely nicotine-containing, federally regulated e-cigarette and vaping products.

With all of these things in mind, it is important to note that still, not all of the illnesses and deaths were linked to marijuana vaping; some patients contracted EVALI by only using nicotine-containing e-cigarettes. Further, just because nicotine-only products did not necessarily cause the hospitalizations does not mean that they are not having other, potentially major, negative impacts on the body. There is currently minimal data regarding the long-term impacts of e-cigarette use, meaning that continuous users could be subject to developing illnesses just as long-term cigarette users are more likely to develop heart disease and lung cancer (CDC, 2006).

Additionally, some studies argue that e-cigarettes actually are effective as a cessation device (Brown et al., 2014; Rahman et al., 2015), implying that changing policies may be causing unnecessary stress to adults who are appropriately using the products to quit traditional cigarette use. Although government-issued policies are making attempts to curb smoking habits, these ventures are focused primarily on youth and young adults, largely ignoring the influence of these products on adults. A lack of research on this area combined with the increasing amount of policy changes makes it difficult to tease out whether or not e-cigarettes are truly effective when used appropriately by adults for cessation purposes. Although laws have largely restricted many aspects of e-cigarettes and their components, these devices still need to be thoroughly analyzed in order to better understand any potential efficacy they have when used appropriately.

In addition, the legislative changes are riddled with loopholes that could potentially diminish their overall effectiveness. For example, just as underage individuals find ways to access alcohol, adolescents can certainly manage to enable the continued use of e-cigarettes and vaping products. Therefore, e-cigarette use still remains a critical issue to be researched, as does tobacco consumption at large. In 2018 alone, United States tobacco farms harvested over 533 million pounds of the crop, showcasing the strong ties the country continues to have with tobacco and its uses (U.S. Department of Agriculture, 2019). The current state of tobacco use and abuse in the United States continues to call for prolonged and intensive research on potential cessation therapies that yield consistently high success rates. With so many unanswered questions still surrounding tobacco, e-cigarette, and nicotine use, there is a great need to further investigate the potential medical consequences and an array of scientific unknowns underlying these products before adopting further policy changes.

Medical Consequences and Scientific Unknowns

E-cigarettes have a significant influence on society, yet numerous aspects of these devices remain unexplored. This lack of research makes it especially difficult to accurately assess the true effects of e-cigarettes on both smoking cessation and general health outcomes. In 1999, the CDC asserted that "research is needed to determine whether new 'highly engineered' products can reduce the harmful effects of tobacco or whether the mistakes associated with low tar and nicotine cigarettes will be repeated." While e-cigarettes have yet to produce as negative of an impact as traditional cigarettes, a troublesome pattern, one of ignoring a need for research until an enormous problem emerges, may still develop. Despite the CDC's early advice, initial research efforts on e-cigarettes were minimal, and the few studies that were published did not receive widespread promulgation. Further, many of these early studies focused on the effectiveness of these products on cessation attempts rather than on the physical effects they have on the body. After the 2019 outbreak of EVALI cases, policy changes trumped over research efforts, leaving many unanswered questions regarding exactly how e-cigarettes impact health. Considering that e-cigarettes containing only nicotine made up a small percentage of the EVALI illnesses and deaths (CDC, 2019a), it is crucial to better understand which aspects of these products could have contributed to the observed negative health outcomes.

On the other hand, some of the unknowns accompanying e-cigarettes could provide useful explanations as to why these products increase the rate of traditional cigarette cessation success among some groups of users. In studies focused on adults who use e-cigarettes as a cessation therapy, numerous researchers report optimistic conclusions about these devices

(Brown et al., 2014; Rahman et al., 2015; Siegal et al., 2011). Unfortunately, many of the legislative changes focused on curbing adolescent e-cigarette use also make it more difficult for adults to obtain their preferred vaping products, especially if they had previously relied on flavoring agents. This difficulty can potentially create a huge hurdle in a person's journey to quit smoking. The tension between observed negative health outcomes within some individuals and observed cessation success in others calls for more refined studies about e-cigarettes to confirm their actual benefits and detriments.

Perhaps one explanation underlying the lack of early research on e-cigarettes and their health impacts stems from how quickly the devices evolved during their first several years on the market (Dinakar & O'Connor, 2016). Some researchers claim that various "generations" of e-cigarettes can produce different effects and consequences. For example, newer brands and models, like JUUL and NJOY, may be more satisfying to use than older models due to their overall appearance and discreteness, but may also produce more harmful effects depending on the contents of the liquid (Dinakar & O'Connor, 2016). An overwhelming number of e-cigarette products currently exist on the market, with one source reporting over 7,000 different kinds of flavors available for purchase, not including products that are inevitably purchased on the black-market (Dinakar & O'Connor, 2016). The chemical makeup of these chemicals varies, and therefore all of the chemicals have different effects on the human body. Therefore, researchers are faced with many choices when deciding which flavors and products to examine. Because the e-cigarette industry is largely dominated by JUUL, as the company holds approximately 75% of market shares, it is important for researchers to prioritize researching this specific device and how its components impact both negative health outcomes and cessation (LaVito, 2018).

JUUL attempts to be transparent about the components of their products, but the company provides minimal information on how these elements and their combination actually impact the human body. According to their website, JUUL pods contain a mixture of propylene glycol and glycerin, which work to create a visible vapor that resembles a smoke cloud (JUUL, Inc, n.d.). Notably, the long-term effects of the aerosolization and inhalation of two major components of all e-cigarettes, propylene glycol or vegetable glycerin are unknown, although their ingestion is FDA approved (Fadus et al., 2019). In addition to the propylene glycol and glycerin mixture, JUULs also contain benzoic acid. JUUL's website states that benzoic acid "is an ingredient that when combined with nicotine as part of our nicotine salts formulation, helps provide cigarette-like satisfaction" (JUUL, Inc, n.d.). While chemists have found that benzoic acid makes nicotine salts easier to inhale, they have also uncovered the compound's relation to several alarming side effects, such as significant nose, throat, and eye irritation when a person is exposed to it in its vapor form (National Center for Biotechnology Information, 2020).

In addition to the propylene glycol and glycerin mixture and benzoic acid, JUUL states that its products contain "pharmaceutical grade nicotine from tobacco plants" (JUUL, Inc, n.d.). JUUL pods can contain up to 5% nicotine, compared to most other devices which hold concentrations of 1-3% (JUUL Labs, Inc., 2020). This high nicotine concentration makes their product especially addictive relative to other alternatives on the market, as nicotine is the active ingredient that triggers addiction (Blaha, 2020). While the mechanisms underlying e-cigarettes are still not completely understood, the negative effects of nicotine have been extensively investigated. One meta-analysis documented several alarming findings concerning the effects of nicotine on various organ systems. The meta-analysis documents several consistent findings that emphasize the widespread effects of nicotine on the human body. According to Mishra et al., nicotine consumption decreases the heart's efficiency, increases the risk of chronic kidney disease, leads to cancers of the lung, pancreas, breast and the gastrointestinal system, causes loss of penile erections and erectile dysfunctions, and causes immunosuppression (2015). Notably, this list is not comprehensive of all of the study's findings, further demonstrating the strong relationship between nicotine and negative health outcomes (Mishra et al., 2015). Due to nicotine's widespread adverse effects on body systems, as well as its addictive properties, the reviewers concluded that nicotine products should be under the control of a trained medical professional (Mishra et al., 2015).

A different publication by Kennedy et al. (2019) reviews experimental studies that have investigated the effects of nicotine on the cardiovascular system. This review explains that the nicotine initiates a metabolic pathway which arouses the sympathetic nervous system, in turn stimulating the cardiovascular system and increasing myocardial contractility, heart rate, blood pressure, and coronary vasoconstriction. Additionally, the reviewers clarify that although e-cigarettes were designed to alleviate the cardiovascular influence of combustible tobacco products, they do not eliminate the problem because they still contain nicotine, which is the true culprit behind the cardiovascular effects observed after prolonged tobacco use. The researchers also mention that smokeless tobacco use is associated with fatal coronary artery disease, although existing research cannot tell whether this relationship is due to nicotine or due to other substances in e-cigarettes (Kennedy et al., 2019).

The documented negative effects of nicotine are especially crucial to keep in mind when analyzing e-cigarettes as some researchers have found that people who use e-cigarette are exposed to more nicotine than people who use other kinds of tobacco products (Blaha, 2020). A report from Johns Hopkins Medicine found that when using e-cigarettes, “you can buy extra-strength cartridges, which have a higher concentration of nicotine, or you can increase the e-cigarette’s voltage to get a greater hit of the substance” (Blaha, 2020). While JUUL pods vary in their nicotine content, most pods contain about the same amount of nicotine as a pack of cigarettes (JUUL, Inc, n.d.). Moreover, according to previous research, smoking an entire JUUL pod is not uncommon amongst users (Jackler & Ramamurthi, 2019). This trend, which includes adolescents and young adults, is especially alarming if the user typically smokes less than a pack of traditional cigarettes a day, and even more troublesome if the user has no previous history with tobacco use, as they are exposing themselves to more nicotine than they would have otherwise.

The final and most ambiguous component of JUUL products is flavor. As stated on the JUUL website, “flavors consist of both natural and artificial flavor ingredients which provide the specific taste profile for each flavor” (JUUL, Inc, n.d.). In other words, the flavors are used to mask unwanted flavors and scents accompanying the other ingredients. JUUL’s website does not include further information concerning the chemical makeup of its flavoring agents but does state that the company uses “tasting panels of trained users to ensure consistent flavor from batch to batch” (JUUL, Inc, n.d.). One study attempting to gain more insight into the actual chemical makeup of these flavoring used gas spectrometry to find that JUUL flavors contain over 50 different substances, with some of these substances existing in high enough concentrations to be considered cytotoxic (Omaiye et al., 2019). That is to say, the substances have the potential to cause severe cell damage (Omaiye et al., 2019). In an effort to elaborate on the cytotoxic nature of these substances, a different study found that JUUL flavors induced oxidative stress and inflammatory responses in lung epithelial cells and macrophages. The results of this study demonstrate that the inclusion of these flavors in JUUL pods is not risk-free (Rahman, Lamb, & Muthumalage, 2019).

Analyzing flavoring agents can be especially complicated because these substances can be toxic in some contexts, but completely harmless in others. Simply put, when substances are inhaled rather than ingested, they undergo different metabolic processes in the body and have the potential to produce different physical effects (Kanwal et al., 2006). This concept was illustrated in the 1990s when a mysterious illness began to emerge among people who worked at a microwave popcorn plant. Many of the factory workers developed a dry cough and shortness of breath, symptoms of an ailment that would later be diagnosed as bronchiolitis obliterans (Kanwal et al., 2006). More colloquially referred to as “popcorn lung,” physicians later found that this condition was associated with exposure to a chemical found in the factory environment (Kanwal et al., 2006). The factory workers were inhaling diacetyl, the substance that gives microwave popcorn a buttery flavor. Although diacetyl is typically safe when ingested, when inhaled, it creates a coating on the inside of the lungs, which results in adverse functioning (Galbraith & Weill, 2009).

One study tested a variety of e-cigarette brands and flavors and found diacetyl in the majority of its samples (Allen et al., 2016). Similar effects have been seen with other flavoring agents, such as cinnamaldehyde, a chemical that produces a cinnamon flavor, and vanillin, a chemical that produces a vanilla flavor (Clapp et al., 2017; Sherwood & Boitano, 2016). Notably, only a small amount of the many different types of vaping liquids have been tested, meaning there is still a lot of work to be done in order to fully understand the full scope of flavoring effects (Kennedy et al. 2019).

In addition to understanding the effects of individual e-cigarettes, it is also critical to understand the general toxicity of e-cigarette vapor when all of these elements are combined. So far, existing studies have raised major concerns about the harmful outcomes of e-cigarette vapors. One study by Lee et al. (2018) measured DNA damage in different organs of mice who were exposed to e-cigarette smoke. Researchers found mutated DNA in the lung, bladder, and heart of mice exposed to e-cigarette smoke. Approximately 22.5% of mice exposed to e-cigarette smoke with nicotine for 54 weeks developed lung adenocarcinomas. This study implies that e-cigarette smoke is carcinogenic and can cause lung and bladder cancer, as well as heart disease.

Another study by Madison et al. (2019) found that when mice were exposed to e-cigarette vapor every day for four months, they exhibited altered lung lipid homeostasis in alveolar macrophages and epithelial cells. That is to say, their lung cells could not function normally after experiencing long-term e-cigarette exposure. This damage was further exemplified when mice who were exposed to these vapors were later infected with a strain of influenza. Mice who had been exposed to the e-cigarette vapors had a more difficult time overcoming the infection in comparison to mice who had never been exposed to the vapors. It should be noted that the mice in Madison et al.'s study were exposed to vapors that did not contain any nicotine, implying that the observed toxicity is driven by a different substance in the product. More extensive research must be published before scientists can deduce whether a single product is linked to the health issues in mice, or if the combination of ingredients is the actual culprit (2019).

Moreover, researchers have found that ingredients found in the devices themselves, rather than just in the vaping liquids, are consumed by users and can have a toxic influence. For example, one study discovered volatile organic compounds (VOCs) and toxic metals in the urine of over 5,000 e-cigarette users (Goniewicz et al., 2018). Acrylonitrile and ethylbenzene, noxious VOCs found in plastics and gasoline, respectively, are components of e-cigarettes. Additionally, dangerous metals like cadmium and lead increase overall toxicity of these products. The study found that e-cigarette users had high levels of biomarkers related to both volatile organic compounds and harmful metals, although these levels were not as high as levels in people who smoke traditional cigarettes (Goniewicz et al., 2018). These findings are significant because they emphasize the wide array of factors that play into e-cigarette toxicity beyond the main ingredients of the vaping liquids.

Although the aforementioned studies are certainly helpful in understanding some of the health effects of e-cigarettes, the studies are accompanied by several limitations that scientists eventually will have to overcome in the future to more accurately assess the impact these devices have on health and wellbeing. For instance, the bulk of the current research on e-cigarettes and their medically-related effects use animal models or *in vitro* cell studies. While animal models can provide scientists with helpful information and give them a rough idea about what effects can potentially manifest in humans, the results of these studies are not directly generalizable. Further, *in vitro* studies are likely exposing cells to concentrations of nicotine that are far higher than the concentrations that humans are exposed to when they are using either e-cigarettes or combustible tobacco products, meaning the effects noted in these studies may appear more severe than they would in the cells of e-cigarette users (Kennedy et al., 2019).

The few studies that do actually focus on human subjects only measure short-term effects, as e-cigarettes are still a relatively new invention. Therefore, subjects who do not experience some of the previously studied short-term effects may not be immune to future risk. At this time we cannot determine whether e-cigarette use influences one's susceptibility to respiratory and heart diseases in the future because many users have not been vaping long enough for studies to be conducted on these associations. Finally, human studies typically only investigate healthy adult patients are used, eliminating any discussion of the impacts of e-cigarettes on high-risk groups (Kennedy et al., 2019). High-risk groups include people with pre-existing medical conditions, dual users (meaning those who smoke both traditional cigarettes as well as e-cigarettes), and adolescents (Schroeder, 2019).

Despite evidence of the harmful consequences of e-cigarette use, a large population of people has still opted to give e-cigarettes a try. Furthermore, a number of reports have described the ability of e-cigarettes to actually help curb smoking habits. For example, a 2011 study by Siegal et al. found that 31% of survey respondents who used e-cigarettes remained abstinent from traditional cigarettes after 6 months, and 34.3% of these respondents were able to quit both e-cigarettes and traditional cigarettes. Although traditional cigarette cessation success was not perfect, 66.8% of respondents had at least reduced the number of cigarettes they used per day (Siegal et al., 2011). Notably, the study found that respondents who used their e-cigarettes at least 20 times a day had a high rate of traditional cigarette cessation, at 70% (Siegal et al., 2011). That being said, Siegal et al. did not elaborate on the health consequences of using e-cigarettes over 20 times a day or whether this kind of e-cigarette use actually reduced the harmful effects of using traditional cigarettes (2011).

A different study by Brown et al. (2014) found that, according to self-reports, smokers were more successful in quitting traditional cigarettes by using e-cigarettes compared to other nicotine-replacement therapies, such as nicotine patches or nicotine gum. With this finding in mind, the study does mention that the success of e-cigarettes may be attributable to how mechanistically similar smoking and vaping are to each other. In other words, smoking an e-cigarette is a very comparable process to smoking a traditional cigarette, which could help better ease the transition to cessation when compared to other cessation therapies that do not give the same psychological and physical sensations of using a cigarette.

Additionally, a meta-analysis conducted by Rahman et al. (2015) found that in six studies examining smoking cessation success, e-cigarette users were the most successful in quitting or reducing traditional cigarette use, echoing the major findings of Brown et al. (2014). Both studies, however, fail to mention how much nicotine is actually delivered to a person through various nicotine-replacement therapies. If e-cigarettes are delivering especially high concentrations of nicotine to the user in comparison to other cessation therapies, it would make sense as to why they were so much more effective at helping smokers quit. On the other hand, high nicotine delivery rates raise concerns about health consequences. It will be essential for future studies to focus on more specific mechanisms underlying e-cigarettes and exactly why some people find these products to be so effective in their cessation journey.

Understanding the rate of e-cigarette nicotine delivery is an especially important goal considering that not all e-cigarette users are vaping in an effort to quit smoking. A study by Nardone et al. (2019) used surveys to gather data from 15 participants regarding their personal e-cigarette habits, specifically as they pertained to JUUL devices. In the survey, participants, who range from 21- to 51-years old with an average age of 29.8 years, were asked to indicate all of the reasons why they began using e-cigarettes. Nine of the 15 participants used JUUL devices to reduce risk from tobacco cigarettes, four to reduce the number of tobacco cigarettes smoked, nine to quit smoking tobacco cigarettes, and one because they enjoyed vaping. Five participants indicated that their use of JUUL devices was completely unrelated to reducing or stopping tobacco cigarette use. Further, many of the participants indicated that they first tried a JUUL from a friend before purchasing their own. Overall, this study serves to express the wide array of reasons why people choose to use e-cigarettes, emphasizing that a significant proportion of users do not link their e-cigarette usage to cessation attempts. On a more optimistic note, however, of the 15 participants, 73% said that they would like to quit JUULing in the future (Nardone et al., 2019). This percentage includes users who did not start their e-cigarette usage with cessation from traditional cigarettes in mind, indicating that users may be gaining awareness of the potential dangers associated with e-cigarettes.

Another major factor to consider when assessing the validity of scientific research on e-cigarettes is the possible presence of conflicts of interest. In the past, the tobacco industry has allocated funding to scientific efforts that focus on their products. Beginning in the 1950s, the tobacco industry used different public relations strategies to combat the emergence of scientific research warning the public about the risks associated with e-cigarettes (Brandt, 2012). The amount of published, peer-reviewed studies linking respiratory and cardiac diseases to smoking significantly increased in the 1950s, although studies had been published for decades prior (Brandt, 2012). Tobacco companies realized that they could not just ignore the science that was targeting their products, so instead, they began to devise ways to control these studies and their outcomes. Companies called for more research and offered to fund these projects, carefully designing studies to counter negative findings. Additionally, they would seek out scientists who were already critical of existing claims and the methods used to reach those claims (Brandt, 2012). Because studies were not designed honestly and ethically, the tobacco industry's involvement in scientific pursuits had the effect of producing scientific uncertainty and therefore undermining "public health efforts and regulatory interventions designed to reduce the harms of smoking" (Brandt, 2012). It is possible that because scientists had financial gain in mind when conducting these studies, they were willing to cut corners and bend data in a way that would benefit the tobacco industry instead of publishing results that could possibly stunt the sale of tobacco products.

Overall, financial conflicts of interest in science can influence the overall integrity of the research in the sense that profit-seeking aspirations can lead to the publication of falsified data (Sax, 2012). For instance, in 1978, Dr. Michael Russell, a British psychologist, stated that "people smoke for the nicotine but they die from the tar" (Glantz, 2018). Russell spent a large majority of his career investigating means of nicotine delivery that could be less toxic and refining the composition of traditional cigarettes (Glantz, 2018). It was later revealed that Russell had strong ties to British American Tobacco (BAT), a cigarette and tobacco manufacturing company based in London, England. His work with the company was used to promote "heat not burn" cigarettes despite having little quantitative data behind the invention (Glantz, 2018). Had Russell received funding from a different source, or had the BAT not emphasized a need to create certain advertising claims, research pointing to the toxic effects of nicotine could have been published sooner and, theoretically, lives could have been saved in the process.

History has the potential to repeat itself, as more recently, in response to studies revealing adverse effects of e-cigarettes and increasing skepticism about their products from lawmakers, JUUL Labs created "JLI Science" in early 2019. According to the JLI Science website, the company's mission is to "better understand the effects and impact vaping products have in the long term, while also discouraging new users, and to share those results with the scientific community" (n.d.). Despite these intentions, JUUL has yet to actually submit evidence that its product is safer or even an effective cessation tool for tobacco use (JUUL, Inc, n.d.). Consequently, upon opening the JLI Science website, the viewer is asked to read and agree to the Terms of Use and Privacy Statement, which concludes with, "No part of the site, including any particular content, represents, or is intended to represent, a claim of reduced exposure or reduced risk" (JLI Science, n.d.). JLI Sciences states that they are interested in financing many different types of research that will help give a more holistic picture of JUUL products and their effects. These different types of research include clinical studies, observational behavior studies, nonclinical toxicology studies, and environmental analyses (JLI Science, n.d.).

Significantly, JLI Science is not ignorant of the long-standing relationship between the tobacco industry and the scientific community. As a result, JLI Science founded its investigator-initiated research (IIR) program which "believes in supporting ethical, independent research conducted by qualified third-party investigators" (JLI Science, n.d.). That is to say, JLI Science claims to favor funding expenditures for scientists who are not affiliated directly with JUUL, Inc. and who initiate the projects independently of the company.

Although JLI Science communicates a seemingly genuine commitment to examining and reevaluating the safety of its product, a 2019 study by Tan et al. found glaring issues in the ethical integrity of studies funded by JLI Science. Researchers used eight criteria to evaluate the research from JUUL Labs-sponsored research (2019). These criteria are transparency and independence, competitive funding process, ownership of data and freedom to publish, independent research agenda, ownership of data and freedom to publish, independent research agenda, governance, protection against conflict of interest, industry public relations gains that counteract public health, and feasibility. Weaknesses were found in JLI Science's research in all eight criteria with the exception of freedom to publish. More specifically, Tan et al. (2019), found that "JUUL Labs promoted research findings favorable to the company's interests at scientific meetings and in press releases and the news media." Therefore, they gained public relations advantages from the research at hand. Further, Tan et al. argue that "meetings with investigators before submitting a proposal might pose a conflict with achieving an independent research agenda" (Tan et al., 2019). In other words, the study claims that the IIR program is not as entirely independent as it lets on, with some researchers discussing project proposals with JLI Science representatives before officially submitting them.

This study also found that studies with a conflict of interest or with substantial bias tend to judge e-cigarettes more favorably, implying that experimenter bias or data falsification could have taken place (Tan et al., 2019). Even the stated goal of JLI Science, which is "to encourage independent, investigator-initiated research studies that validate or advance the science of electronic nicotine delivery systems (ENDS)" (JLI Science, n.d.), is rather problematic, as it does not mention the value of studies that shed light on the dangers or inefficacy of e-cigarette products. Instead, there is an obvious objective to prove the safety and effectiveness of these devices. If JLI Science truly desires to be a harmless conflict of interest, they should place more emphasis on publishing the results of all studies related to e-cigarettes, regardless of their outcome.

When it comes to the existing literature concerning e-cigarettes, it is obvious that many unknowns still remain, including the relative novelty of e-cigarettes, a large amount of unstudied but widely available products, the presence of integrity-threatening conflicts of interest, and the combination of different chemicals within the wide array of products, just to name a few obstacles. Within each of these issues, future scientists should also consider assessing variations within individual risk, as every person's propensity to become addicted to nicotine and develop medical consequences is different (Benowitz, 2010). The effects of a person's exposure to different genetic and environmental factors should also be considered in future studies. Finally, and perhaps most importantly, there needs to be honest motivation in studying e-cigarettes. None of the unknowns can be properly investigated unless the science underlying their investigation is good and sincere.

This type of science cannot be produced unless its underlying motives are completely forthright. One of the greatest roadblocks to honest science is financial motivation. Economic interest pertaining to e-cigarettes can easily complicate the scientific process and prevent the spread of important discoveries as researchers wrestle with monetary incentives and a desire to be published in academic journals, desires that may sometimes be stronger than the aspiration to conduct honest research (Brandt, 2012). In order to better understand the influence of economics on scientific endeavors related to e-cigarettes, it is critical to analyze the bidirectional relationship between the tobacco industry and the economy.

Marketing Strategies and Economic Impacts

In the United States, a great deal of tension exists between public health advocates and the tobacco industry. At one end, the public health community seeks to protect individuals from the known dangers of smoking cigarettes, regardless of whether they are traditional cigarettes or e-cigarettes. On the other hand, the tobacco industry seeks to protect its products and its capital in an effort to maintain both financial gain and the investments of stockholders. This manifests itself in a series of assiduous marketing strategies intended to attract new customers and maintain the loyalty of existing customers. While many people would argue that, according to general principles of ethics, protecting human lives is undoubtedly more important than making money, the United States government has historically hesitated when it comes to controlling the tobacco industry, implementing only minimal interventions (Mendes, 2014). Such hesitance can largely be attributed to the enormous influence that the tobacco industry has on the economy at large.

While tobacco possesses a long history of economic influence, its first major "spotlight moment" occurred in 1890 when the American Tobacco Company was founded by James Buchanan Duke, combining the five biggest manufacturers in the industry (Porter, 1969). These manufacturers included W. Duke & Sons, Allen & Ginter, W.S. Kimball & Company, Kinney Tobacco, and Goodwin & Company. Merging these producers lessened the production costs of individual companies, resulting in a greater pool of money for advertising campaigns.

According to a report by the United States Department of Health and Human Services, "Duke's marketing practices included setting relatively low prices, providing sophisticated packaging, carrying out promotions such as including picture cards in cigarette packs and sponsoring various public events, and paying distributors and retailers to promote his brands" (2012). One of the most interesting of these advertising expenditures was the inclusion of trading cards "clearly aimed at young collectors" (Blum, 1995) in each pack of cigarettes. During the late nineteenth century, cigarettes were sold in a paper wrapping, so the trading cards helped to stiffen the packaging. In general, customers would want to complete the set, whether it was for themselves or for children in their lives (Blum, 1995). Cigarette companies only produced a few cards in each series, which helped retain customers' loyalty to the brandby offering a topic of interest for everyone. While some people, like I.O. Evans, an author and cigarette-card enthusiast, believed that "Card collecting had little vogue among American youth...as the cards appeared intermittently, and were often exchangeable, sometimes for albums and sometimes for boxes of sweetmeats," other claims state that cigarette cards were a part of everyday life for American children who were constantly exposed to salespeople on the streets emphasizing the cards and how fun they were to collect (Broom, 2018). Regardless, cigarette trading cards certainly appealed to many users and sold many packs of cigarettes during their time while simultaneously demonstrating innovative efforts to advertise tobacco products (Broom, 2018).

Within the first year of its existence, the American Tobacco Company manufactured nearly 90% of all tobacco products in the United States, classifying the company as a monopoly (Porter, 1969). Therefore, the American Tobacco Company was found to be in violation of the Sherman Antitrust Act during a 1911 United States Supreme Court case, *The United States, v. American Tobacco Company* (Porter, 1969). The lawsuit forced the lawsuit monopoly to split into an oligopoly (Porter, 1969). However, these consequences of the trial were minimal as the United States perceived tobacco sales to be incredibly important to bolstering the company.

Notably, the court case made no mention of the medical consequences of such large-scale tobacco production despite the fact that several studies had been published with warnings of the dangers of tobacco and nicotine. Furthermore, the government did not raise any concerns with the industry's marketing efforts in light of the newfound scientific data (Porter, 1969).

The tobacco industry did not face massive government confrontation again until 1964, following Surgeon General Luther Terry's landmark announcement warning the United States of the dangers of e-cigarettes (CDC, 2006). At this point, the government began to express concerns about allowing the industry to freely advertise products without acknowledging their harmful effects. Consequently, just days after Terry's 1964 announcement, the FTC proposed a policy change that required cigarette manufacturers to add warning labels to their products disclosing links between tobacco products and negative health consequences (CDC, 2006). Before this law was officially enacted, however, Congress intervened by passing the Cigarette Labeling and Advertising Act of 1965. The Act was far less stringent than the changes proposed by the FTC. For instance, under Congress's law, the warnings were not required to be placed on tobacco advertisements like the FTC recommended, only on the products themselves. Additionally, Congress barred the FTC from requiring advertisement warnings until July 1, 1969, giving the tobacco industry plenty of time to devise plans to protect themselves from the impending changes (CDC, 2006).

In 1969, the Public Health Cigarette Smoking Act finally tightened restrictions on the industry, banning cigarette advertising in the broadcasting media and called for the first mandated annual report on the health consequences of smoking (CDC, 2006). In 1972, the FTC was finally allowed to require manufacturers to place the same warnings on advertisements.

Terminology on warning labels was also clarified, stating “Warning: The Surgeon General Has Determined that Cigarette Smoking is Dangerous to Your Health” instead of the original “Caution: cigarette smoking may be dangerous to your health” (CDC, 2006). Although no conclusive evidence proves that Congress was acting with a financial interest in mind, it is hard to believe that they were not hesitant about the influence of tobacco laws on the economy and the job market.

As more scientific evidence became available to the public regarding the dangers of tobacco and nicotine, tobacco manufacturers began to create and advertise harm reduction products, such as filtered cigarettes and low-tar options (Warner, 2000). Companies also began to simply refute scientific claims. As one review states, “In the years following the release of 1964 Surgeon General’s report the tobacco industry also stepped up its public relations campaign aimed at reassuring the public, especially smokers, that there was no real link between smoking and disease” (Cummings & Proctor, 2015). These advertising efforts were closely related to the unethical research practices mentioned in the previous section of the current thesis, as businesses desperately attempted to protect themselves.

Companies also sought out more cheerful and vivacious means of promoting their products. Camel, a popular American cigarette brand, introduced a cartoon camel to help mascot its products in 1987. Also known as “Joe Camel” or “Old Joe,” the smiling camel appeared in advertisements until 1997. Like the cigarette trading cards, Old Joe appealed to young children. One article concluded that “Old Joe Camel cartoon advertisements are far more successful at marketing Camel cigarettes to children than to adults… consistent with tobacco industry documents that indicate that a major function of tobacco advertising is to promote and maintain tobacco addiction among children" (DiFranza et al., 1991). Another study found that "30% of 3-year-old children and 91.3% of 6-year-old children correctly matched Old Joe with a picture of a cigarette (Fischer et al., 1991). Old Joe not only drew customers' attention away from medical warnings on packaging and in advertisements, but he also served as a memorable connection for kids between cartoons and tobacco products.

Several more decades passed before the United States government implemented more strict tobacco policies. In June 2009, President Barack Obama signed the Family Smoking Prevention and Tobacco Control Act, which granted the FDA the right to regulate all tobacco products (Hecht, 2011). Essentially, the new law restricted tobacco marketing and sales to youth, required smokeless tobacco product warning labels, ensured harm reduction claims are supported by scientific evidence, and required disclosure of ingredients in tobacco products (Hecht, 2011). For the first time in history, the FDA was granted the right to implement standards for tobacco products to protect public health and regulate nicotine and ingredient levels. Although on the surface level, the Family Smoking Prevention and Tobacco Control Act appears to significantly reduce the power of the tobacco industry, it also imposes some limitations on the FDA's powers. Under this act, the FDA was still not granted the ability to require prescriptions to purchase tobacco products, require the reduction of nicotine yields to zero, ban face-to-face sales in a particular category of retail outlet, or ban certain classes of tobacco products. Additionally, the language of the Family Smoking Prevention and Tobacco Control Act is relatively ambiguous in terms of specific details, allowing for the tobacco industry to continue to find cracks in the policy that called for only slightly modified practices.

Unfortunately, the Family Smoking Prevention and Tobacco Control Act could not have foreseen the impact that e-cigarettes would have on society just a few years after the act's implementation, and therefore many of its restrictions did not apply to the regulation of these products. For instance, the act bans cigarettes with flavors other than menthol and tobacco, raising concern about their role in increasing youth use 10 years prior to the Trump administration's flavor ban. This rule, however, did not apply to e-cigarettes in 2009. As a result, JUUL, Inc. and other e-cigarette companies have been able to continuously utilize marketing strategies, such as including kid-friendly flavors, for their devices that have previously been banned for more traditional tobacco products.

As JUUL, Inc. partakes in these questionable practices, they demonstrate their positionality as a business that ultimately seeks to gain capital and protect itself from legislative threats despite the company's many claims of human interest and well-being. One of JUUL, Inc.'s most contentious operations is its marketing strategies that appeal to youth. As demonstrated in the American Tobacco Company's use of trading cards, advertising to youth has become a common practice within the tobacco industry. According to a 2012 report by the Surgeon General regarding the use of tobacco in youth and young adults, tobacco manufacturers refute the idea that they directly influence youth use, stating that their "marketing efforts do not increase the overall demand for tobacco products and have no impact on the initiation of tobacco use among young people." Instead, the companies argue that their advertising claims are purely designed to help in "competing with other companies for market share" (United States Department of Health and Human Services, 2012).

As far back as 1999, the United States filed a lawsuit against several major tobacco companies, claiming that they had deceived the public with their advertising claims (United States Department of Health and Human Services, 2012). Despite the companies' claims stating their advertising efforts did little to promote the initiation of tobacco use and acting purely out of competitiveness, Judge Gladys Kessler ruled that the tobacco companies were guilty of deceiving the public and targeting youth. In her verdict, Judge Kessler stated:

> Cigarette marketing, which includes both advertising and promotion, is designed to play a key role in the process of recruiting young, new smokers by exposing young people to massive amounts of imagery associating positive qualities with cigarette smoking. Research in psychology and cognitive neuroscience demonstrates how powerful such imagery can be, particularly for young people, in suppressing perception of risk and encouraging behavior.

Although Judge Kessler's ruling included many strong statements about the role of the tobacco industry in creating young smokers, the outcome of the court case focused largely on forcing the companies to make corrective statements regarding the potential risk of their products (United States Department of Health and Human Services, 2012). The court case did not implement any consequences in terms of promoting youth use, giving the companies no more than a gentle slap on the risk. Consequently, e-cigarette companies have mirrored the previous actions of the tobacco industry, adopting bold approaches in their advertising strategies and clearly targeting youth and young adults in their promotions while stating they are only trying to compete with other e-cigarette companies.

Social media has played an enormous role in documenting both the advertising efforts of e-cigarette companies and the influence these efforts have on youth. The CDC has collected its own data on youth use of e-cigarettes. According to their website, in 2014, “more than half of high school students (about 8 million) saw e-cigarette ads in retail stores, and more than 6 million saw them on the Internet” (2017b). In a sense, JUUL’s use of social media advertisements was the “cigarette trading card” or “Old Joe Camel” of the 21st century. Each strategy appeared in the guise of a light-hearted, fresh approach to advertising. In actuality, however, the advertisements appeal to young people who consequently view the product as safe and friendly. In turn, tobacco and e-cigarette companies create young users who will potentially become loyal to their brand for many years. Unfortunately, these young users may also be subjecting themselves to a lifetime of health complications.

Although e-cigarette companies deny the notion that their social media campaigns target adolescents and young adults, numerous studies have found that the social media posts youth see on the Internet are often created with young people in mind. One study by Jackler et al. (2019) analyzed 2,691 Twitter posts, 248 Facebook posts, 187 Instagram posts, and 171 customer directed marketing emails from JUUL in order to further investigate the role of social media advertisements and youth tobacco use. The study found that in social media posts, JUUL, Inc. commonly “featured models in their 20s appearing in trendy clothes engaged in poses and movements more evocative of underage teens than mature adults” (Jackler et al., 2019). The company also created relationships with popular social media influencers and paid them to review their products, although JUUL, Inc. later denied this relationship (Jackler et al., 2019). The researchers also claim that JUUL’s design and kid-friendly flavors, such as mango, bubble gum, and cake, influence interest from a younger population and easily tie into the company's social media presence. Overall, the study argues that JUUL and its affiliated marketers created a niche media environment influencing a young adult demographic.

Social media has also proven to be a helpful tool in analyzing how youth are responding to these ads. More specifically, research characterizing JUUL perceptions on Instagram, Twitter, and Reddit has been conducted. These types of studies have been particularly helpful in understanding how JUUL's online advertising created a foundation of young users that carry on the popularity of the products despite JUUL having since halted online advertising in response to the 2019 EVALI outbreak (Fadus et al., 2019). More specifically, a 2019 study by Brett et al. investigated youth perceptions of JUUL use by analyzing Reddit posts created between January 2015 and May 2017. Researchers found that 60% of overall posts displayed negative perceptions of JUULing, but only 37% of content posted specifically by young people expressed these negative sentiments. The posts also revealed that a majority of youth claimed that they began using JUUL because of its popularity amongst other peers, implying that adolescent users who JUUL in response to social media advertisements continue to influence the consumption of these products (Brett et al., 2019).

All of JUUL's early advertising efforts helped the company gain significant traction in the e-cigarette market. A study by Huang et al. (2014) found that from 2011-2017, sales of e-cigarettes increased exponentially across the United States, with JUUL specifically increasing the most from 2016-2017. In 2017, JUUL accounted for 40% of e-cigarette retail market shares. By 2019, JUUL took up more than half of the market share, despite eliminating almost all notable marketing expenditures in fear of government intervention. Further, the researchers noted that “because of JUULs growth, the e-cigarette brands owned by the tobacco industry, which had dominated the e-cigarette retail market since 2013, no longer had the majority of the market share at the end of 2017.” In other words, JUUL, an independent business with no direct ties to larger partnerships, was outperforming e-cigarettes backed by incredibly wealthy and notable names in tobacco manufacturing. It was not long, however, until JUUL, Inc. would join forces with the tobacco industry.

In response to the 2019 EVALI cases, the United States’ government devised several plans designed to more strictly restrict and regulate e-cigarette companies. Additionally, JUUL, Inc. was undergoing several lawsuits in response to their advertising expenditures and was in desperate need of executive and financial support. As legislators toughened e-cigarette policies and courts of law continued to press JUUL for more information on their true motives, the company underwent several administrative modifications to respond to the changing environment. For example, the CEO of JUUL, Inc., Kevin Burns, stepped down from his position, and KC Crosthwaite assumed the role (Diaz, 2019). Before becoming CEO, Crosthwaite was the chief strategy and growth officer for Altria, the company behind Marlboro cigarettes (Abbott, 2019). Furthermore, Altria bought a 35% stake in JUUL for nearly $13 billion (Mitchell & Young, 2019). JUUL needed the money, expertise, and political clout of Altria in order to maintain a successful business, regardless of whether their mission was to sell a tobacco product or to curb smoking habits. Altria also needed the relationship with JUUL in order to gain access to the vaping market. After Altria’s investment, JUUL was valued at $38 billion (Mitchell & Young, 2019). Shortly after the investment, Altria suggested merging with Philip Morris, another giant name in tobacco, in order to help “create incremental revenue and cost synergies”

(Linnane & Kilgore, 2019). As the EVALI cases became more numerous, however, talks of combining forces were called off.

All of these plans, which occurred despite full knowledge of connections between e-cigarettes and negative health outcomes, demonstrate JUUL's primary interest in defending its business, prioritizing increasing wealth as over protecting people. Up until recently, the United States government was also extremely interested in protecting the wealth of companies like JUUL, Altria, and Philip Morris, due to these businesses' perceived financial contribution to the economy. The tobacco industry often hired consulting firms to create presentations for legislators that emphasized statistics on the industry's important participation in the economy. These statistics included information on the industry's income, the number of jobs they provided, and how much tax revenue they generated (Warner & Fulton, 1995). According to a 1994 report by the United States Department of Agriculture, the tobacco industry reported that 47 million people depended directly on the sale of tobacco for their employment. Additionally, 10 million people were supported indirectly by the sale of tobacco. A different report states that "In 1994, an estimated 5.34×10^{12} cigarettes were produced worldwide, roughly 1000 cigarettes for every man, woman, and child" (Warner & Fulton, 1995). Further, the tobacco industry claims that as cigarette sales have been declining over the past decade, e-cigarettes have been creating new jobs and making up for the loss in revenue, heralding e-cigarettes as just as important to the economy as traditional tobacco products. These bold claims and large numbers have led policymakers to act with reservations when considering legislation that would potentially restrict the tobacco industry. Big numbers, however, can be deceiving.

Despite the tobacco industry's data and large calculations, other sources estimate that the tobacco industry accounts for only about 1% of the United States' Gross Domestic Product (GDP) (Ekpu & Brown, 2015). Another study found that "Almost half of the tobacco counties in the US derive less than 1% of their income from tobacco farming work off their farms, most holding full-time jobs elsewhere. Indeed, there is only a single farm dependent county in the entire US that derives a majority of its farm revenues from tobacco" (Warner, 2000). This finding implies that if the United States would stop subsidizing tobacco crops, or if sales of tobacco were to significantly decrease, farms would have other sources of income to fall back on. Further, the same researchers believe that the tobacco industry uses variables that can deceptively boost their statistical estimates. For example, the study claims that "by including 'expenditure included employment' in addition to core tobacco sector jobs and those of industry suppliers" (Warner, 2000). Expenditure induced employment refers to jobs that are created in all economic sectors as the result of tobacco workers spending their wages. In other words, the tobacco industry claims that it helps employ people who receive the money of tobacco farmers, regardless of whether or not those transactions are for tobacco. If expenditure induced employment was removed from the equation, the impact the tobacco industry has on employment would prove to be much smaller.

Other studies have argued that if the United States further restricted cigarettes, the economy would lose revenue from cigarette excise taxes, which was equivalent to $13.75 billion in 2006. These tobacco sales advocates claim that "if Americans stopped smoking altogether, states could see a 1.4 percent decrease in revenue" (Kliff, 2011). Other researchers argue, however, that the economy would be able to fully adjust as people reallocated their spending on other things. The researchers state that "this alternative spending would generate employment and tax revenues associated with the production, distribution, and sale of purchased goods and services" (1995). It appears that policymakers have been significantly deceived by the tobacco industry and their claims of economic importance, consequently resulting in negative health outcomes for millions of people. Still, regardless of evidence proving that the tobacco industry makes negligible and replaceable contributions to the economy, the federal government still gives tobacco farmers more than $60 million in subsidies a year (Faber, 2018). According to one study, these subsidies are "hypocritical and damaging to the health of the nation. By encouraging tobacco growing, the subsidy encourages smoking" (Warner, 2000). Despite statements expressing a commitment to the health of citizens, the government has demonstrated the prioritization of its perceived economic benefits in conjunction with the tobacco industry.

All of the efforts the United States government has taken to prevent the demise of the tobacco industry are especially surprising when considering the striking economic consequences associated with the medical impacts of tobacco products and e-cigarettes. Before the introduction of JUUL to the market, some investigators, such as Hajek et al. (2014), believed that the introduction of e-cigarettes to the market may decrease the incidence of smoking-related death and disease, therefore eliminating the amount of economic damage caused by smoking. Estimates quantifying this damage vary. According to The World Bank's Global Tobacco Control Program, "Worldwide, the total economic damage of smoking has been estimated at more than US $1.4 trillion per year, equivalent to 1.8 percent of the world's annual GDP" (2018). This number includes both medical costs and productivity losses from death and disability and is a total estimate, not an annual estimate. The World Health Organization (WHO) projects that globally, tobacco use causes $500 billion in economic damage each year (WHO, 2013). Notably, all of these conjectures are much larger than the numbers produced by the tobacco industry regarding its economic contributions.

The conclusion of Hajek et al.'s (2014) article states, "Regulating [e-cigarettes] as strictly as cigarettes, or even more strictly as some regulators propose, is not warranted on current evidence" (Hajek et al., 2014). These authors believed in the potential of e-cigarettes and wanted the public to have widespread access to them, assuming that they would be used appropriately. Unfortunately, this was not the case, as many people initiated nicotine use for the first time with e-cigarettes. Additionally, there is not enough research on e-cigarettes to conclude whether or not they truly improve the medical and economic impact of cigarettes.

Although the United States has gone to great lengths to preserve tobacco companies and e-cigarettes companies in order to maintain the country's economic status, many studies denounce the positive impacts of these products. In fact, traditional tobacco products and e-cigarettes are likely generating more economic harm than good. With all of this in mind, it is again necessary to emphasize the need for further research on e-cigarettes. Such research could help better inform how e-cigarettes can be more appropriately advertised, if they should even be marketed at all. More research must be conducted to better understand how these products work and what they do to the health of users, especially considering how these products remain a point of contention in the economic sectors of both the United States and the world at large.

Seeking-Behavior in Larval Zebrafish Exposed to Vaping Liquids

Background

More research must be conducted concerning e-cigarette products in order to better understand their medical consequences and guide potential legislative changes that seek to regulate and/or restrict these devices. In an effort to expand the current pool of literature on this topic, the current study analyzes how vaping liquids and their components (specifically cinnamaldehyde and nicotine) influence seeking behavior in larval zebrafish (*Danio rerio*), a popular model organism for drug discovery research (Klee et al., 2011).

To those unfamiliar with the potential of zebrafish, these animals may seem like an unusual candidate for research of this nature. Zebrafish, however, have an array of qualities that make them an ideal model organism for studies on psychopharmacology (Klee et al., 2011). For instance, each time zebrafish mate, they typically produce between 100 to 300 embryos. Moreover, females can mate once a week, therefore supplying researchers with a large volume of subjects at a relatively low cost (Klee et al., 2011). Further, zebrafish embryos are transparent, allowing for simplified, noninvasive observation of developmental changes beginning from fertilization. Although humans are more closely related to rodents, we share greater genetic commonalities with zebrafish than we do with other popular model organisms, such as fruit flies (Klee et al., 2011). Like traditional rodent models, zebrafish also demonstrate a variety of robust behaviors that emphasize their usefulness in drug discovery studies (Klee et al., 2011). Additionally, zebrafish develop to maturity more quickly than rodent models, meaning that developmental studies can occur more rapidly in zebrafish than in other popular model organisms (Klee et al., 2011).

These qualities are especially well-suited for research on e-cigarettes and their components for several reasons. First, a person's susceptibility to becoming addicted to nicotine, alongside the other components of e-cigarettes, is largely dependent on their genetic makeup (Jain & Mukherjee, 2003). Because zebrafish share many genetic commonalities with humans and can be genetically modified with relative ease, they are fitting for studies concerning some of the biological underpinnings of e-cigarette dependence and addiction. Previous studies have even used the zebrafish to characterize specific genetic mutations that modulate nicotine responses (Petzold, 2009). Moreover, because zebrafish reproduce and develop so quickly, a large sample size can be incorporated in experiments, and studies can be conducted quickly. These are incredibly advantageous qualities as they allow for results to be reported with high confidence and can increase the rate by which studies are conducted and then published. Considering how important research on e-cigarettes is during a time when people are experiencing medical consequences alongside a large flux of government interventions, an increase of publications with high confidence in their results is crucial.

The current study seeks to better understand how vaping liquids influence nicotine-seeking behavior in zebrafish. Additionally, the current study aims to explore whether zebrafish are suitable for this type of research. The behavioral effects of combinations of nicotine, flavoring agents, and other components of vaping liquids have not been studied in zebrafish. However, the developmental effects of these substances, both individually and when combined, are somewhat better understood in zebrafish. For instance, one study exposed zebrafish to low concentrations of isolated propylene glycol. When zebrafish were exposed to propylene glycol as embryos, they experienced significant developmental impairments, such as small size, tissue swelling, and hyperactivity, when compared to young fish who had never been exposed to the compound. (Massarsky et al., 2017). A different study investigating nociception classified cinnamaldehyde as a chemical that produces pain in zebrafish. Here, zebrafish injected with cinnamaldehyde exhibited reduced movement in comparison to control zebrafish (Taylor et al., 2017). This implies that some chemical sensory system in the zebrafish is able to detect cinnamaldehyde and trigger a behavioral response.

Additionally, one study sought to understand the developmental effects of e-cigarette flavor mixtures on zebrafish (Holden et al., 2020). Out of nine tested flavors, seven caused inauspicious developmental effects. Interestingly, the study found that cinnamaldehyde drove toxicity in these chemicals. In other words, the hazards detected by this study were not necessarily from nicotine, but rather from the flavoring additives (Holden et al., 2020). This study is currently the only published paper on this specific topic, although other labs are investigating similar phenomena in zebrafish using vaping liquids.

The current study compares nicotine, cinnamaldehyde, and different concentrations of vaping liquids to better understand how these substances influence seeking behavior in zebrafish. This study has the potential to inform future studies about how combinations of different substances can modulate seeking-behavior in other animals, including humans. In vaping liquids, flavoring agents are incorporated in order to mask the irritating effects of pure nicotine so that consumption is more tolerable (JUUL, Inc, n.d.) E-cigarette flavors cover up both the smell and taste of nicotine. With this in mind, it is important to note that at this time, it is unclear whether zebrafish and humans share similar taste and smell preferences. Additionally, it is unclear if zebrafish detect the substances in question through taste and/or smell, or rather some different chemical sensory mechanism. Larval zebrafish do, however, have both taste buds and an olfactory bulb to facilitate their chemical senses.

The zebrafish olfactory bulb develops in the first few hours after fertilization, and previous studies have found that zebrafish can detect smells as early as two or three days past fertilization (Hansen & Zeiske, 1993; Li et al., 2005; Mack-Bucher et al., 2007). Like in the human olfactory system, in zebrafish, the onset of olfactory receptor expression is asynchronous, so it is possible that zebrafish may have not developed the ability to smell certain substances at certain developmental timepoints (Barth, Justice, & Ngai, 1996). Additionally, scientists have found that zebrafish develop taste buds between four and five days past fertilization, although taste receptor expression is also asynchronous, although not to the extent of olfactory receptor expression (Hansen, Reutter, & Zeiske, 2002). Further, it is likely that zebrafish have more limited tasting and smelling abilities than mammals as zebrafish have fewer taste buds and a comparatively simple olfactory apparatus (Hansen, Reutter, & Zeiske, 2002; Hansen & Zeiske, 1993).

The current study seeks to fill in some of the scientific gaps to get a more comprehensive picture of how vaping liquid components drive the behavior of larval zebrafish. Developing a robust test which allows zebrafish to show choice-behavior would allow future studies to compare preferences of zebrafish and preferences of humans. More generally, such a test would permit future studies to decide whether the zebrafish is an appropriate model organism for studies on specific flavors depending on zebrafish preferences versus human preferences. With all of this in mind, the current study hypothesizes that individual vaping liquid components, as well as vaping liquid mixtures, will change rates of seeking-behavior in larval zebrafish relative to those in larval zebrafish who were never exposed to vaping liquids or their components.

Methodology

The current study assesses the responses of zebrafish to nicotine, cinnamaldehyde, two different concentrations of a cinnamon-flavored vaping liquid, and water. The vaping liquids were prepared by Dr. Maria Bondesson's lab at Indiana University - Bloomington's Luddy School of Informatics, Computing, and Engineering. The liquids tested were cinnamon-flavored "vape juice" containing nicotine, cinnamaldehyde, and a mixture of humectant propylene glycol and vegetable glycerine diluted. Further, in the laboratory, the solution was dissolved in dimethyl sulfoxide (DMSO). While most vaping liquids do not contain DMSO, the solution would not be water-soluble otherwise. Additionally, it is highly likely that these products contain some sort of benzene compounds, such as the benzoic acid found in JUUL devices. Unfortunately, at this time, we cannot say for certain as to what these benzene compounds are exactly, and in what concentration, as many vaping liquid companies do not disclose their exact components or their concentrations. The vaping liquid stock solution was ordered from an online retailer that is not associated with JUUL, Inc.

The zebrafish used in the current study were all six to eight days past fertilization. This age range was chosen because during this specific span of time, zebrafish are considered larvae, and few confounding variables exist in their environment. At six days post-fertilization, the zebrafish's swim bladder has fully inflated, marking its transition from an embryo stage to a larval stage (Robertson et al., 2007). At eight days post-fertilization, the zebrafish uses up its yolk sac and must switch to a brine shrimp diet, which could introduce confounding variables if some of the tested zebrafish still receive nutrients from their yolk sac whereas others were fed brine shrimp (Jardine & Litvak, 2003). Further, we know that zebrafish have developed some level of taste and olfactory receptor expression at this age, meaning that they can potentially detect the substances in question through these chemical sensory mechanisms (Hansen, Reutter, & Zeiske, 2002; Hansen & Zeiske, 1993).

Standard animal research protocols were used in accordance with the DePauw University Institutional Animal Care and Use Committee requirements (fulfilled and approval was given for research, IACUC date: 2013-06-03). Wildtype fish were housed at DePauw University at approximately 28°C and on a 14/10 hour light-dark cycle. In the tests involving the vaping liquids, a new group of transgenic fish was introduced. These fish were provided by the Bondesson Lab at Indiana University - Bloomington. These fish derived from line Tg(fli:GFP) express green fluorescent protein (GFP) in their blood vessels, which the Bondesson Lab uses to study the developmental and morphological effects of vaping liquids on the zebrafish vascular system. The transgenic fish were housed at Indiana University - Bloomington until they were five days past fertilization. Then, they were brought to DePauw University for testing on their sixth- and/or seventh-day post-fertilization. At DePauw University, the transgenic fish were exposed to the same housing conditions as the wildtype fish.

Two basic types of behavioral assays were employed in the current study to measure larval zebrafish responses to the various vaping liquid components. The first test, an acute response test, has been widely used to analyze a zebrafish's initial response to a high concentration of each liquid, measuring their general responsiveness (Egan et al., 2009;

Sackerman et al., 2010). In the test, zebrafish were placed into a 12-well plate, with one zebrafish per well. Each well contained 2.8 ml of water. The fish are left in the 12-well plate to acclimate to this novel environment for one hour in order to eliminate any potential confounding variables associated with an anxious response to the environment. After this hour-long period, the fish were video recorded for two minutes. At the two minute mark, the camera was stopped. At this time, 200 µl of the substance of interest was added to each well. This substance was either nicotine, cinnamaldehyde, water, or vaping liquids, depending on which test was being run at that time. After the substance of interest was added to each well, the fish were again video recorded, this time for five minutes. Data were analyzed using EthovisionXT (Noldus). The two measures recorded in this analysis are distance traveled (measured in centimeters) and velocity (measured in centimeters per second). Velocity was calculated throughout the video as a rolling average with the frame rate, then an average of these calculations is presented. Static subtraction tracking methods were used. The average distance traveled and velocity for each group of fish were obtained and standard error of means was calculated.

The second test, a four-arm maze test, analyzes seeking behavior in a choice task. Four-arm mazes were made of 2% agarose in a Petri dish with a diameter of 100 cm (see **Figure 1**). Behind each arm is a trough that the substance of interest can be placed. Over time, the substance diffuses through the trough into the arm. This slow diffusion helps to keep a high concentration of the substance in that particular arm.

On the day of an experiment, zebrafish were put into a Petri dish and then placed onto a lightbox in a dark room and allowed to acclimate to this novel environment for one hour. Wildtype zebrafish were not retested in the current study, but the same transgenic fish were used in both the acute response tests and four-arm maze tests due to a limited supply of these fish. After one hour, one zebrafish was added to a four-arm maze and video-recorded for 30 minutes. This 30 minute time block was used to establish whether the fish had a baseline preference for any area in the maze. After this period, 40 µl of the substance of interest was added to one trough of the four-arm maze. This substance could be nicotine, cinnamaldehyde, water, or vaping liquids, depending on which test is being run at that time. and the zebrafish were recorded for two hours.

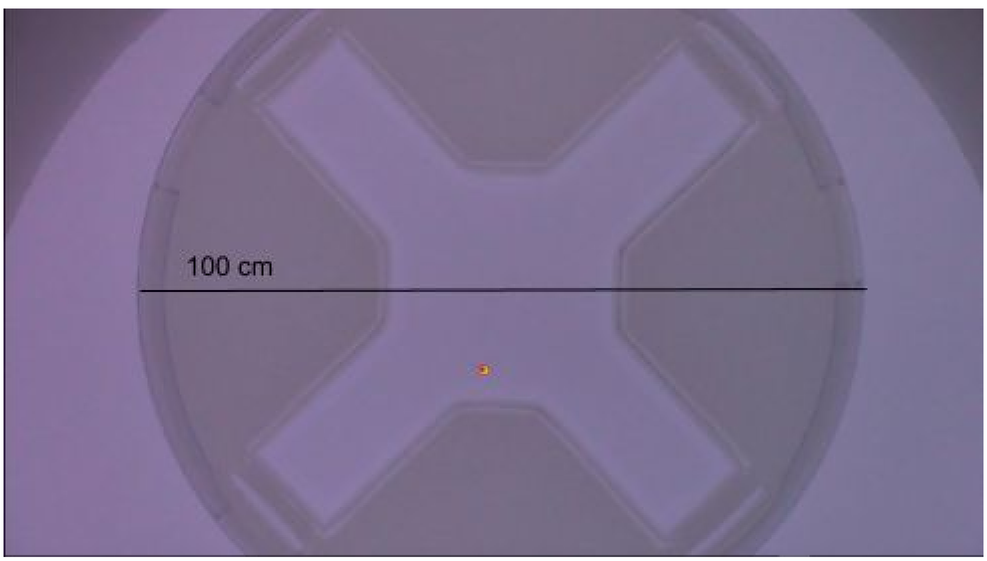


Figure 1. A four-maze containing a larval zebrafish. The substance of interest is placed in the trough in the upper left arm.

Data were analyzed using EthovisionXT (Noldus). This program allows us to divide the maze into five different zones for analysis in order to measure how much time a fish spends in a particular part of the maze, and how frequently they visit a certain part of the maze (see **Figure 2**). The two measures recorded in this analysis are cumulative duration (measured in seconds; indicates how much time a fish spent in a zone) and frequency (how many times a fish entered a zone during a testing period). Static subtraction tracking methods were used. The average cumulative duration and frequency for each group of fish were obtained and standard error of means was calculated.

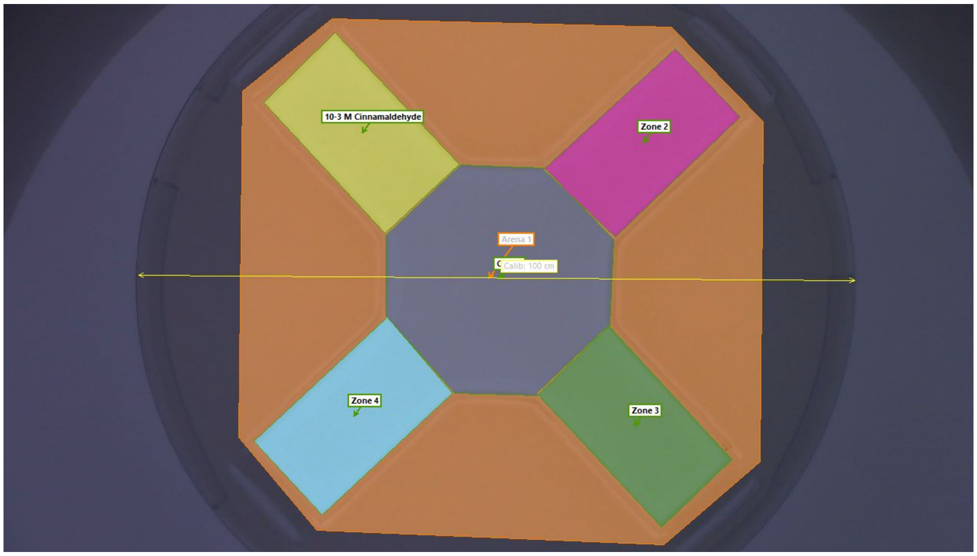


Figure 2. A picture of the four-arm maze in EthovisionXT. The image shows the zones used for data analysis. The upper left arm is not always labeled as containing cinnamaldehyde, but rather is labeled with respect to the substance and concentration during that particular experiment.

Fish were classified as a "Duration Seeker" if they spent the most amount of their time in the zone containing the substance of interest compared to the other three zones of the maze. Fish were classified as a "Frequency Seeker" if they visited the zone containing the substance of interest more often in comparison to the other three zones of the maze. If a fish met the qualifications for both a Duration Seeker and a Frequency Seeker, they were further classified as a "Dual Seeker." The center zone was not taken into consideration when classifying seekers, as it is expected that the fish will spend a significant amount of time in the center zone since it must spend time in this area in order to access other zones.

The different seeker classifications arose because it remains unknown how much of a substance a zebrafish can tolerate at a time, or how long each substance remains in the zebrafish nervous system. In other words, it remains unknown whether zebrafish prefer to constantly sit in a chemical to experience its effects, or if they would rather revisit the area with the chemical as to not overstimulate their systems. Further, the answer to this question may not be the same for each substance of interest. Therefore, the current model of classifying seekers allows for flexibility in seeking behavior and recognizes that how a zebrafish seeks one substance may not be the same as how the same zebrafish seeks a different chemical.

Notably, all transgenic fish in the four-arm maze tests were later tested in the acute response tests, meaning they had experienced previous, although not acute, exposure to either 2% or 20% vaping liquids.

Finally, after testing, fish were returned to their housing environments. Methods of euthanasia were unnecessary to utilize in the current study as the zebrafish were not subject to long-term discomfort or pain.

Results

Acute Response Tests

Acute response tests measure the initial response of zebrafish to a high concentration of the substance of interest. This information is helpful because it helps determine whether zebrafish are responsive to certain substances, and at what concentrations these responses occur. Acute response tests were carried out for nicotine, cinnamaldehyde, vaping liquids, and water. For each acute response test, track map visualizations were generated to help visually quantify the changes in swimming patterns of each zebrafish before and after exposure to the substance of interest (see **Figure 3**; also see **Appendix**).

In order to establish a baseline, acute response tests were conducted using water as the substance of interest. Only wildtype fish were used in these experiments. After the water was introduced to the wells, the fish increased their distance traveled over a two minute period (136.2% increase, from $M = 29.8$ cm to $M = 70.4$ cm), but their velocity remained the same ($M = 0.3$ cm/s) (see **Figures 3-5**, **Table 1**). These changes are likely due to the surprise the fish experiences when the water is inserted into the well with a pipette. Further, the force of the water entering the well may be causing the fish to move a bit.

Because nicotine is the known addictive chemical in vaping liquids, the influence of nicotine was tested in an acute response test. A concentration of 10^{-3} M nicotine was used in this particular test. Only wildtype fish were used in these experiments. It is important to note that in the current study, zebrafish were not retested unless otherwise indicated. This means that “Fish 1” in one experiment is not the same as “Fish 1” in another. Further, this means that fish were only exposed to one substance of interest. Additionally, EthovisionXT had difficulties detecting Fish 8 and Fish 12 in the before trial, so the track map visualizations in **Figure 3** are not accurate for those two subjects and were omitted from the acute response test analyses.

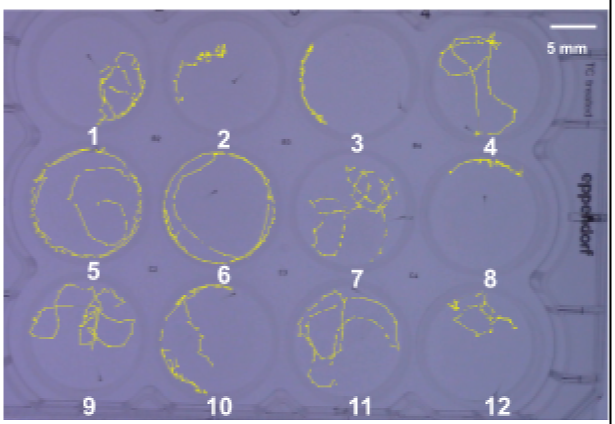
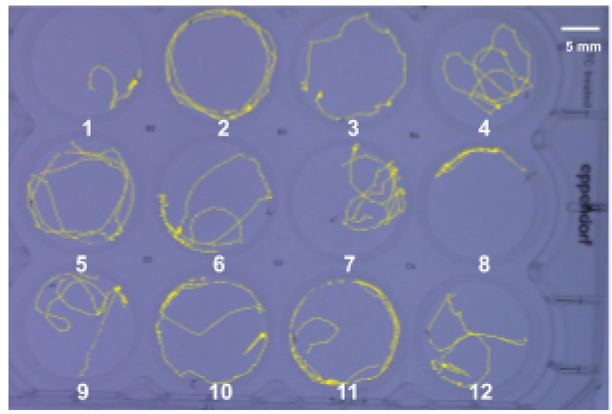
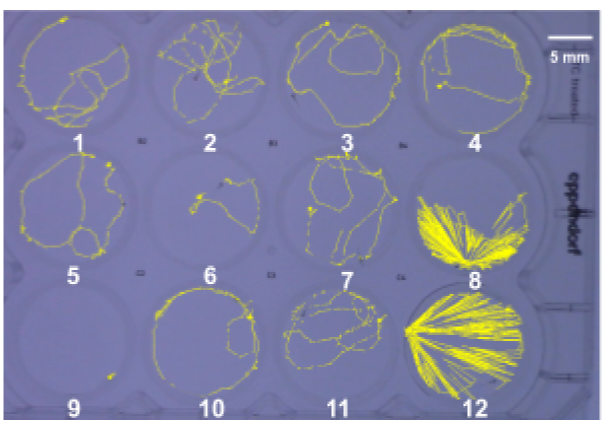
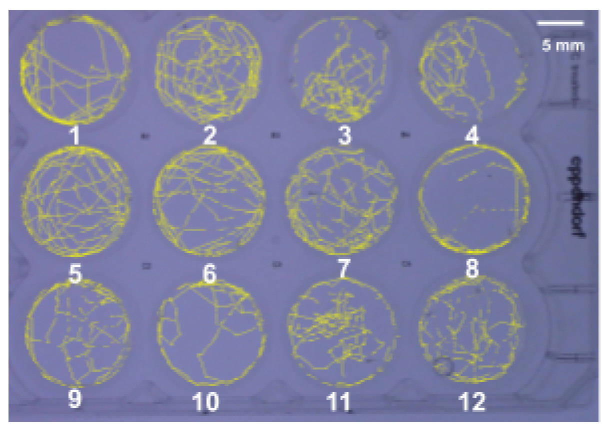
ACUTE RESPONSE TEST TRACK MAP VISUALIZATIONS	
BEFORE ADDITION WATER	**AFTER ADDITION OF WATER**
	
BEFORE ADDITION OF NICOTINE (1 MILLIMOLAR)	**AFTER ADDITION OF NICOTINE (1 MILLIMOLAR)**
	

Figure 3. *Top Left:* A track map visualization of the two minutes before the application of 200 µl water. *Top Right:* A track map visualization of the two minutes after the application of 200 µl water. The yellow track shows the last two minutes of a five-minute recording. *Bottom Left:* A track map visualization of the two minutes before the application of 200 µl 10^{-3} M nicotine. *Bottom Right:* A track map visualization of the two minutes after the application of 10^{-3} M nicotine. The yellow track shows the last two minutes of a five-minute recording.

Fish exposed to nicotine showed increased distance traveled (310.4% increase, from *M* = 30.8 cm to *M* = 126.4 cm) and velocity (100% increase, *M* = 0.3 cm/s to *M* = 0.6 cm/s) compared to fish exposed to water (see **Figures 3-5**, **Table 1**).

Next, the influence of two concentrations of cinnamaldehyde was tested in acute response tests (see **Table 1**; **Appendix**). Concentrations of both 10^{-3} M and 10^{-6} M cinnamaldehyde were used to determine if the two concentrations had different effects on behavior. Only wildtype fish were used in these experiments. When fish were exposed to the 10^{-3} M concentration, distance traveled increased by 151.9% (from *M* = 21.2 cm to *M* = 53.4 cm; see **Figure 4**, **Table 1**). When fish were exposed to the 10^{-6} M concentration, distance traveled increased by 111.3% (from *M* = 20.4 cm to *M* = 43.1 cm; see **Figure 4**, **Table 1**). Velocity remained the same for fish exposed to the 10^{-6} M concentration (*M* = 0.2 cm/s) but increased by 50% for fish exposed to the 10^{-3} M concentration (from *M* = 0.2 cm/s to *M* = 0.3 cm/s; see **Figure 5**, **Table 1**).

Then, the influence of two concentrations of vaping liquids was tested in acute response tests. Concentrations of both 2% and 20% were used to determine if the two concentrations had different effects on behavior. In experiments concerning vaping liquids, transgenic fish from the Bondesson Lab were also studied in addition to wildtype fish. For both concentrations and in all fish, distance traveled increased (see **Figure 4**, **Table 1**), although the amount of increased varied depending on the line of fish, whether it was pre-treated or not, and, if pre-treatment, what substance it was exposed to prior to testing. Wildtype fish showed a 131.7% increase in distance traveled when exposed to 2% vaping liquid (from *M* = 18.9 cm to *M* = 43.8 cm), but a 57.9% increase in distance traveled when exposed to 20% vaping liquid (from *M* = 17.1 cm to *M* = 27.0 cm). Fish pre-treated with 0.01% vaping liquid prior to testing showed a 404.3% increase in distance traveled when exposed to 2% vaping liquid (from *M* = 13.8 cm to *M* = 69.6 cm) and a 1642.6% increase in distance traveled when exposed to 20% vaping liquid (from *M* = 14.1 cm to *M* = 245.7 cm). Fish pre-treated with DMSO prior to testing showed an 874.2% increase in distance traveled when exposed to 2% vaping liquid (from *M* = 9.3 cm to *M* = 90.6 cm) and a 666.6% increase when exposed to 20% vaping liquid (from *M* = 29.9 cm to *M* = 229.2 cm).

Velocity increased for all fish except for wildtype fish exposed to 2% vaping liquid (20% decrease, from *M* = 0.5 cm/s to *M* = 0.4 cm/s). Otherwise, increases in velocity were consistently greater for the 20% concentration than the 2% concentration (see **Figure 5**, **Table 1**). Wildtype fish exposed to 20% vaping liquid showed a 650% increase in velocity (from *M* = 0.2 cm/s to *M* = 1.5 cm/s). Fish pre-treated with 0.01% vaping liquid prior to testing showed a 300% increase in velocity when exposed to 2% vaping liquid (from *M* = 0.1 cm/s to *M* = 0.4 cm/s). This increase was 2600% when exposed to 20% vaping liquid (from *M* = 0.1 cm/s to *M* = 2.7 cm/s). Fish pre-treated with DMSO prior to testing showed a 300% increase in velocity when exposed to 2% vaping liquid (from *M* = 0.1 cm/s to *M* = 0.4 cm/s). This increase tripled when fish were exposed to 20% vaping liquid (900% increase, from *M* = 0.3 cm/s to *M* = 3.70 cm/s).

Notably, the fish exposed to the 20% concentration initially showed rapid bursts of swimming and then were paralyzed shortly after exposure to this substance, whereas fish exposed to the 2% concentration demonstrated preserved mobility after acute exposure.

In summary, acute response tests showed that only nicotine (with respect to wildtype larval zebrafish) and 20% vaping liquids (with respect to 0.01% vaping liquid pre-treated and DMSO pre-treated larval zebrafish) caused the strongest increase in both distance traveled and velocity.

ACUTE RESPONSE TEST RESULTS				
CONDITION (line/substance)	**DISTANCE TRAVELED BEFORE (cm)**	**DISTANCE TRAVELED AFTER (cm)**	**VELOCITY BEFORE (cm/s)**	**VELOCITY AFTER (cm/s)**
Wildtype / Water	29.8 ± 1.88	70.4 ± 3.66	0.3 ± 0.02	0.3 ± 0.02
Wildtype / 10^{-3} Nicotine	30.8 ± 3.14	126.4 ± 10.72	0.3 ± 0.02	0.6 ± 0.04
Wildtype / 10^{-3} Cinnamaldehyde	21.2 ± 1.91	53.4 ± 4.84	0.2 ± 0.01	0.3 ± 0.03
Wildtype / 10^{-6} Cinnamaldehyde	20.4 ± 2.88	43.1 ± 8.78	0.2 ± 0.02	0.2 ± 0.03
Wildtype / 2% Vaping Liquid	18.9 ± 2.67	43.8 ± 6.09	0.5 ± 0.27	0.4 ± 0.05
Wildtype / 20% Vaping Liquid	17.1 ± 3.50	27.0 ± 10.22	0.2 ± 0.03	1.5 ± 0.74
0.01% VL Pre-treated / 2% Vaping Liquid	13.8 ± 5.63	69.6 ± 28.41	0.1 ± 0.05	0.4 ± 0.17
0.01% VL Pre-treated / 20% Vaping Liquid	14.1 ± 5.74	245.7 ± 100.32	0.1 ± 0.06	2.7 ± 1.12
DMSO Pre-treated / 2% Vaping Liquid	9.3 ± 3.78	90.6 ± 36.99	0.1 ± 0.03	0.4 ± 0.18
DMSO Pre-treated / 20% Vaping Liquid	29.9 ± 12.21	229.2 ± 93.56	0.3 ± 0.13	3.0 ± 1.24

Table 1. A table showing distance traveled, measured in centimeters, and velocity, measured in centimeters in seconds, for both before and after trials of each experiment. Concentrations of added solutions are shown. Calculated means and standard errors of means are presented.

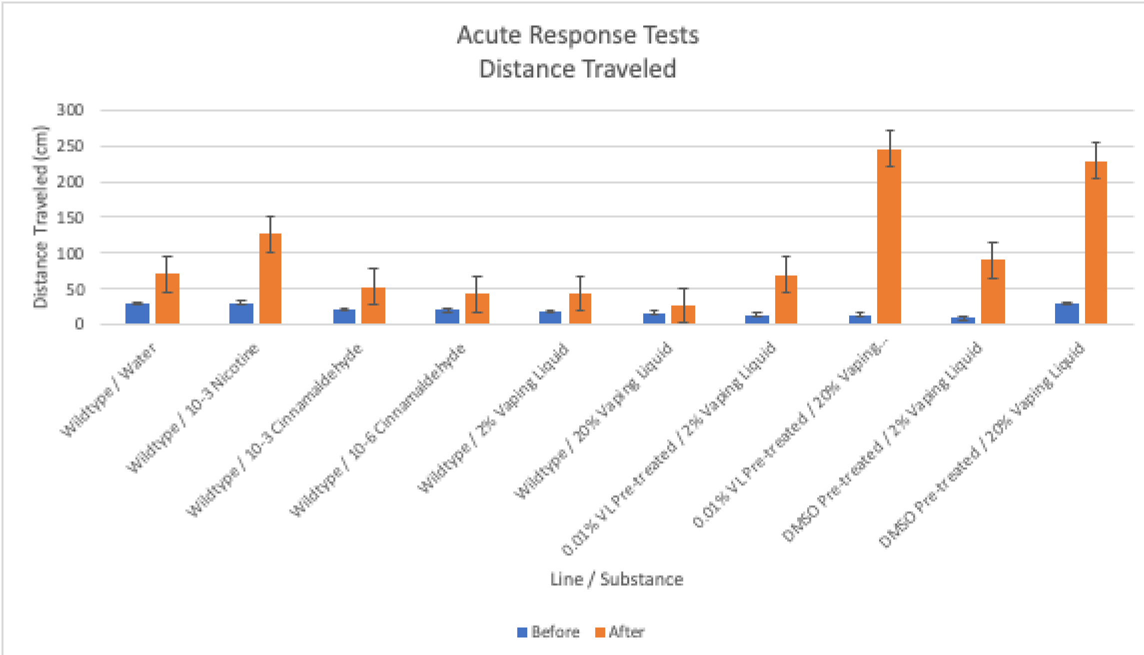


Figure 4. A histogram showing distance traveled, measured in centimeters, for each experimental condition. All bars show the average of 12 fish except for the wildtype fish exposed to nicotine (10 fish) and the pre-treated fish exposed to different concentrations of vaping liquids (6 fish each). Error bars show standard error of means.

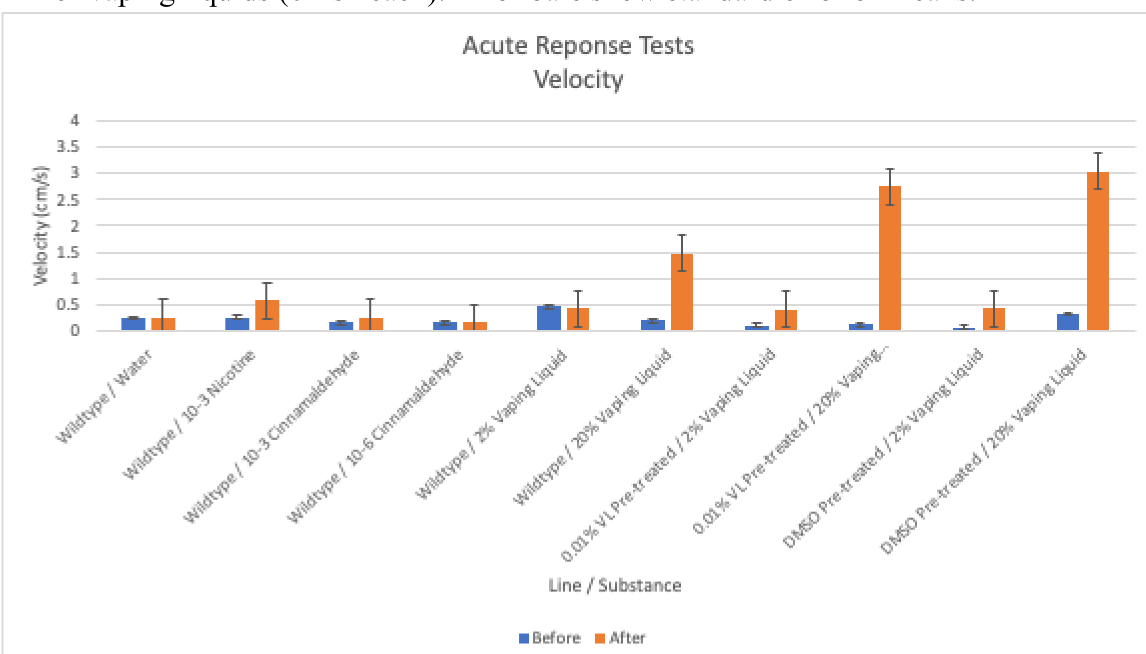


Figure 5. A histogram showing velocity, measured in centimeters per second, for each experimental condition. All bars show the average of 12 fish except for the wildtype fish exposed to nicotine (10 fish) and the pre-treated fish exposed to different concentrations of vaping liquids (6 fish each). Error bars show standard error of means.

Four-Arm Maze Tests

Four-arm maze tests determine seeking behavior in larval zebrafish by allowing freely swimming larval zebrafish to make a choice between chemicals presented in different zones. For each four-arm maze test, cumulative duration and frequency data were used to classify fish as seekers (see **Figure 6**).

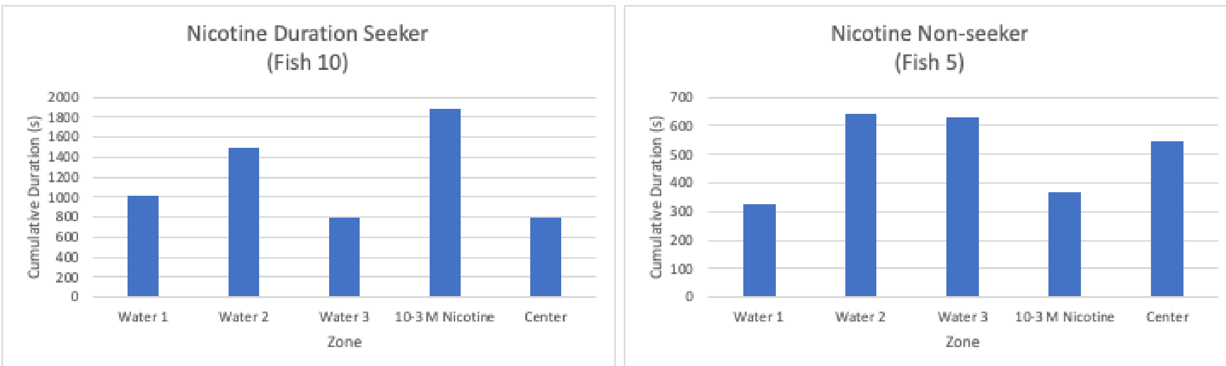


Figure 6. *Left:* A histogram showing cumulative duration data (measured in seconds) for a fish classified as a duration seeker when exposed to nicotine in a four-arm maze test. Note that the center compartment is not taken into account when classifying seekers. *Right:* A histogram showing cumulative duration data (measured in seconds) for a fish that does not classy as a duration seeker when exposed to nicotine in a four-arm maze test. Note that the center compartment is not taken into account when classifying seekers.

In order to establish baseline seeking behaviors, four-arm maze tests were conducted using water as the substance of interest. Out of twelve wildtype fish tested, four were found to be duration seekers (33.3%) and two were found to be frequency seekers (16.7%). Of these fish, two were classified as dual seekers (16.7%).

Next, four-arm maze tests were conducted with 10^{-3} M nicotine as the substance of interest to compare levels of nicotine-seeking with levels of water-seeking. In this set of experiments, three out of the twelve wildtype fish (25%) were duration seekers and two were frequency seekers (16.7%). Of these fish, two were classified as dual seekers (16.7%).

Then, 22 wildtype fish were tested with 10^{-3} M cinnamaldehyde. Six of these fish were duration seekers (27%) and six were frequency seekers (27%). Four out of the 22 wildtype fish were dual seekers (18.2%).

Finally, fish were tested with 2% and 20% vaping liquids. In the tests with vaping liquids, transgenic fish were also tested. Of the 12 wildtype fish tested with 2% vaping liquid, there were no duration seekers but there was one frequency seeker (16.7%). Because there were no duration seekers, there were no dual seekers in this experimental group. Comparatively, of the 12 wildtype fish tested with 2% vaping liquid, one fish was classified as a duration seeker (16.7%), two as frequency seekers (33.3%), and zero as dual seekers.

In the six fish that were pre-treated with 0.01% vaping liquid prior to testing and exposed to 2% vaping liquid in the four-arm maze test, two duration seekers were found (33.3%), but no frequency or dual seekers. When six different 0.01% vaping liquid pre-treated fish were exposed to 20% vaping liquid, one frequency seeker was found (16.7%), but no duration or dual seekers.

In the six fish that were pre-treated with DMSO prior to testing and exposed to 2% vaping liquid in the four-arm maze test, one duration seeker and two frequency seekers were found (16.7% and 33.3%, respectively), but no frequency or dual seekers. When six different DMSO pre-treated fish were exposed to 20% vaping liquid, there were no seekers.

In summary, amid all of the conditions, no group of fish had a percentage of seekers higher than 33.3%. With this in mind, duration seeking behavior was highest in the water condition (33.3%). Frequency seeking and dual seeking behavior were highest in the 10^{-6}

cinnamaldehyde condition (27% and 18.2%, respectively). In the 2% and 20% vaping liquid conditions, however, rates of seeking decreased, especially in terms of the number of dual seekers. Zero percent of fish were dual seekers in both the 2% and 20% vaping liquid conditions, whereas 16.7%, 16.7%, and 18.2% of fish were dual seekers in the water, nicotine, and cinnamaldehyde conditions, respectively. Additionally, of all the larval zebrafish exposed to either 2% or 20% vaping liquid, the 0.01% vaping liquid pre-treated fish exposed to 2% vaping liquid had the highest rate of duration seekers (33.3%). Wildtype fish exposed to 20% vaping liquid and DMSO pre-treated fish exposed to 2% vaping liquid had the highest rates of frequency seekers (both 33.3%). Importantly, considering the limited sample size of each group these outcomes only indicate general trends.

Further, within each group of seekers, fish sought the substances at different levels (see **Figures 7-8**). For instance, while both Fish 4 and Fish 11 were considered duration seekers after exposure to 10^{-3} M nicotine in a four-arm maze test, Fish 4 spent about 1850 seconds in the nicotine zone, but Fish 11 only spent about 1450 seconds in the nicotine zone (**Figure 7**). Further, both Fish 4 and Fish 11 were also considered frequency seekers in the same test, but Fish 4 visited the nicotine zone about 350 times, whereas Fish 11 visited the nicotine zone only about 250 times (**Figure 8**). Similar observations regarding levels of seeking behavior were made in all experimental groups with multiple duration and/or frequency seekers.

FOUR-ARM MAZE TESTS			
CONDITION (line/substance)	**DURATION SEEKERS**	**FREQUENCY SEEKERS**	**DUAL SEEKERS**
Wildtype / Water	4/12 33.3%	2/12 16.7%	2/12 16.7%
Wildtype / 10^{-3} Nicotine	3/12 25%	2/12 16.7%	2/12 16.7%
Wildtype / 10^{-6} Cinnamaldehyde	6/22 27%	6/22 27%	4/22 18.2%
Wildtype / 2% Vaping Liquid	0/6 0%	1/6 16.7%	0/6 0%
Wildtype / 20% Vaping Liquid	1/6 16.7%	2/6 33.3%	0/6 0%
0.01% VL Pre-treated / 2% Vaping Liquid	2/6 33.3%	0/6 0%	0/6 0%
0.01% VL Pre-treated / 20% Vaping Liquid	0/6 0%	1/6 16.7%	0/6 0%
DMSO Pre-treated / 2% Vaping Liquid	1/6 16.7%	2/6 33.3%	0/6 0%
DMSO Pre-treated / 20% Vaping Liquid	0/6 0%	0/6 0%	0/6 0%

Table 2. A table showing the number and percentage of duration seekers, frequency seekers, and dual seekers for each experimental group in the four-arm maze tests.

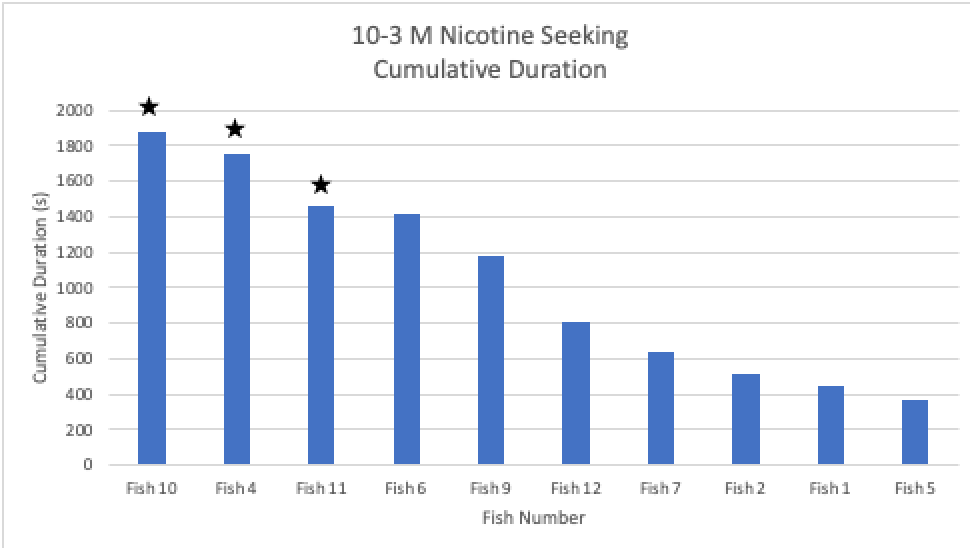


Figure 7. A histogram showing the distribution of nicotine-seeking behavior across 12 fish with respect to cumulative duration (measure in seconds). Bars marked with stars indicate fish that were classified as a duration seeker.

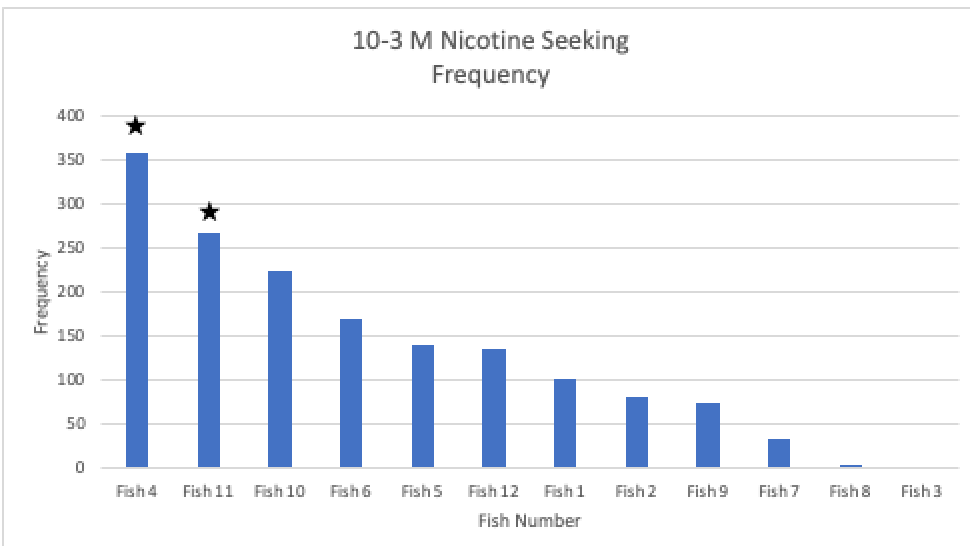


Figure 8. A histogram showing the distribution of nicotine-seeking behavior across 12 fish with respect to frequency. Bars marked with stars indicate fish that were classified as a frequency seeker.

Discussion

Overall, the acute response tests show that larval zebrafish are especially sensitive to the nicotine and the 20% vaping liquid conditions when compared to the water condition as a baseline. The heightened sensitivity of larval zebrafish to the 20% vaping liquid could be attributable to the concentration of nicotine in the vaping liquid, although a comprehensive chemical analysis of the vaping liquid would have to be conducted before this conclusion was made for certain. On a similar note, the 2% vaping liquid may not have enough nicotine to evoke a response. Future studies should conduct liquid chromatography-mass spectrometry or gas chromatography-mass spectrometry to conclusively identify the components and the concentrations of those components in the vaping liquids.

Additionally, the acute response tests reveal that vaping liquids have a toxic effect on larval zebrafish. Right after fish were exposed to the 20% vaping liquid, fish moved much further and much more quickly than fish exposed to the 2% vaping liquid. Notably, fish exposed to the 20% concentration in the acute response test were almost instantly paralyzed and did not survive. Such rapid swimming behavior followed by paralysis has been modeled in other studies where larval zebrafish are exposed to toxins (Jin et al., 2015; Chen, Wang, & Wu, 2011). In the current study, it appears that zebrafish were especially overwhelmed by acute exposure to the higher concentration of vaping liquid.

Furthermore, both DMSO pre-treated and 0.01% vaping liquid pre-treated larval zebrafish swam further and faster than wildtype fish exposed to 2% and 20% vaping liquids. These results could be due to genetic differences between the wildtype and transgenic lines, so further testing should be conducted on the transgenic fish with the other substances of interest in order to better understand these differences. Additionally, undergoing pre-treatment, whether it was with DMSO or 0.01% vaping liquid, could have influenced the behavior of transgenic fish in the acute response tests. Therefore, other studies should consider conducting acute response tests with more pre-treated fish. Previously, Petzold et al. (2009) conducted acute nicotine response tests with zebrafish that had been pre-treated with nicotine as embryos. During an acute nicotine response test, the fish that had been pre-treated with nicotine moved about 25% more than fish with no prior exposure to nicotine. Future studies should consider investigating the behavioral effects of cinnamaldehyde pre-treatments. Such research could begin to give insight into the influence of repeated e-cigarette use in humans. Further, the timing of pre-treatment may also have significant impacts on behavior, as pre-treatment during the embryo stage may produce different effects than pre-treatment at a later developmental stage, as the age of exposure to nicotine has been found to be extremely critical in whether humans develop an addiction (Jordan & Andersen, 2017).

The results of the four-arm maze tests indicate that larval zebrafish tend to avoid, rather than seek, vaping liquids and several of their components. Additionally, the four-arm maze tests emphasize the toxicity of the vaping liquids demonstrated in the acute response tests, as in the four-arm maze test, fish avoid both the 2% and 20% vaping liquids. All fish (including wildtype, 0.01% vaping liquid pre-treated, and DMSO pre-treated) exposed to 20% vaping liquid show more variation than the fish exposed to 2% vaping liquid, implying that they are having a more difficult time adjusting. It is possible that zebrafish pre-treated with .01% vaping liquid have been desensitized to the vaping liquids and therefore show less dramatic behavioral responses to the 20% vaping liquid in the four-arm maze test, but more testing would have to be conducted in order to further tease apart this trend.

That being said, it may be that the 20% vaping liquid has more toxic effects than the 2% vaping liquid. Preliminary studies conducted in Maria Bondesson's lab at Indiana University suggest that the effects observed in the current study have health-related explanations. After pre-treating zebrafish embryos with vaping liquids, fish were found to have morphological alterations with respect to their bone, cartilage, and vascular development (B. Bhattacharya, personal communication, October 30, 2019). Additionally, zebrafish embryos pre-treated with 0.02% vaping liquid did not survive to develop into larvae. These trends show that the higher the concentration of vaping liquid and the longer a fish undergoes exposure, the more deadly the outcome. Even the wildtype larval zebrafish in the current study fall into this trend, as they avoid the 20% vaping liquid more than the 2% vaping liquid. These observations point towards the conclusion that vaping liquid is unhealthy and produces detrimental effects in larval zebrafish.

Interestingly, there was less avoidance behavior found in the cinnamaldehyde condition than in any other condition. This is surprising as previous studies have found cinnamaldehyde to drive toxicity in vaping liquids (Holden et al, 2020) and cause pain in zebrafish (Taylor et al., 2017). It is likely that fish are absorbing much less cinnamaldehyde in the current study compared to in previous studies. Further, there may be a positive smell or taste associated with cinnamaldehyde that attracts zebrafish despite its negative effects.

Future four-arm maze tests could further study avoidance behavior by using mustard oil, a known irritant that healthy zebrafish tend to avoid, to assess health (Taylor et al., 2017). Such experiments could also help confirm rates of avoidance in larval zebrafish and could serve as a baseline for comparative analyses between other substances of interest. Additionally, like with acute response tests, future four-arm maze tests should consider looking into the effects of pre-treatment in order to better understand how prior exposure influences seeking behavior. Repeated incubation of larval zebrafish in the substance of interest would likely change their seeking behavior and give valuable insight into repeated exposure in humans.

Altogether, the findings of the current study provide an interesting complement to previous work by Holden et al. (2020), the only published study to have tested the influence of vaping liquids on zebrafish. Holden et al. (2020) predominantly reported developmental effects of vaping liquids. The current study implies that larval zebrafish would actually avoid the vaping liquids at the concentrations in the Holden et al. (2020) study. The four-arm maze test should be used with other flavors and concentrations in order to better understand the kinds of liquids zebrafish would show preference towards in a choice-test.

Although there are many clear advantages to using zebrafish in high-throughput toxicology screens, there are several persistent limitations. When conducting animal studies, there are always translational gaps. For example, zebrafish undergo a short period of adolescence in comparison to humans, making it difficult to tap into the same developmental sensitive periods that humans experience (Jordan & Andersen, 2017). Additionally, zebrafish were not inhaling the substances as a human does, and previous studies have found that mechanisms of ingestion versus inhalation are different in how they influence the mind and body (Kanwal et al., 2006; Galbraith & Weill, 2009).

Furthermore, the study is limited in its use of transgenic animals. There is no baseline information on the seeking behavior of these animals, and their genetic alterations may influence their general seeking behavior. Therefore, the performance of transgenic fish in the vaping liquid tests is not necessarily comparable to data obtained regarding wildtype zebrafish in other experiments. Finally, the transgenic fish could have also been influenced by their travel from Indiana University - Bloomington to DePauw University. The travel could have induced stress or anxiety, both of which could have impacted their behavior in experiments.

In the future, it will be important for researchers to consider accessing fish individually instead of in terms of averages. Just like people have different levels of susceptibility to nicotine addiction, zebrafish may also exhibit varying likelihoods of becoming addicted. In humans, studies focus a great deal on genetic differences and variability (Bruijnzeel, 2017; Mansvelder & McGehee, 2002), so the same focus should exist in animal studies.

Previous studies (Petzold et al., 2009; Schneider, 2017) have tested the response of larval fish always ends up as combining individuals and getting a mean. High-throughput screening, however, erases individual differences. While testing in large volumes, as demonstrated in the acute response tests, reveals general trends in zebrafish behavior, it does not reflect choice behavior. By using the four-arm maze, the current study begins to tease apart these individual differences. That being said, future studies should also focus on expanding the sample size of these tests in order to produce results with higher confidence. A much larger number of animals must be used in order to access the trends found in the current study.

Future Directions and Conclusions

The current thesis outlined patterns in how the tobacco industry and the United States government have perpetuated tobacco and nicotine use. More specifically, this thesis has explained how these institutions tend to emphasize the hypothetical economic importance of these substances while simultaneously minimizing their scientifically-supported dangers. When cigarettes were at their peak popularity decades again, the government chose to turn its head while the tobacco industry covered up evidence of health problems in smokers. Up until late 2019, e-cigarettes were taking a similar path, as the government largely allowed these products to go unregulated, costing thousands of people their health, or even taking their lives. Although more stringent restrictions have been set in place for e-cigarettes since the outbreak of EVALI cases in 2019, the tobacco industry is still finding loopholes in current restrictions and regulations that allow both traditional cigarette and e-cigarette manufacturers to continue to make financial gains. In order to soften the impact of this public health crisis, new cessation therapies need to be evaluated and legislative policies need to be evaluated for effectiveness. Most importantly, scientific research needs to continue to study e-cigarettes and their effects on health so that new cessation therapies and legislative policies can be based on strong empirical investigation.

Currently, there is simply not enough data to make a scientifically sound conclusion regarding the efficacy and effects of e-cigarettes and vaping devices. Although neither product should be regarded as safe, many medical professionals emphasize that combustible tobacco products are still considered more dangerous than cigarettes (Blaha, 2020). This conclusion could be due in part to the lack of long-term research on e-cigarette use. In January of 2020, the Surgeon General of the United States, Jerome Adams, released a statement regarding e-cigarette and vaping devices. In his statement, he explained that part of the difficulty of making a more decisive conclusion about e-cigarettes despite increasing research efforts is due to the quickly changing composition of e-cigarettes and the lack of research on the long-term effects of e-cigarettes (Craver, 2020). He further communicated that while we do not have all of the answers about e-cigarettes, we do know a great deal about cigarettes. One of the things we know right now is that, at least in the short-run, traditional cigarettes seem to have more detrimental effects on health than e-cigarettes. Therefore, one must access their own risk accordingly. In other words, for traditional cigarette smokers, the choice is really about picking the lesser of two evils: the well-documented harms of traditional cigarettes versus the unknown risk of e-cigarettes.

The Surgeon General further explains that "Among those who have switched completely [from traditional cigarettes to e-cigarettes], the ultimate goal should be to also stop using e-cigarettes completely" (Craver, 2020). Surgeon General Adams recognizes the negative health outcomes associated with nicotine regardless of its method of delivery and encouraged all smokers to completely rid tobacco and nicotine products from their lifestyle. He ended his report with a call to action, urging "healthcare professionals, health systems, employers, insurers, public health professionals, and policymakers to take action to put an end to the staggering, and completely preventable, human and financial tolls that smoking takes on our country" (Craver, 2020).

One potential answer to the Surgeon General's call to action is to further study other potential cessation therapies for smoking beyond e-cigarettes. Offering a safe, reliable alternative to smoking would likely become the largest public health improvement in the United States, ever. Eighty percent of people who attempt to quit smoking return to their habits within one month. Of these people, only 3% are successful (Benowitz, 2010). Currently, two major cessation aids exist: nicotine-replace therapies (such as patches, gum, and lozenges) and medication (CDC, 2019b). Medication has been found to be the most successful means of breaking nicotine addiction (CDC, 2019b). Due to these low success rates in cessation attempts, some researchers emphasize the importance of viewing nicotine addiction as a chronic disorder that needs long-term treatment (Ekpu & Brown, 2015). Interestingly, with this view in mind, antidepressants such as bupropion (Wellbutrin) and varenicline (also known as Chantix or Champix) have become increasingly common prescriptions for smoking cessation.

Bupropion, a drug that inhibits both dopamine and norepinephrine release, is also commonly prescribed to patients with major depression or seasonal affective disorder (Dwoskin et al., 2007). With respect to smoking cessation, this drug reduces withdrawal symptoms associated with cessation attempts, although finding the right dosage can prove to be a difficult task, as too low of a dose has actually been found to enhance the feelings of pleasure associated with nicotine (Dwoskin et al., 2007). Clinical studies have confirmed that higher doses of bupropion tend to produce higher cessation rates, and have confirmed that all tested doses produce higher cessation rates than a placebo. Still, one year after the trial, only 23.1% of participants exposed to the highest dose of bupropion were still successful in their cessation attempt. At this one year mark, rates of cessation success decreased as dose decreased (Hurt et al., 1997). Notably, the effectiveness of bupropion has been found to increase when the drug is combined with other cessation therapies, such as a nicotine-replace patch, although this increase is slight (Jorenby et al., 1999).

Varenicline has been found to be slightly more effective at long-term cessation success than bupropion, with roughly 34.6% of patients maintaining cessation after one year (Kaur, Kaushal, & Chopra, 2009; Garrison & Dugan, 2009). This drug is a nicotinic acetylcholine receptor partial agonist designed to reduce the pleasurable feelings associated with nicotine consumption by inhibiting dopamine release (Kaur, Kaushal, & Chopra, 2009). Like with bupropion, clinical studies have found that higher doses of varenicline are associated with higher rates of successful cessation attempts, but there is no evidence stating that lower doses of varenicline increase the pleasure effects associated with nicotine (Oncken et al., 2006).

Although bupropion and varenicline are two of the most effective treatments for nicotine addiction, their success rates are still far below 50% (Oncken et al., 2006; Garrison & Dugan, 2009). Therefore, future studies are still working to further increase success rates. One study suggests that scientists target neuropeptide systems due to their role in systems associated with the initiation of nicotine use, withdrawal symptoms, and relapse (Bruijnzeel, 2017). More specifically, two neuropeptide receptor genes: hypocretin and galanin, influence an individual's risk of nicotine dependence (Bruijnzeel, 2017). On a similar note, genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) in the human acetylcholine receptor that are indicators of one's risk to use nicotine-containing products. These receptor genes also influence how likely an individual is to relapse (Mansvelder & McGehee, 2002). By modulating the expression of these genes and SNPs, scientists may be able to increase the effectiveness of already existing cessation therapies (Bruijnzeel, 2017; Mansvelder & McGehee, 2002).

Importantly, as these alternative cessation therapies are studied, there should be more regulations in place to prevent the influence of conflicts of interest. History shows that studies funded by the tobacco industry are prone to experimental bias and unethical practices (Tan et al., 2019). In order to guarantee the publication of honest science that seeks to improve public health, sources of funding should be unbiased. At a minimum, conflicts of interest should be more clearly stated so that readers know how to better interpret findings supported by the tobacco industry.

Another potential solution to the Surgeon General's aforementioned call to action is to refine current policies regarding e-cigarettes and to access potential new policies that could help reduce the number of e-cigarette users, especially among youth and young adults. Currently, it is too early to assess the effectiveness of the Trump administration's decisions to both raise the legal age to buy tobacco and nicotine products as well as ban most flavored products. In the meantime, new research on e-cigarettes should help to inform potential legislative changes that can help to support existing ones, especially considering that although the intentions of these policy changes are to prevent children from initiating vaping, these changes may only steer adolescents and young adults to black-market products, such as the dank vapes that have been strongly tied to the EVALI outbreak.

Some of the new legislative changes could involve mandatory education on the risks and dangers associated with e-cigarettes. These changes could potentially steer youth away from e-cigarettes at large as opposed to directing them to purchase different brands under the table.

Because schools are an environment in which youth spend a large amount of time, they are an ideal place for prevention and intervention efforts to take place with regard to e-cigarette use. A 2019 study by Schillo et al. found that less than half of the teachers surveyed could identify a photo of a JUUL device, although most respondents did indicate that they had heard of the brand before. Further, many of the schools involved in the study had e-cigarette policies, but many of the policies were specifically about JUUL or specifically mentioned JUUL products (Schillo et al., 2019). According to respondents who worked at school with an e-cigarette policy, these expectations are difficult to enforce because of the product's design and the difficulty in detecting a scent or visible vapor-like in traditional vaping devices (Schillo et al., 2019).

Schillo et al.'s study (2019) gives helpful insight into where schools can begin implementing change in order to help prevent youth smoking. First, teachers must be made more aware of the products and of their risks. The more aware teachers are of these products, the more likely they will be able to detect them and enforce school policies. Additionally, policies should become broader to cover the use of devices that may not be JUUL-specific products. Finally, schools should take more care to educate students on risks associated with e-cigarettes and vaping. None of the schools surveyed by Schillo et al. (2019) mentioned any specific educational tools they were taking advantage of to teach youth about e-cigarettes. On this note, schools should conduct research on the best way to present this information to students in a meaningful way. A 1995 article by Dr. Alan Blum, the director for the Center for the Study of Tobacco & Society at the University of Alabama, states:

> All too many efforts to educate young people about tobacco have relied on pamphlets, posters, and preaching on the dangers of nicotine. In the future,

> strategies must increasingly focus on observing and listening to teenagers in their natural mass media-saturated habitat and learning from them about the important issues and images in their world.

In order to achieve maximum effectiveness in providing educational resources and circulating warnings about e-cigarettes, schools should consider addressing students through forms of media that they are most familiar and comfortable with, such as social media. After all, these are the sources by which many of them are learning about e-cigarettes (CDC, 2017a), so it may be helpful for youth to receive information through these means.

Yet another answer to the Surgeon General's call to action is to reframe how the government understands the tobacco industry's actual role in the economy. One potential solution would be to increase taxes, which would be a possible means of taking advantage of the tobacco industry economically while still decreasing the number of smokers in the United States. According to the United States Department of Health and Human Services, the most effective way to reduce nicotine consumption is to increase the price of products, and that price increases especially influencing the purchasing behaviors of youth and young adults (2012). More specifically, the CDC has also found that the single most effective means of reducing tobacco and nicotine consumption is through price increases, stating that "A 10% increase in price has been estimated to reduce overall cigarette consumption by 3–5%. Research on cigarette consumption suggests that both youth and young adults are two to three times more likely to respond to increases in price than adults" (CDC, 2019a).

Not only it is likely that the number of users would significantly decrease if e-cigarettes were taxed at a similar rate as traditional cigarettes, but the taxes would also generate revenue for both state and federal governments. Some states are already seeing this benefit. While there is currently no federal e-cigarette tax, 24 states have passed excise taxes on e-cigarettes (Maher & Gilmore, 2020). That being said, there is great variation within how much each state has chosen to tax these products, and the total estimated potential revenue generated by these taxes ranges from $1 million to $10 million (Maher & Gilmore, 2020).

As policymakers wait on more conclusive scientific evidence about the effects of e-cigarettes, policy changes keep rolling out on any different levels. Some of these policy changes have proven to be more controversial and less effective at detouring smoking and vaping. For instance, in 21 states, it is now legal for companies to stop hiring smokers. In early January of 2020, U-Haul announced a new "wellness policy" that would prevent recruiters from hiring smokers. The World Health Organization is another company that refuses to hire smokers as a symbolic salute to their commitment to reducing smoking (Schmidt et al., 2013). By having such a ban, companies can reduce the cost of healthcare expenditures. According to Schmidt et al. (2013), these companies are communicating to its employees that "smokers should be responsible for the consequences of their smoking, such as higher costs for health insurance claims, higher rates of absenteeism, and lower productivity."

Although on the surface this practice appears to be a valiant effort to reduce rates of smoking, it is not necessarily ethical. By refusing a smoker a job, these companies are choosing to not provide that person access to needed smoking cessation therapies. In fact, refusing to hire smokers further distances these people from access to health insurance and medical services. Even more alarming, these policies tend to be exclusionary, as "rates of tobacco use vary markedly among sociodemographic groups, with higher rates in poorer and less-educated populations" (Schmidt et al., 2013). Therefore, these policies are more likely to influence certain minority groups and socioeconomic groups that are more prone to nicotine addiction.

Punishment is not the answer to curbing smoking, as instead of encouraging smokers to quit, these companies are leaving people without access to the means to quit smoking. These ineffective and problematic policies further emphasize a dire need to increase research efforts on e-cigarettes. In response to these exclusionary policies, other employers, like Walgreens, have gone as far as to "provide free nicotine-replacement therapy and smoking-cessation counseling to employees" (Schmidt et al., 2013). These kinds of policies do not condone smoking, but rather provide employees with resources to quit and improve their health.

Evaluations of current and future policies should happen urgently, as the tobacco industry will surely continue to adapt and find loopholes in the current approaches. These adaptations have already begun to manifest. Despite the heightened scrutiny that e-cigarette companies have begun to face from both the government and the general public, tobacco companies chose to take advantage of a compromised customer base during the COVID-19 pandemic of 2020. Companies used strategies such as, "offering freebie protective gear, doorstep deliveries, and festive pandemic-themed discounts. Some players have donated ventilators and mounted charity campaigns" (Baumgaertner, 2020). These procedures came even after the National Institute on Drug Abuse released a statement reporting, "Because it attacks the lungs, the coronavirus that causes COVID-19 could be an especially serious threat to those who smoke tobacco or marijuana or who vape" (National Institute on Drug Abuse, 2020). None of the tobacco industry's actions can be deemed illegal, but they can certainly be considered dishonorable. These strategies encourage customers to initiate or continue vaping practices during the pandemic and cover up the heightened risk customers face of contracting and suffering from COVID-19.

Such unethical practices could be a desperate attempt for e-cigarette manufacturers to profit after a year of significant economic loss. Since its investment in JUUL, Altria's share price has lost ~19% (Kary & Levito, 2020). In less than a year, Altria lost over $30 billion due to the FDA's investigation into vaping, as well as state and personal lawsuits, following the EVALI outbreak (Reinicke, 2019). The CEO of Altria, Howard Willard, stepped down from his position in April of 2020 because of the failed JUUL investment which he had initiated at the beginning of his term in 2018 (Kary & Levito, 2020). Certainly, Altria, amongst other struggling e-cigarette manufacturers, is looking toward novel means of making up this financial deficit and will act in ways that protect their businesses.

In summary, if there are benefits to e-cigarettes and vaping devices, they are few and far between. The current research has struggled to find such benefits without raising more concerns about their medical impact. People should refrain from using e-cigarettes, especially if they have never smoked before. Furthermore, if people are planning to use e-cigarettes as a cessation tool, they should absolutely reconsider, especially because there are many other cessation devices that have been shown to be less dangerous, such as nicotine patches, nicotine gum, bupropion, and varenicline.

Additionally, although the literature on e-cigarettes is starting to experience a sort of "boom" in regards to the number of published studies on the topic, there are still gaping holes pertaining to the long-term effects of e-cigarettes. The United States government should prioritize supporting research on these products until current legislative practices can be evaluated for efficacy and reconsidered. Despite the fact that there are so many unanswered questions about these products, e-cigarettes have clearly demonstrated their ability to cause negative health outcomes and should henceforth be approached with heightened caution.

References

Abbott, B. (Producer). (2019, October 01). How Doctors Uncovered the Vaping Crisis [Audio podcast]. Retrieved from https://gimletmedia.com/shows/the-journal/gmh33n/how-doctors-uncovered-the-vaping-crisis

Allen, J. G., Flanigan, S. S., LeBlanc, M., Vallarino, J., MacNaughton, P., Stewart, J. H., & Christiani, D. C. (2016). Flavoring chemicals in e-cigarettes: diacetyl, 2, 3-pentanedione, and acetoin in a sample of 51 products, including fruit-, candy-, and cocktail-flavored e-cigarettes. *Environmental Health Perspectives*, *124*(6), 733-739. https://doi.org/10.1289/ehp.1510185

Alltucker, K. (2020, January 2). FDA bans mint- and fruit-flavored vaping products but exempts menthol and tobacco. Retrieved from https://www.usatoday.com/story/news/health/2020/01/02/vaping-ban-fda-strikes-mint-and-fruit-flavored-products/2796299001/

Altman, A. (2009, January 2). The Cigar. Retrieved from http://content.time.com/time/nation/article/0,8599,1869320,00.html

American Heart Association. (2018, October 17). Why it's so hard to quit smoking. Retrieved from https://www.heart.org/en/news/2018/10/17/why-its-so-hard-to-quit-smoking

Barth, A. L., Justice, N. J., & Ngai, J. (1996). Asynchronous onset of odorant receptor expression in the developing zebrafish olfactory system. *Neuron*, *16*(1), 23-34. https://doi.org/10.1016/S0896-6273(00)80020-3

Baumgaertner, E. (2020, April 17). Tobacco, vaping industries seize opportunities in coronavirus with freebies, donations. Retrieved from https://www.latimes.com/science/story/2020-04-17/coronavirus-tobacco-vaping-industries-opportunities

Benowitz, N. L. (2010). Nicotine addiction. *New England Journal of Medicine*, *362*(24), 2295-2303. https://doi.org/10.1056/NEJMra0809890

Blaha, M. J. (2020, January 21). 5 Vaping Facts You Need to Know. Retrieved from https://www.hopkinsmedicine.org/health/wellness-and-prevention/5-truths-you-need-to-know-about-vaping

Blum, A. (1995). DOC's Deck-O-Butts trading cards: using humour to change youth attitudes about tobacco. *Tobacco Control*, *4*(3), 219. https://doi.org/10.1136/tc.4.3.219

Brandt, A. M. (2012). Inventing conflicts of interest: a history of tobacco industry tactics. *American Journal of Public Health, 102*(1), 63-71. https://doi.org/10.2105/AJPH.2011.300292

Brandt, A. M. (2007). *The cigarette century: The rise, fall, and deadly persistence of the product that defined America*. Basic Books.

Brett, E. I., Stevens, E. M., Wagener, T. L., Leavens, E. L., Morgan, T. L., Cotton, W. D., & Hébert, E. T. (2019). A content analysis of JUUL discussions on social media: Using Reddit to understand patterns and perceptions of JUUL use. *Drug and Alcohol Dependence,* 194, 358-362. https://doi.org/10.1016/j.drugalcdep.2018.10.014

Broom, J. (2018). *A history of cigarette and trade cards: The magic inside the packet*. Grub

Street Publishers.
Brown, J., Beard, E., Kotz, D., Michie, S., & West, R. (2014). Real‐world effectiveness of e‐cigarettes when used to aid smoking cessation: a cross‐sectional population study. *Addiction*, *109*(9), 1531-1540. https://doi.org/10.1111/add.12623
Bruijnzeel, A. W. (2017). Neuropeptide systems and new treatments for nicotine addiction. *Psychopharmacology*, *234*(9-10), 1419-1437. https://doi.org/10.1007/s00213-016-4513-5
Burns, D. M., Lee, L., Shen, L. Z., Gilpin, E., Tolley, H. D., Vaughn, J., & Shanks, T. G. (1997). Cigarette smoking behavior in the United States. In National Institutes of Health, Department of Health & Human Services, National Cancer Institute *Changes in cigarette-related disease risks and their implications for prevention and control* (pp. 13-42). Createspace Independent Pub.
Butala, N. M., Secemsky, E. A., Wasfy, J. H., Kennedy, K. F., & Yeh, R. W. (2018). Seasonality and Readmission after Heart Failure, Myocardial Infarction, and Pneumonia. *Health Services Research, 53*(4), 2185-2202. https://doi.org/10.1111/1475-6773.12747
Charlton, A. (2004). Medicinal uses of tobacco in history. *Journal of the Royal Society of Medicine*, *97*(6), 292-296. https://doi.org/10.1258/jrsm.97.6.292
Chen, T. H., Wang, Y. H., & Wu, Y. H. (2011). Developmental exposures to ethanol or dimethylsulfoxide at low concentrations alter locomotor activity in larval zebrafish: implications for behavioral toxicity bioassays. *Aquatic Toxicology*, *102*(3-4), 162-166. https://doi.org/10.1016/j.aquatox.2011.01.010
Centers for Disease Control and Prevention (CDC). (1999). Tobacco use--United States, 1900-1999. MMWR. *Morbidity and Mortality Weekly Report, 48*(43), 986.
Centers for Disease Control and Prevention (CDC). (2017a). Current Cigarette Smoking Among U.S. Adults Aged 18 Years and Older. Retrieved from https://www.cdc.gov/tobacco/campaign/tips/resources/data/cigarette-smoking-in-united-states.html
Centers for Disease Control and Prevention (CDC). (2017b). E-cigarette Ads and Youth. Retrieved from https://www.cdc.gov/vitalsigns/ecigarette-ads/index.html
Centers for Disease Control and Prevention (CDC). (2019a). Outbreak of Lung Injury Associated with the Use of E-Cigarette, or Vaping, Products. Retrieved from https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html
Centers for Disease Control and Prevention (CDC). (2019b). Quitting Smoking. with the Use of E-Cigarette, or Vaping, Products. Retrieved from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/cessation/quitting/index.htm
Centers for Disease Control and Prevention (CDC). (2006, December). History of the Surgeon General's Report on Smoking and Health. Retrieved from https://www.cdc.gov/tobacco/data_statistics/sgr/history/index.htm
Clapp, P. W., Pawlak, E. A., Lackey, J. T., Keating, J. E., Reeber, S. L., Glish, G. L., & Jaspers, I. (2017). Flavored e-cigarette liquids and cinnamaldehyde impair respiratory innate immune cell function. *American Journal of Physiology-Lung Cellular and Molecular Physiology, 313*(2), L278-L292. https://doi.org/10.1152/ajplung.00452.2016
Craver, R. (2020, January 23). U.S. Surgeon General update has mixed message on e-cigarette

effectiveness. Retrieved from https://www.journalnow.com/business/u-s-surgeon-general-update-has-mixed-message-on-e/article_d1c44960-9730-5beb-b9e6-1ed889e10593.html

Creswell, J., & Kaplan, S. (2019, November 23). How Juul Hooked a Generation on Nicotine. Retrieved from https://www.nytimes.com/2019/11/23/health/juul-vaping-crisis.html

Cummings, K. M. (2002). Programs and policies to discourage the use of tobacco products. *Oncogene*, *21*(48), 7349-7364. https://doi.org/10.1038/sj.onc.1205810

Cummings, K. M., & Proctor, R. N. (2014). The changing public image of smoking in the United States: 1964–2014. *Cancer Epidemiology and Prevention Biomarkers*, *23*(1), 32-36. https://doi.org/10.1158/1055-9965.EPI-13-0798

Ducharme, J. (2019, November 6). How Lawsuits and Regulations Are Chipping Away at Juul. Retrieved from https://time.com/5713970/juul-lawsuits-regulations-bans/

Diaz, A. (2019, September 25) Juul CEO steps down as worries about vaping grow. *The Los Angeles Times*. Retrieved from https://www.latimes.com/business/story/2019-09-25/juul-labs-ceo-kevin-burns-is-stepping-down

DiFranza, J. R., Richards, J. W., Paulman, P. M., Wolf-Gillespie, N., Fletcher, C., Jaffe, R. D., & Murray, D. (1991). RJR Nabisco's cartoon camel promotes Camel cigarettes to children. *JAMA*, *266*(22), 3149-3153. https://doi.org/10.1001/jama.1991.03470220065028

Dwoskin, L. P., Rauhut, A. S., King‐Pospisil, K. A., & Bardo, M. T. (2006). Review of the pharmacology and clinical profile of bupropion, an antidepressant and tobacco use cessation agent. *CNS Drug Reviews*, *12*(3‐4), 178-207.

Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., ... & Mohnot, S. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, *205*(1), 38-44. https://doi.org/10.1111/j.1527-3458.2006.00178.x

Ekpu, V. U., & Brown, A. K. (2015). The economic impact of smoking and of reducing smoking prevalence: a review of evidence. *Tobacco Use Insights*, *8*, TUI-S15628. https://doi.org/10.4137/TUI.S15628

Faber, S. (2018, May 16). Thank You for (Subsidizing) Smoking. Retrieved from https://www.ewg.org/agmag/2018/05/thank-you-subsidizing-smoking

Fadus, M. C., Smith, T. T., & Squeglia, L. M. (2019). The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. *Drug and Alcohol Dependence. 201*, 85-93 https://doi.org/10.1016/j.drugalcdep.2019.04.011

Food and Drug Administration (2009). *Evaluation of e-cigarettes.* St. Louis: Food and Drug Administration. Retrieved from http://www.casaa.org/wp-content/uploads/Evalution-of-electronic-cigarettes.pdf

Federal Trade Commission. (2019). *Federal Trade Commission Smokeless Tobacco Report for 2017* Washington: Federal Trade Commission. Retrieved from https://www.ftc.gov/system/files/documents/reports/federal-trade-commission-cigarette-report-2017-federal-trade-commission-smokeless-tobacco-report/ftc_smokeless_tobacco_report_2017.pdf

Fischer, P. M., Schwartz, M. P., Richards, J. W., Goldstein, A. O., & Rojas, T. H. (1991). Brand logo recognition by children aged 3 to 6 years: Mickey Mouse and Old Joe the Camel. *JAMA*, *266*(22), 3145-3148. https://doi.org/10.1001/jama.266.22.3145

Food and Drug Administration. (2009). *FDA Warns of Health Risks Posed by E-Cigarettes*. Atlanta: U.S. Department of Health and Human Services, 2009. Retrieved from www.fda.gov/NewsEvents/Newsroom/ PressAnnouncements/ucm173222.htm

Galbraith, D., & Weill, D. (2009). Popcorn lung and bronchiolitis obliterans: a critical appraisal. *International Archives of Occupational and Environmental Health*, *82*(3), 407. https://doi.org/10.1007/s00420-008-0337-x

Garrison, G. D., & Dugan, S. E. (2009). Varenicline: a first-line treatment option for smoking cessation. *Clinical Therapeutics*, *31*(3), 463-491. https://doi.org/10.1016/j.clinthera.2009.03.021

Glantz, S. A. (2018, June 11). Michael Russell, the father of tobacco harm reduction, had ties with BAT. Retrieved from https://tobacco.ucsf.edu/michael-russell-father-tobacco-harm-reduction-had-ties-bat

Goniewicz, M. L., Kuma, T., Gawron, M., Knysak, J., & Kosmider, L. (2013). Nicotine levels in electronic cigarettes. *Nicotine & Tobacco Research*, *15*(1), 158-166. https://doi.org/10.1093/ntr/nts103

Goniewicz, M. L., Smith, D. M., Edwards, K. C., Blount, B. C., Caldwell, K. L., Feng, J., ... & van Bemmel, D. (2018). Comparison of nicotine and toxicant exposure in users of electronic cigarettes and combustible cigarettes. *JAMA Open Network*, *1*(8), e185937-e185937.

Goodman, J. (2005). *Tobacco in history: The cultures of dependence*. Routledge.

Grana, R., Benowitz, N., & Glantz, S. A. (2014). E-cigarettes: a scientific review. *Circulation*, *129*(19), 1972-1986. https://doi.org/10.1161/CIRCULATIONAHA.114.007667

Grunwald, D. J., & Eisen, J. S. (2002). Headwaters of the zebrafish—emergence of a new model vertebrate. *Nature Reviews Genetics*, *3*(9), 717. https://doi.org/10.1038/nrg892

Hajek, P., Etter, J. F., Benowitz, N., Eissenberg, T., & McRobbie, H. (2014). Electronic cigarettes: review of use, content, safety, effects on smokers and potential for harm and benefit. *Addiction, 109*(11), 1801-1810. https://doi.org/10.1111/add.12659

Hall, K. (2017, July 25). Do Filtered Cigarettes Lower the Risk of Lung Cancer? Retrieved from https://health.usnews.com/health-care/patient-advice/articles/2017-07-25/do-filtered-cigarettes-lower-the-risk-of-lung-cancer

Hammond, E. C., & Horn, D. (1958). Smoking and Death Rates—Report on 44 Months of Follow-up of 187,783 Men: I. Total Mortality. *JAMA*, *166*(11), 1159-1172.

Hammond, S. K. (2009). Global patterns of nicotine and tobacco consumption. In *Nicotine Psychopharmacology* (pp. 3-28). Springer, Berlin, Heidelberg.

Hansen, A., Reutter, K., & Zeiske, E. (2002). Taste bud development in the zebrafish, *Danio rerio*. *Developmental Dynamics: An Official Publication of the American Association of Anatomists, 223*(4), 483-496. https://doi.org/10.1002/dvdy.10074

Hansen, A., & Zeiske, E. (1993). Development of the olfactory organ in the zebrafish, *Brachydanio rerio*. Journal of Comparative Neurology, 333(2), 289-300.

Hatsukami, D. K., Stead, L. F., & Gupta, P. C. (2008). Tobacco addiction. *The Lancet*, *371*(9629), 2027-2038. https://doi.org/10.1016/S0140-6736(08)60871-5

Hecht, S. S. (2011). Research opportunities related to establishing standards for tobacco products under the Family Smoking Prevention and Tobacco Control Act. *Nicotine & Tobacco Research*, *14*(1), 18-28. https://doi.org/10.1093/ntr/ntq216

Holden, L. L., Truong, L., Simonich, M. T., & Tanguay, R. L. (2020). Assessing the hazard of E-Cigarette flavor mixtures using zebrafish. *Food and Chemical Toxicology*, *136*, 110945. https://doi.org/10.1016/j.fct.2019.110945

Howard, J. (2019, December 27). The US officially raises the tobacco buying age to 21. Retrieved from https://www.cnn.com/2019/12/27/health/us-tobacco-age-21-trnd/index.html

Huang, J., Duan, Z., Kwok, J., Binns, S., Vera, L. E., Kim, Y., ... & Emery, S. L. (2019). Vaping versus JUULing: how the extraordinary growth and marketing of JUUL transformed the US retail e-cigarette market. *Tobacco Control, 28*(2), 146-151. https://doi.org/10.1136/tobaccocontrol-2018-054382

Hurt, R. D., Sachs, D. P., Glover, E. D., Offord, K. P., Johnston, J. A., Dale, L. C., ... & Croghan, I. T. (1997). A comparison of sustained-release bupropion and placebo for smoking cessation. *New England Journal of Medicine*, *337*(17), 1195-1202. https://doi.org/10.1056/NEJM199710233371703

Jackler, R. K., Chau, C., Getachew, B. D., Whitcomb, M., Lee-Heidenreich, J., Bhatt, A., & Ramamurthi, D. (2019). JUUL advertising over its first three years on the market. *SRITA White Paper*.

Jackler, R. K., & Ramamurthi, D. (2019). Nicotine arms race: JUUL and the high-nicotine product market. *Tobacco Control*, *28*(6), 623-628. https://doi.org/10.1136/tobaccocontrol-2018-054796

Jain, R., & Mukherjee, K. (2003). Biological basis of nicotine addiction. *Indian Journal of Pharmacology*, *35*(5), 281-289. http://medind.nic.in/ibi/t03/i5/ibit03i5p281.pdf

Jardine, D., & Litvak, M. K. (2003). Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. *Journal of Fish Biology*, *63*(2), 388-397. https://doi.org/10.1046/j.1095-8649.2003.00161.x

Jin, Y., Liu, Z., Peng, T., & Fu, Z. (2015). The toxicity of chlorpyrifos on the early life stage of zebrafish: a survey on the endpoints at development, locomotor behavior, oxidative stress, and immunotoxicity. *Fish & Shellfish Immunology*, *43*(2), 405-414. https://doi.org/10.1016/j.fsi.2015.01.010

JLI Science. (n.d.) Retrieved from https://jliscience.com/about-us

Jordan, C. J., & Andersen, S. L. (2017). Sensitive periods of substance abuse: Early risk for the transition to dependence. *Developmental Cognitive Neuroscience*, *25*, 29-44. https://doi.org/10.1016/j.dcn.2016.10.004

Jorenby, D. E., Leischow, S. J., Nides, M. A., Rennard, S. I., Johnston, J. A., Hughes, A. R., ... & Fiore, M. C. (1999). A controlled trial of sustained-release bupropion, a nicotine patch, or both for smoking cessation. *New England Journal of Medicine*, *340*(9), 685-691. https://doi.org/10.1056/NEJM199903043400903

JUUL, Inc. (n.d.) Retrieved from https://www.juul.com/our-story

Kaisar, M. A., Prasad, S., Liles, T., & Cucullo, L. (2016). A decade of e-cigarettes: limited research & unresolved safety concerns. *Toxicology, 365*, 67-75. https://doi.org/10.1016/j.tox.2016.07.020

Kanwal, R., Kullman, G., Piacitelli, C., Boylstein, R., Sahakian, N., Martin, S., ... & Kreiss, K. (2006). Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. *Journal of Occupational and Environmental Medicine*, *48*(2), 149-157. https://doi.org/10.1097/01.jom.0000194152.48728.fb

Kaplan, S. (2020, January 23). Surgeon General Says 'Shocking' Portion of People Aren't Told to Stop Smoking. Retrieved from https://www.nytimes.com/2020/01/23/health/surgeon-general-smoking-e-cigarettes.html?searchResultPosition=8

Kary, T., & LaVito, A. (2020, April 17). Altria Names Billy Gifford as CEO After Howard Willard Steps Down. Retrieved from https://www.bloomberg.com/news/articles/2020-04-17/altria-names-gifford-as-ceo-after-willard-steps-dowm

Kaur, K., Kaushal, S., & Chopra, S. C. (2009). Varenicline for smoking cessation: a review of the literature. *Current Therapeutic Research*, *70*(1), 35-54. https://doi.org/10.1016/j.curtheres.2009.02.004

Kelly, J. (2019, November 19). President Trump Backtracks On Flavored Vaping Ban-Fearing Potential Job Losses. Retrieved from https://www.forbes.com/sites/jackkelly/2019/11/19/president-trump-backtracks-on-flavored-vaping-ban-out-of-fear-of-jobs-losses/#602565eb1812

Kennedy, C. D., van Schalkwyk, M. C., McKee, M., & Pisinger, C. (2019). The cardiovascular effects of electronic cigarettes: A systematic review of experimental studies. *Preventive Medicine*, 105, 770. https://doi.org/10.1016/j.ypmed.2019.105770

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253-310. https://doi.org/10.1038/nrg2091

Klee, E. W., Ebbert, J. O., Schneider, H., Hurt, R. D., & Ekker, S. C. (2011). Zebrafish for the study of the biological effects of nicotine. *Nicotine & Tobacco Research, 13*(5), 301-312. https://doi.org/10.1093/ntr/ntr010

Kliff, S. (2011, September 19). What would happen if Americans stopped smoking? Retrieved from https://www.washingtonpost.com/blogs/ezra-klein/post/what-would-happen-if-americans-stopped-smoking/2011/09/19/gIQAIpXBfK_blog.html?wprss=ezra-klein

LaVito, A. (2018, December 20). Tobacco giant Altria takes 35% stake in Juul, valuing e-cigarette company at $38 billion. Retrieved from https://www.cnbc.com/2018/12/20/altria-takes-stake-in-juul-a-pivotal-moment-for-the-e-cigarette-maker.html

Lee, H. W., Park, S. H., Weng, M. W., Wang, H. T., Huang, W. C., Lepor, H., ... & Tang, M. S. (2018). E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. *Proceedings of the National Academy of Sciences, 115*(7), E1560-E1569. https://doi.org/10.1073/pnas.1718185115

Li, J., Mack, J. A., Souren, M., Yaksi, E., Higashijima, S. I., Mione, M., ... & Friedrich, R. W. (2005). Early development of functional spatial maps in the zebrafish olfactory bulb. *Journal of Neuroscience*, *25*(24), 5784-5795. https://doi.org/10.1523/JNEUROSCI.0922-05.2005

Linnane, C., & Kilgore, T. (2019, September 28). Philip Morris and Altria merger talks are off as Juul CEO steps down amid vaping fears. Retrieved from https://www.marketwatch.com/story/philip-morris-and-altria-merger-talks-are-off-as-juul-ceo-steps-down-amid-vaping-fears-2019-09-25

Little, B. (2018, September 13). When Cigarette Companies Used Doctors to Push Smoking. Retrieved from https://www.history.com/news/cigarette-ads-doctors-smoking-endorsement

Mack‐Bucher, J. A., Li, J., & Friedrich, R. W. (2007). Early functional development of interneurons in the zebrafish olfactory bulb. *European Journal of Neuroscience*, *25*(2), 460-470. https://doi.org/10.1111/j.1460-9568.2006.05290.x

Madison, M. C., Landers, C. T., Gu, B. H., Chang, C. Y., Tung, H. Y., You, R., ... & Putluri, N. (2019). Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. *The Journal of Clinical Investigation*, *129*(10). https://doi.org/10.1172/JCI128531

Maher, E., & Gilmore, S. (2020, April 6). E-Cigarette & Vaping Product Taxes. Retrieved from https://www.ncsl.org/research/fiscal-policy/electronic-cigarette-taxation.aspx

Mansvelder, H. D., & McGehee, D. S. (2002). Cellular and synaptic mechanisms of nicotine addiction. *Journal of Neurobiology*, *53*(4), 606-617. https://doi.org/10.1002/neu.10148

Massarsky, A., Abdel, A., Glazer, L., Levin, E. D., & Di Giulio, R. T. (2017). Exposure to 1, 2-propanediol impacts early development of zebrafish (*Danio rerio*) and induces hyperactivity. *Zebrafish*, *14*(3), 216-222. https://doi.org/10.1089/zeb.2016.1400

Mendes, E. (2014, January 9). The Study That Helped Spur the U.S. Stop-Smoking Movement. Retrieved from https://www.cancer.org/latest-news/the-study-that-helped-spur-the-us-stop-smoking-movement.html

Miech, R., Johnston, L., O'Malley, P. M., Bachman, J. G., & Patrick, M. E. (2019). Trends in adolescent vaping, 2017–2019. *New England Journal of Medicine*, *381*(15), 1490-1491. https://doi.org/10.1056/NEJMc1910739

Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., & Garg, A. (2015). Harmful effects of nicotine. *Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical & Paediatric Oncology, 36*(1), 24–31. https://doi.org/10.4103/0971-5851.151771

Mitchell, A. & Young, A. L. (Producers). (2019, October 19). *The Promise and Peril of Vaping, Part 2: The Story of Juul* [Audio podcast]. https://www.nytimes.com/2019/10/30/podcasts/the-daily/juul-dangers.html

Nardone, N., Helen, G. S., Addo, N., Meighan, S., & Benowitz, N. L. (2019). JUUL electronic cigarettes: Nicotine exposure and the user experience. *Drug and Alcohol Dependence, 203,* 83-87. https://doi.org/10.1016/j.drugalcdep.2019.05.019

National Center for Biotechnology Information. (2020). PubChem Database. Benzoic acid, CID=243, Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Benzoic-acid

National Institute on Drug Abuse. (2020, April 6). COVID-19: Potential Implications for Individuals with Substance Use Disorders. Retrieved from https://www.drugabuse.gov/about-nida/noras-blog/2020/04/covid-19-potential-implications-individuals-substance-use-disorders

National Parks Service (NPS). (2019). *Tobacco: The Early History of a New World Crop.*

Retrieved from https://www.nps.gov/jame/learn/historyculture/tobacco-the-early-history-of-a-new-world-crop.htm

NJOY. (n.d.). Retrieved from https://shop.njoy.com/about-us/

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

Oncken, C., Gonzales, D., Nides, M., Rennard, S., Watsky, E., Billing, C. B., ... & Reeves, K. (2006). Efficacy and safety of the novel selective nicotinic acetylcholine receptor partial agonist, varenicline, for smoking cessation. *Archives of Internal Medicine*, *166*(15), 1571-1577. https://doi.org/10.1001/archinte.166.15.1571

Peltz, J. F. (2019, September 11) Trump to propose ban on flavoring used in e-cigarettes. *The Los Angeles Times.* Retrieved from https://www.latimes.com/politics/story/2019-09-11/trump-propose-ban-on-flavorings-e-cigarettes

Petzold, A. M., Balciunas, D., Sivasubbu, S., Clark, K. J., Bedell, V. M., Westcot, S. E., ... & Ekker, S. C. (2009). Nicotine response genetics in the zebrafish. *Proceedings of the National Academy of Sciences*, *106*(44), 18662-18667. https://doi.org/10.1073/pnas.0908247106

Porter, P. G. (1969). Origins of the American Tobacco Company. *Business History Review*, *43*(1), 59-76. https://doi.org/10.2307/3111987

Preston, A. (Producer). (2019, June 28). Raising the Age to Purchase Tobacco: Will it solve the problem and is vaping to blame? [Audio podcast]. Retrieved from https://omny.fm/shows/newscycle/raising-the-age-to-purchase-tobacco-will-it-solve

Proctor, R. N. (2012). The history of the discovery of the cigarette–lung cancer link: evidentiary traditions, corporate denial, global toll. *Tobacco Control*, *21*(2), 87-91. https://doi.org/10.1136/tobaccocontrol-2011-050338

Rahman, I., Lamb, T., & Muthumalage, T. (2019). JUUL e-cigarette and vape pen flavors impose oxidative stress and inflammatory responses in lung epithelial cells and macrophages. *American Thoracic Society, 107*, A4186-A4186. https://doi.org/10.1164/ajrccm-conference.2019.199.1_MeetingAbstracts.A4186

Rahman, M. A., Hann, N., Wilson, A., Mnatzaganian, G., & Worrall-Carter, L. (2015). E-cigarettes and smoking cessation: evidence from a systematic review and meta-analysis. *PLOS One*, *10*(3). https://doi.org/10.1371/journal.pone.0122544

Reinicke, C. (2019, September 20). Top Juul investor Altria has seen $30 billion - almost a third of its market value - erased since the FDA launched a vaping investigation in April (MO) | Markets Insider. Retrieved from https://markets.businessinsider.com/news/stocks/juul-investor-altrias-stock-price-has-fallen-amid-vaping-crackdown-2019-9-1028541639

Robertson, G. N., McGee, C. A. S., Dumbarton, T. C., Croll, R. P., & Smith, F. M. (2007). Development of the swimbladder and its innervation in the zebrafish, *Danio rerio*. *Journal of Morphology*, *268*(11), 967-985. https://doi.org/10.1002/jmor.10558

Rodrigues, J. (2009, March 31). When smoking was cool, cheap, legal and socially acceptable.

Retrieved from https://www.theguardian.com/lifeandstyle/2009/apr/01/tobacco-industry-marketing

Sawrey, K. (Producer). (2019, October 24). Vaping: What the Hell is Going On?! [Audio podcast]. Retrieved from https://gimletmedia.com/shows/science-vs/n8hw5a/vaping-what-the-hell-is-going-on

Sackerman, J., Donegan, J. J., Cunningham, C. S., Nguyen, N. N., Lawless, K., Long, A., ... & Gould, G. G. (2010). Zebrafish behavior in novel environments: effects of acute exposure to anxiolytic compounds and choice of *Danio rerio* line. *International Journal of Comparative Psychology/ISCP; sponsored by the International Society for Comparative Psychology and the University of Calabria*, *23*(1), 43. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2879659/

Sax, J. K. (2012). Financial conflicts of interest in science. *Annals Health L.*, *21*, iv.

Schillo, B. A., Cuccia, A. F., Patel, M., Simard, B., Donovan, E. M., Hair, E. C., & Vallone, D. (2019). JUUL in School: Teacher and Administrator Awareness and Policies of E-Cigarettes and JUUL in US Middle and High Schools. *Health Promotion Practice*. https://doi.org/10.1177/1524839919868222

Schmidt, H., Voigt, K., & Emanuel, E. J. (2013). The ethics of not hiring smokers. *New England Journal of Medicine*, *368*, 1369-1371. https://doi.org/10.1056/NEJMp1301951

Schneider, H. (2017). Zebrafish neurobehavioral assays for drug addiction research. In *The rights and wrongs of zebrafish: Behavioral phenotyping of zebrafish* (pp. 171-205). Springer, Cham.

Schneider, H., Pearson, A. C., & Harris, D. (June 2018). *Nicotine-seeking behavior and gene expression in juvenile zebrafish.* Poster presented at the Society for Neuroscience Conference, San Diego, CA.

Schroeder, M. O. (2019, October 10). Smoking and Vaping – and Not Quitting Either? You're Not Alone. Retrieved from https://health.usnews.com/wellness/articles/smoking-and-vaping

Sherwood, C. L., & Boitano, S. (2016). Airway epithelial cell exposure to distinct e-cigarette liquid flavorings reveals toxicity thresholds and activation of CFTR by the chocolate flavoring 2, 5-dimethypyrazine. *Respiratory Research*, *17*(1), 57. https://doi.org/10.1186/s12931-016-0369-9

Siegel, M. B., Tanwar, K. L., & Wood, K. S. (2011). Electronic cigarettes as a smoking-cessation tool: results from an online survey. *American Journal of Preventive Medicine*, *40*(4), 472-475. https://doi.org/10.1016/j.amepre.2010.12.006

Stewart, A. M., Nguyen, M., Wong, K., Poudel, M. K., & Kalueff, A. V. (2014). Developing zebrafish models of autism spectrum disorder (ASD). *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *50*, 27-36. http://dx.doi.org/10.1016/j.pnpbp.2013.11.014

Stolerman, I. P., & Jarvis, M. J. (1995). The scientific case that nicotine is addictive. *Psychopharmacology*, *117*(1), 2-10. https://doi.org/10.1007/BF02245088

Tan, A. S., Soneji, S., Moran, M. B., & Choi, K. (2019). JUUL Labs' sponsorship and the scientific integrity of vaping research. *The Lancet, 394*(10196), 366-368. https://doi.org/10.1016/S0140-6736(19)31718-0

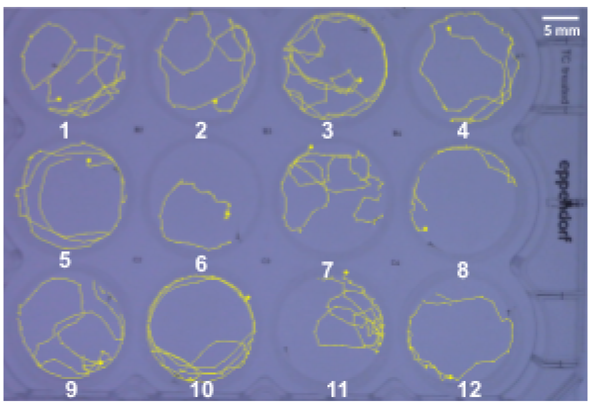
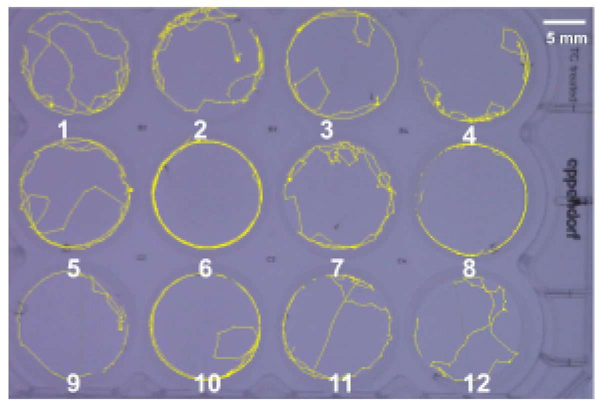
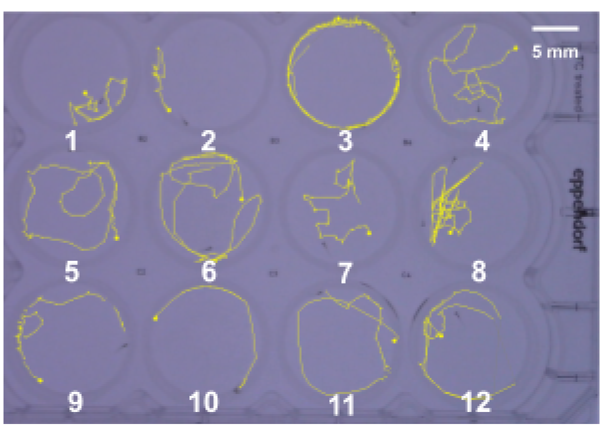
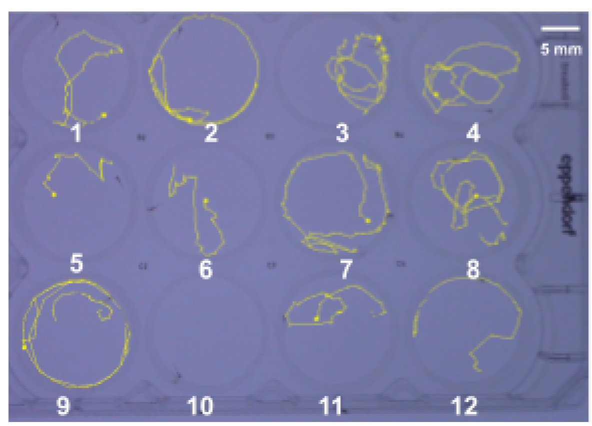
Taylor, J. C., Dewberry, L. S., Totsch, S. K., Yessick, L. R., DeBerry, J. J., Watts, S. A.,

& Sorge, R. E. (2017). A novel zebrafish-based model of nociception. *Physiology & Behavior, 174*, 83-88. https://doi.org/10.1016/j.physbeh.2017.03.009
U.S. Department of Agriculture. (1994). *Tobacco World Markets and Trade*. Washington: U.S. Department of Agriculture, National Agricultural Statistics Service, 1994
U.S. Department of Agriculture. (2019). *Crop Production 2018 in 2015 Summary*. Washington: U.S. Department of Agriculture, National Agricultural Statistics Service, 2019
U.S. Department of Health and Human Services. (2012). *Preventing Tobacco Use Among Youth and Young Adults: 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, 2012.
U.S. Department of Health and Human Services. (2014). The health consequences of smoking—50 years of progress: a report of the Surgeon General. Atlanta: U.S. Department of Health and Human Services, 2014.
U.S. Department of Health Human Services. *Women and smoking: A report of the Surgeon General.* Rockville, MD: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease, Prevention and Health Promotion, Office on Smoking and Health, 2001.
Viswesvaran, C., & Schmidt, F. L. (1992). A meta-analytic comparison of the effectiveness of smoking cessation methods. *Journal of Applied Psychology*, *77*(4), 554. https://doi.org/10.1037/0021-9010.77.4.554
Warner, K. E. (2000). The economics of tobacco: myths and realities. *Tobacco Control*, *9*(1), 78-89. https://doi.org/10.1136/tc.9.1.78
Warner, K. E., & Fulton, G. A. (1995). Importance of tobacco to a country's economy: an appraisal of the tobacco industry's economic argument. *Tobacco Control*, *4*(2), 180. https://doi.org/10.1136/tc.4.2.180
Witschi, H. (2001). A short history of lung cancer. *Toxicological Sciences*, *64*(1), 4-6. https://doi.org/10.1093/toxsci/64.1.4
World Health Organization (2006). Tobacco: deadly in any form or disguise. [Press release] Retrieved from https://www.who.int/tobacco/communications/events/wntd/2006/Tfi_Rapport.pdf
World Health Organization (2008) Marketers of electronic cigarettes should halt unproved therapy claims [Press release]. Retrieved from https://www.who.int/mediacentre/news/releases/2008/pr34/en/
World Health Organization (2013) WHO report on the global tobacco epidemic: enforcing bans on tobacco advertising, promotion, and sponsorship. Retrieved from http://apps.who.int/iris/bitstream/10665/85380/1/9789241505871_eng.pdf
World Health Organization (2013) Taxing Tobacco: A win-win for public health outcomes and mobilizing domestic resources. Retrieved from https://www.worldbank.org/en/topic/tobacco/brief/taxing-tobacco-a-win-win-for-public-health-outcomes-mobilizing-domestic-resources

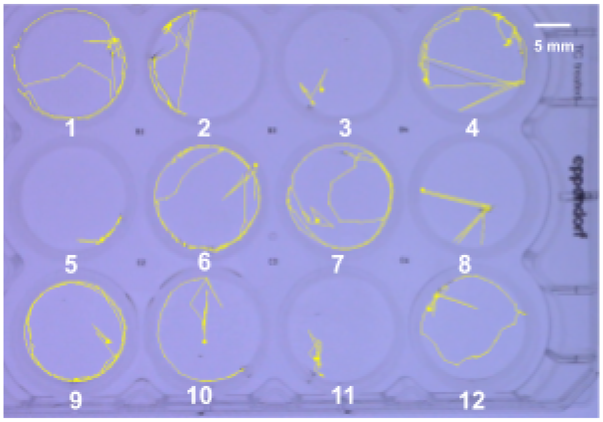
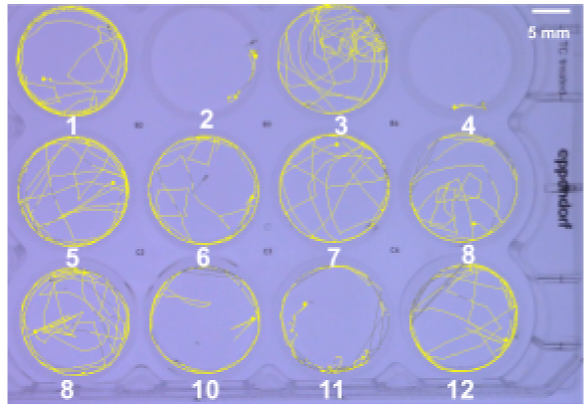
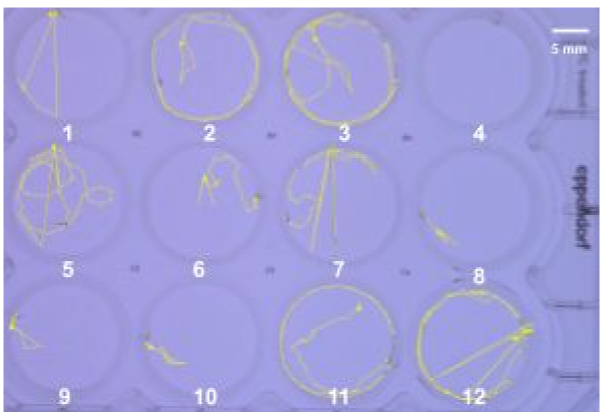
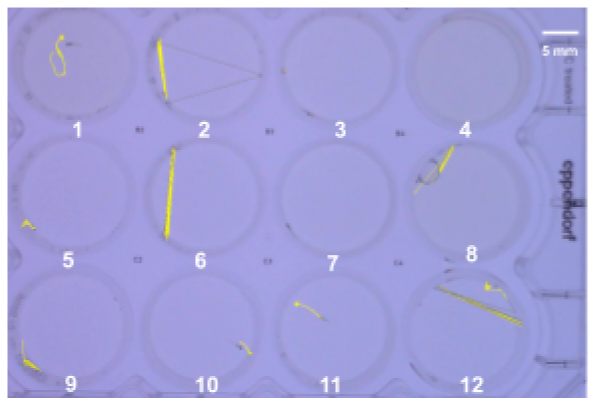
Appendix

Acute Response Test Track Map Visualizations

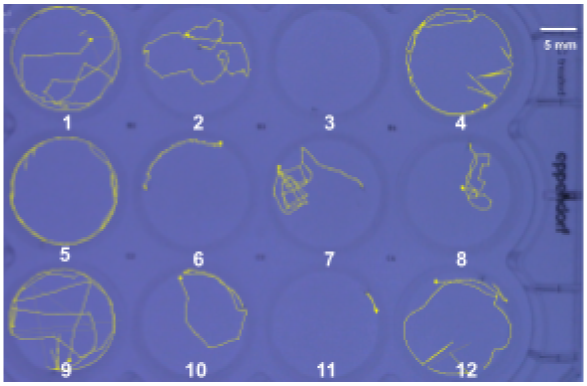
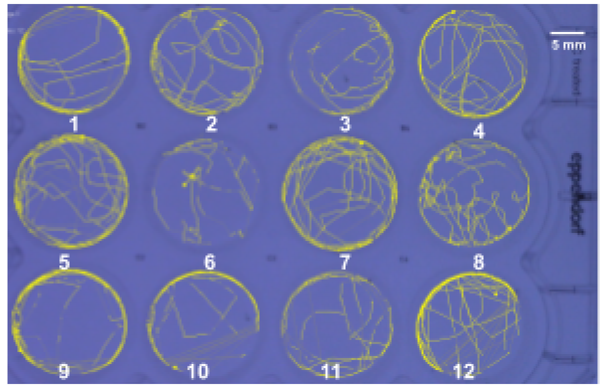
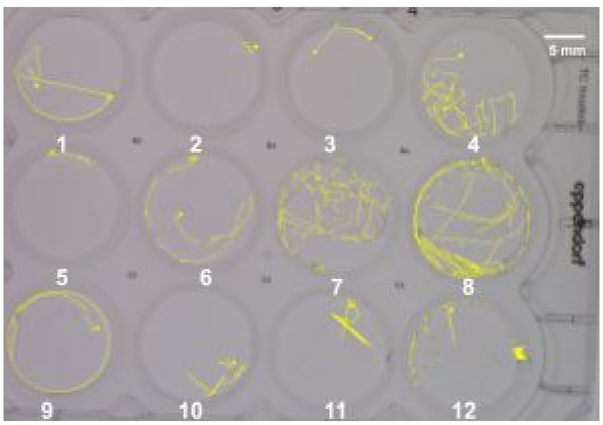
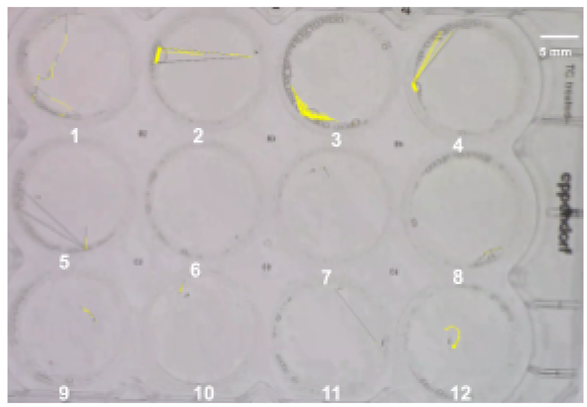
All supplementary images show track map visualizations of acute response tests. The panels to the right show a yellow track of the two minutes before application of 200 µl of the substance of interest, which is indicated above the image. The panels to the left show a yellow track of the two minutes after application of 200 µl of the substance of interest. The "after" tracks show the last two minutes of a five-minute recording.

<table>
<tr><td>BEFORE ADDITION OF CA (1 MILLIMOLAR)</td><td>AFTER ADDITION OF CA (1 MILLIMOLAR)</td></tr>
<tr><td>
</td><td>
</td></tr>
<tr><td>BEFORE ADDITION OF CA (1 MICROMOLAR)</td><td>AFTER ADDITION OF CA (1 MICROMOLAR)</td></tr>
<tr><td>
</td><td>
</td></tr>
</table>

Supplementary Figure 1. Track map visualizations of larval zebrafish exposed to cinnamaldehyde. In all images, fish are wildtype.

BEFORE ADDITION OF VAPING LIQUID (2%)	AFTER ADDITION OF VAPING LIQUID (2%)
	
BEFORE ADDITION OF VAPING LIQUID (20%)	AFTER ADDITION OF VAPING LIQUID (20%)
	

Supplementary Figure 2. Track map visualizations of larval zebrafish exposed to vaping liquids. In all images, all fish are wildtype.

<table>
<tr><td>BEFORE ADDITION OF VAPING LIQUID (2%)</td><td>AFTER ADDITION OF VAPING LIQUID (2%)</td></tr>
<tr><td>
</td><td>
</td></tr>
<tr><td>BEFORE ADDITION OF VAPING LIQUID (20%)</td><td>AFTER ADDITION OF VAPING LIQUID (20%)</td></tr>
<tr><td>
</td><td>
</td></tr>
</table>

Supplementary Figure 3. Track map visualizations of larval zebrafish exposed to vaping liquids. In all images, Fish 1, 2, 5, 6, 9, and 10 were pre-treated with .01% vaping liquid and Fish 3, 4, 7, 8, 11, and 12 were pre-treated with DMSO.