

Neurological and Physiological Effects of Vape Smoke Inhalation

Honors Undergraduate Senior Thesis

By

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Abstract:

The rise in electronic cigarette usage amongst adolescents and adults throughout the recent years has given led to experimental research studying the various chemicals found in these devices as well as the physiological implications they pose on the human body. This research plans to perform a thorough differential analysis of chemicals found within various types of currently, and popularly, used electronic cigarettes. Liquid samples taken from the reservoirs of electronic cigarettes were prepped where chemical results were determined via Gas Chromatography – Mass Spectrometry (GC-MS). A literature review of the found chemicals was referenced with different studies on neurological and physiological pathways within the human body where potential adverse, and unforeseen, effects of the chemicals on the body were stated and explained.

Introduction:

Cigarettes have been the main source of nicotine for Americans and others around the world. The emergence of the technological revolution of the 21st century infiltrated electronic innovation branched into almost every facet of human daily lives. One byproduct of this spread of technology was the mass production and commercialization of electronic cigarettes. Even though iterations of the “smokeless” tobacco pens have been around since the 1920s, it was not until the production of the first commercialized “e-cigarette” in 2003 by Chinese pharmacist, Hon Lik, that sparked the mass interest and widespread use throughout the rest of the world. The dangers of smoking tobacco have been noted ever since the 18th century, when doctors like Dr. Benjamin Rush noted how smoking was "offensive" and “a-moral”, suggesting that smoking

could cause "incurable diseases" and "cancers". Today, the use of electronic cigarettes and vapes is now considered an epidemic among teens in the US, where a 2019 study found over 5 million high schoolers and middle schoolers admitted to having smoked vapes within the last month of being surveyed. Results also included around 60% of the students preferring the JUUL vape as their e-cig of choice. Little was known about the compounds within the vapes, despite being sold on a mass scale. Throughout the past several years, the emergence of a pulmonary illness has been observed in a significant number of young people using these electronic cigarettes. This pulmonary injury was named EVALI, or e-cigarette or vaping product use-associated lung injury. "In August of 2019, the first case of EVALI (e-cigarette, or vaping, product use-associated lung injury) was reported to the US Centers for Disease Control and Prevention. The number of cases peaked in September 2019, and as of Feb 18, 2020, 2807 EVALI cases had been reported with 68 deaths" (CDC). Since the emergence of the COVID-19 pandemic, EVALI cases have not been nearly as high. However, the CDC stopped updating case information in February of 2020. With the emergence of EVALI, the compounds found within different vapes and their flavors have begun to be researched and the chemical effects on different areas of the body are still under extensive research to this day. Current research has been centered around analyzing these various vape chemical compounds and different physiological implications from their use. Much of the present research, however, focuses on respiratory and pulmonary effects, considering these are the result of smoke inhalation. Little research is available on the neurological effects imposed from vape smoke inhalation, as not all chemical constituents within all different types of vape e-liquid (electronic liquid) are fully known. The aim of this research is to collect various e-liquid samples from differently styled and flavored vapes throughout the SUNY Brockport campus. The collected samples were-tested on a gas chromatograph with mass

spectrometry detection (GC-MS) to determine their specific chemical compositions. Literature research was also conducted to determine the chemical constituents with potential neurological effects.

Mechanics of Electronic Cigarettes/Vapes:

To understand how electronic cigarettes, or vapes, contribute to the overall health of the user, the mechanism of vape action must be understood. E-cigarettes are normally filled with a liquid that contains nicotine and other preservative chemicals. This liquid is then heated and combusted by a heating mechanism within the device, and an aerosol vapor is then released for inhalation by the user. The vapor travels into the lungs, where it is then absorbed and processed.

Generally, vapes contain four different structural components that contribute to their overall functionality, as shown in Figure 1. They contain a battery as a power source; a cartridge or reservoir pod, which holds the liquid used for combustion; a heating element, known as an atomizer; and lastly, a mouthpiece where the vapor is released and inhaled by the user (NIH). Electronic liquids are initially heated up by the atomizer, through the pressing of a button, or activation due to sensors detecting the user inhaling onto the device. The vapor produced is drawn up the device and through the mouthpiece, where it is inhaled by the user. Heating and combustion are terminated once suction on the mouthpiece is stopped, or the user releases from a button that is used to activate the atomizer. These simple mechanistic components allow vapes to be so inexpensive and able to be mass produced.

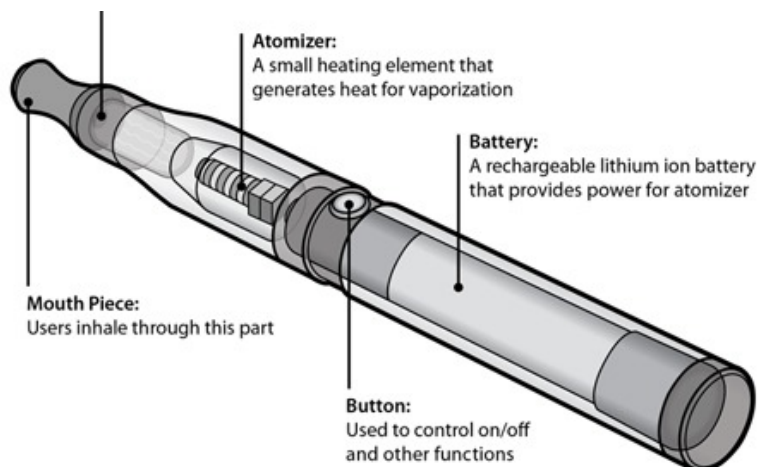


Figure 1. The Components to a conventional vape. The overall mechanics are not complex, making the device easy to use, inexpensive to manufacture, and easily accessible. (NIH)

Different Types of Electronic Cigarettes/Vapes:

As of January of 2020, there are 460 differently branded electronic cigarettes on the market, with practically all vapes falling into three categories based on functionality. There are tanks & mods, rechargeable e-cigarettes (such as *JUUL* brand), and disposable e-cigarettes (such as *Hyde* brand). Tanks and mods are larger in size and served as the main attraction for early recreational vapers, originating in the early to mid-2010s. Tanks and mods contain larger battery and heating compartments, which allows users to more finely control inputs on power and temperature. Most users will vape compounds between 350-500 degrees Fahrenheit, or 176-260 degrees Celsius, with the most preferred temperature falling around 420 degrees Fahrenheit, or 215 degrees Celsius. This precision control in tanks and mods allows the user to produce different consistencies of vapor tethered to their preference in flavor. Users generally change the temperature that their tank combustion based on the types of e-liquid they use to adjust for the flavoring they want. These vapes generally contain screw-on pods that allow the user to refill them with the e-liquid of their choice. Tanks and mods can be recharged using common charging cables, such as micro-USB and C-USB.

The charging properties of rechargeable vapes parallel that of tanks and mods. However, there are other differences within the rechargeable vapes that allow for them to be in a category of their own. Rechargeable vapes are notably much smaller than tanks and mods. Unlike the ability for users to manipulate power and temperature on tanks and mods, most rechargeable vapes are manufactured with a set temperature at which they will combust the liquid inside. This also means they are manufactured with a set, unchangeable, value at which power will be expended. Rechargeable vapes, such as the JUUL, operate at a temperature of 420 degrees Fahrenheit, or 215 degrees Celsius (BMJ). The JUUL, like many other rechargeable vapes, does not contain re-fillable pods, meaning users can select from a variety of flavors pre-loaded into disposable pods that are interchangeable with the vape unit. Other vape companies, such as Suorin, produce rechargeable vapes that also come with pods, or cartridges, that users can refill themselves with their own preference of vape juice. Despite not being as personalized as tanks and mods, rechargeable vapes allow users with more convenience, being cheaper and easier to use in many different situations/locations when compared to the much larger tanks and mods. As popularity around vaping progressed, these types of vapes began to dominate as the device of use for vapers around the world.

The last style of vapes are known as disposable vapes, made to eventually be completely thrown out after a single use. These vapes operate in almost the same exact manner as rechargeable ones, yet do not have the ability to be recharged. They usually contain a fixed pod with a pre-loaded flavor that cannot be refilled. Like rechargeable vapes, disposable vapes are produced with a set temperature at which they will burn. Disposable vapes have become the dominant style preferred in recent years, over both rechargeable vapes and tanks and mods. Disposable vapes are generally hastily produced, thus prone to breaking and leaking a significant

amount of the time. It has also been anecdotally observed that the battery within disposable vapes will die before the vape has ran out of juice. This has led users to taking apart these vapes and trying to manually charge the batteries, leading to potential harm from doing so. Disposable vapes are marketed as incredibly colorful devices filled with fruity flavors, enticing for younger crowds. There is no surprise that disposable vapes are as widely used today. In 2015, there had been more 58 million vapes sold in U.S. grocery and convenience stores, with around 19.2 million of those units designed for single use (Yogi). Figure 2 below shows the different types of vapes widely available on the market today.

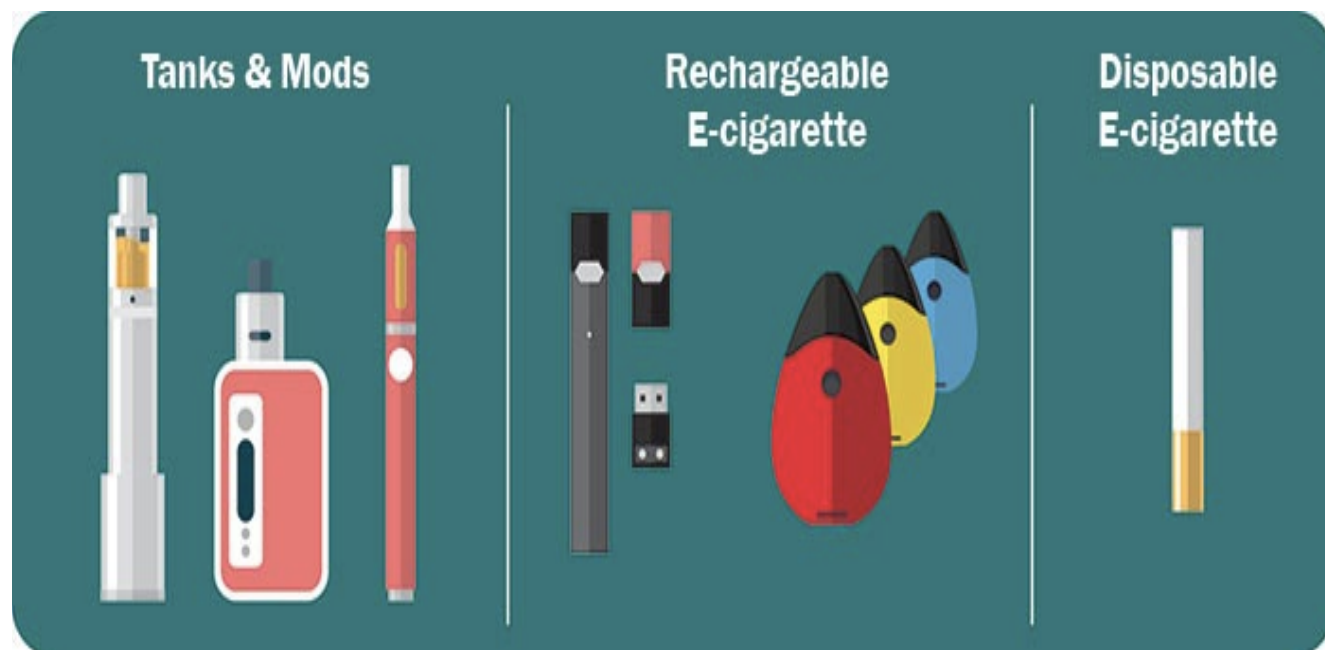


Figure 2. Seen, left to right, are the three main categories of vapes on the market: Tanks and Mods, Rechargeable E-cigarettes, and Disposable E-cigarettes. (CDC)

Different Types of E-liquids:

Not all electronic cigarettes, however, use the same liquid within their pods. Due to the variety of styles of vapes produced, users also have a choice to choose their own style of e-liquid, or purchase disposable vapes that contain different flavors. The main types of e-liquid

available contain a mix of nicotine, vegetable glycerin, propylene glycol, and flavoring. There are four major types of e-liquids, or vape juices, which are used in vapes today. These liquids are known as regular, or freebase nicotine, vape juice, nicotine salt vape juice, sub-ohm salt vape juice, and tobacco-free nicotine vape juice. Regular, or freebase nicotine, vape juice is rather a mixture of propylene glycol, vegetable glycerin, flavoring, and a form of nicotine, called “freebase”. Nicotine naturally found in tobacco leaves are known to protonate, meaning an extra hydrogen atom, having one proton and one electron, has attached at either nitrogen found within the chemical structure of nicotine. Naturally occurring nicotine has been seen to be both monoprotinated and di-protonated in nature, meaning either one, or two, hydrogen atoms have bonded to the nitrogen atoms on the molecule (Seeman). Freebase nicotine can be produced through the addition of ammonia to protonated nicotine solutions to remove hydrogen protons already bonded to the nitrogen atoms within the molecule. Figure 3 demonstrates the differences in chemical structure of protonated and deprotonated nicotine being acted on by ammonia.

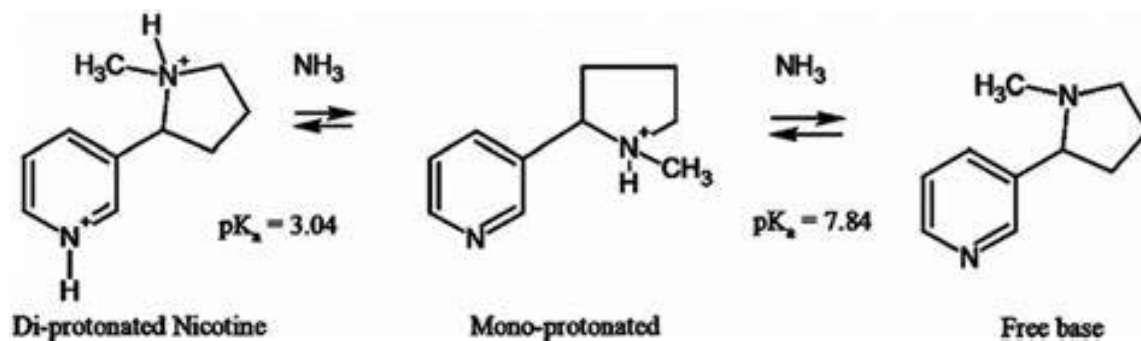


Figure 3. De-protonation of nicotine involves the mixing of ammonia into nicotine solutions to create *freebase* nicotine (Mitra)

Removing extra protons from nicotine increases potency and bodily absorption, without increasing concentration. Absorption rate of nicotine increases with pH, as more protons are removed, nicotine becomes more basic (Holloway). Freebase nicotine is known to be more potent than its protonated derivatives, allowing vape manufacturers to have stronger products

without having to increase the concentration of liquid used (K). Freebase nicotine serves as the main form of nicotine found in vapes where other vape juices use this form of nicotine, along with other types, such as nicotine salts. Figure 4 below shows how increasing the pH of nicotine, through deprotonation, increases its rate of absorption within the human body.

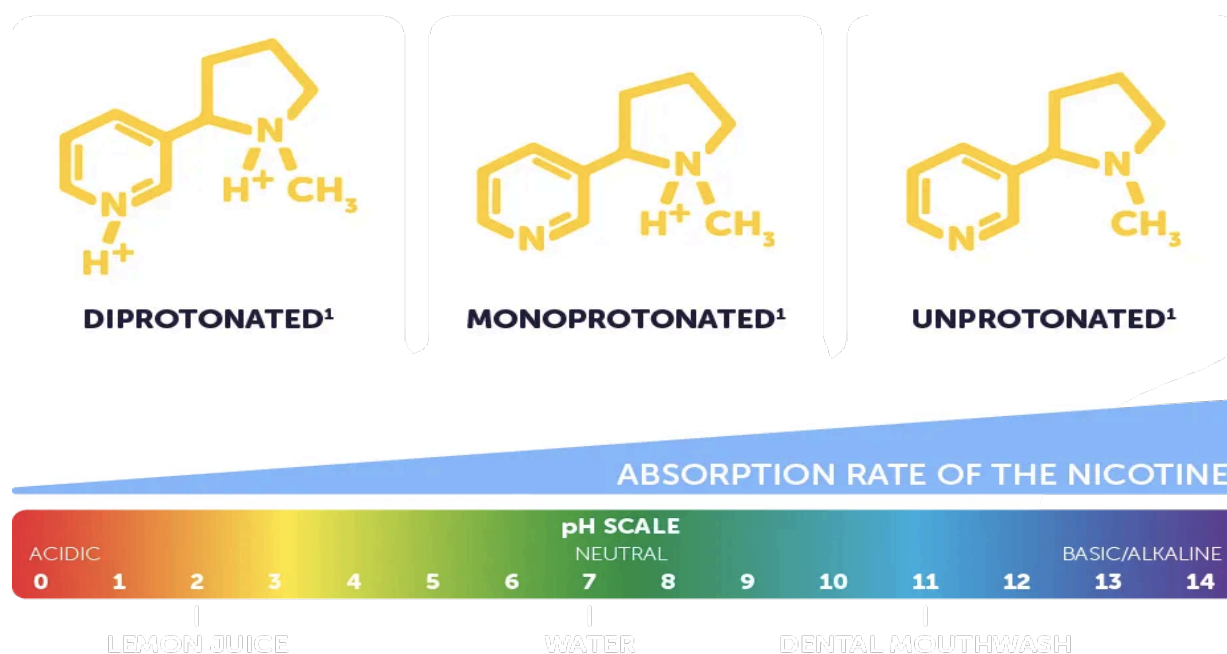


Figure 4. With the deprotonation of nicotine, absorption rate and pH increase directly as well. (Holloway)

Nicotine salt vape juice is served with freebase nicotine as the other dominant type of vape juice. Some nicotine salt vape juices have similar concentrations of propylene glycol, vegetable glycerin, and flavoring as freebase nicotine juices. However, some nicotine salt vape juices have been noted to have a 50/50 propylene glycol: vegetable glycerin concentration mix, whereas others have had a slightly higher amount of propylene glycol (K, Lam). Both vegetable glycerin and propylene glycol play roles in flavoring, preservation, and vapor production. Nicotine salts, however, model the protonated nicotine derivatives, with protons bonded to nitrogen from the addition of organic acids, such as benzoic acid, citric acid and saccharic acid. Organic acids are compounds bonded with a carboxylic group, which can readily donate protons

from an extending hydroxyl substituent at the end of the carboxylic acid. Having nicotine bonded with protons from organic acids lowers its pH and notably allows for a smoother, less harsh, draw when inhaling. Figure 5 below shows the differences in chemical structures and transitions of protonated nicotine to nicotine salts. Salt nicotine is more commonly found in low-powered devices, such as rechargeable and disposable vapes, whereas higher-powered devices, such as tanks and mods, use freebase nicotine liquids.

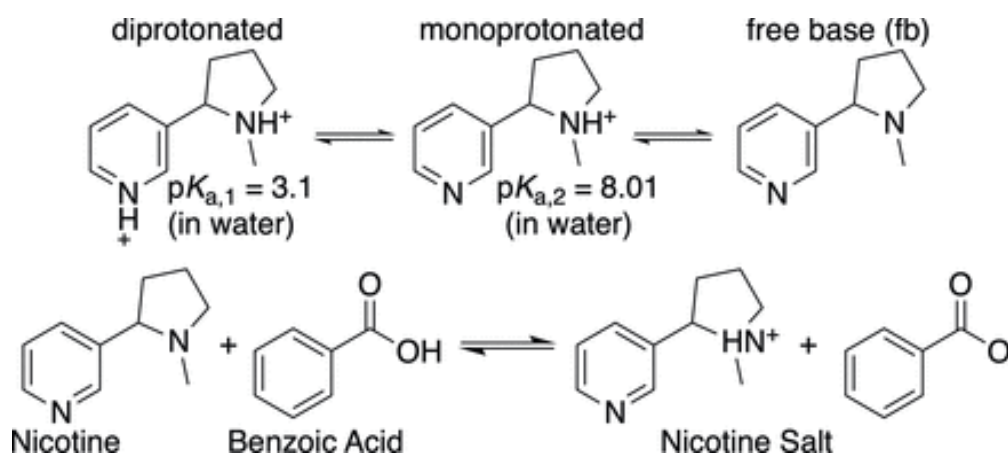


Figure 5. Depicted above are the chemical reactions between protonated nicotine derivatives along with nicotine salts. (Duell)

The next form of e-liquid is known as sub-ohm salt vape juice, a mixture of nicotine salts, propylene glycol, vegetable glycerin, and flavoring. Sub-ohm salt juices normally contain a higher amount of vegetable glycerin than propylene glycol, allowing thicker vapor production (Lam). Before sub-ohm vape juices had been produced, salt nicotine could only be found in pod-based rechargeable vapes, such as JUUL and BO. Sub-ohm vapes now allows users to use tanks and mods and be able to inhale nicotine salts, rather than freebase nicotine. This form of juice is better suited for high-powered devices, such as tanks and mods, allowing for a smoother draw during inhalation.

The last form of vape juice on the market has also gained much interest within the vaping industry for companies, such as Next Generation Labs, who have been producing synthetic, or tobacco-free, nicotine vape juice. This type of e-liquid is similar to the other main types in propylene glycol, vegetable glycerin, and flavoring concentrations. However, lab-made nicotine is substituted for nicotine extracted from tobacco leaves. Vaping companies purchasing and using these types of liquids include NKTR, Cypher, Origins, Defiant, and Klir (Johnson). The production of synthetic nicotine is thought to be cheaper and easier than conventional tobacco extraction, though some evidence shows that tobacco-free nicotine juice production has not been widely pursued due to the cost of extraction and production, which rivals that of conventional extraction methods. In addition, a pure, pharmaceutical-grade form of synthetic nicotine is hard to produce, leaving companies to produce industrial-grade solutions, which are rather harmful for human consumption. Synthetic nicotine is mostly made from niacin, ethanol, and sulfuric acid, in combination with other chemical additives (Berkey). The health effects from the heating and combustion of these and other compounds have raised health concerns, stimulating research into the production and use of vapes and their chemical vape juice substituents.

Physiological Effects of Vape Smoke:

Current information on prolonged vape usage includes multiple studies on current negative health implications on lung, and overall respiratory physiology. Many pathways involved within pulmonary respiration have been studied in how it interacts with vape smoke. Structures affected during vaping range from nasal epithelia to bronchial epithelia to mechanisms within alveoli. Prolonged vape usage has seen an inhibition of ciliary beating and a downregulation of immune genes within nasal epithelia (Gotts). Ciliary beating, otherwise known as Mucociliary Clearance (MCC), is the biomechanical process of cellular ciliary

beating/moving in rhythm to remove trapped particles and pathogens within the mucous layers of the nasal passages (Bustamante-Marin). Inhibition of MCC increases risk of pathogenic infection, as it serves as an innate defense mechanism, and overall dysfunction in particle clearance of nasal epithelium, as trapped particles are removed less efficiently. Ciliary beating inhibition can also be observed within bronchial epithelia, acting the same as it does within nasal epithelia. Excessive vape usage has shown the downregulation of immune genes within nasal epithelia, such as EGR1 (*Early Growth Response 1*), having implications in regulating the expression of other immune genes involved in cytokine/chemokine production, adhesion molecules, proteases, and autophagy genes (Martin).

Prolonged vape usage also has negative implications within bronchial epithelia. Effects within bronchial epithelia include, but are not limited to, increased MUC5AC expression, CFTR inhibition, ciliary beating inhibition, and increased cytokine secretion. The MUC5AC protein has many effects within pulmonary functionality. One primary function is to bind to inhaled particles, which are then removed in MCC. Increased MUC5AC levels have been observed during lung inflammation, while many lung diseases, including COPD (Chronic Obstructive Pulmonary Disease), share increased expression of the protein (Figueiredo). This is seemingly a normal immune response in lung infection, as increased mucin levels increase the capture, suspension, and removal of pathogens within epithelia. Additionally, a study on the effects of nicotine-free electronic vapor saw an increase in MUC5AC levels, as well as the levels of the cytokine, IL-6. Interleukin-6 (IL-6) is a pro-inflammatory cytokine with high levels seen in acute and chronic pulmonary infections, including COPD. The exposure of nicotine-free vape smoke on human small airway epithelial cells (SAEC) resulted with a correlated increase of MUC5AC and IL-6, suggesting similarities within the activating pathways of the two molecules (Gellatly).

Extended release of IL-6 can lead to tissue damage within lung epithelia, as pro-inflammatory signaling has been associated with a multitude of diseases, including arthritis, heart disease, Alzheimer's disease, and cancer (Ricciotti). The inhibition of CFTR (cystic fibrosis transmembrane conductance regulator) from vape smoke serves to increase airway dehydration on ASL (airway surface liquid) due to the dysfunction within the CFTR protein. CFTR is an ion channel protein that functions to pass sodium and chloride ions across the airway epithelial membrane (Kalininskiy). Dysfunction of the CFTR protein leads to reduced sodium and chloride ion exchange, increasing viscosity of the mucous found on the ASL, thereby increasing airway dehydration. A more viscous mucous layer may have implications on innate immune responses, as inhaled particles and pathogens have an easier time passing deeper into the respiratory tract to initiate an infection.

Lastly, bronchial epithelia experience an increase in stiffness and impairment of vasoconstriction and overall gas exchange resulting from extended vape usage. The increase in alveolar stiffness has been seen in many breathing disorders, such as pulmonary fibrosis, a disease involving respiratory dysfunction resulting from damaged or scarred lung tissue. Additionally, stiffness of lung parenchyma (alveoli and other structures) leads to the increase in TGF- β (transforming growth factor beta) and HIF-1 α (hypoxia-inducible factor 1 subunit alpha). Increased expression of both proteins on alveolar endothelia has been associated with the formation of pulmonary fibrosis (Yang). Cases of pulmonary arterial hypertension (PAH) can also be observed from extended vape usage, as increased pressure can be due to a result of vasoconstriction within pulmonary arteries and arterioles. Increased arterial pressure forces the heart to increase cardiac output to compensate for increased resistance within pulmonary arteries. Vape-induced vasoconstriction decreases rates of gas exchange from alveoli to pulmonary

circulation, decreasing the rate of oxygenation of red blood cells. Decreased levels of gas exchange, including oxygenation of RBCs (red blood cells) and release of carbon dioxide, may have implications on acute, and even chronic, respiratory acidosis, in which the lungs have difficulty removing adequate carbon dioxide from the body. The implications of prolonged vape use on many different aspects of pulmonary function have been studied extensively and can be seen below in Figure 6. Many of these implications share congruencies in symptoms of a multitude of pulmonary diseases and dysfunctions.

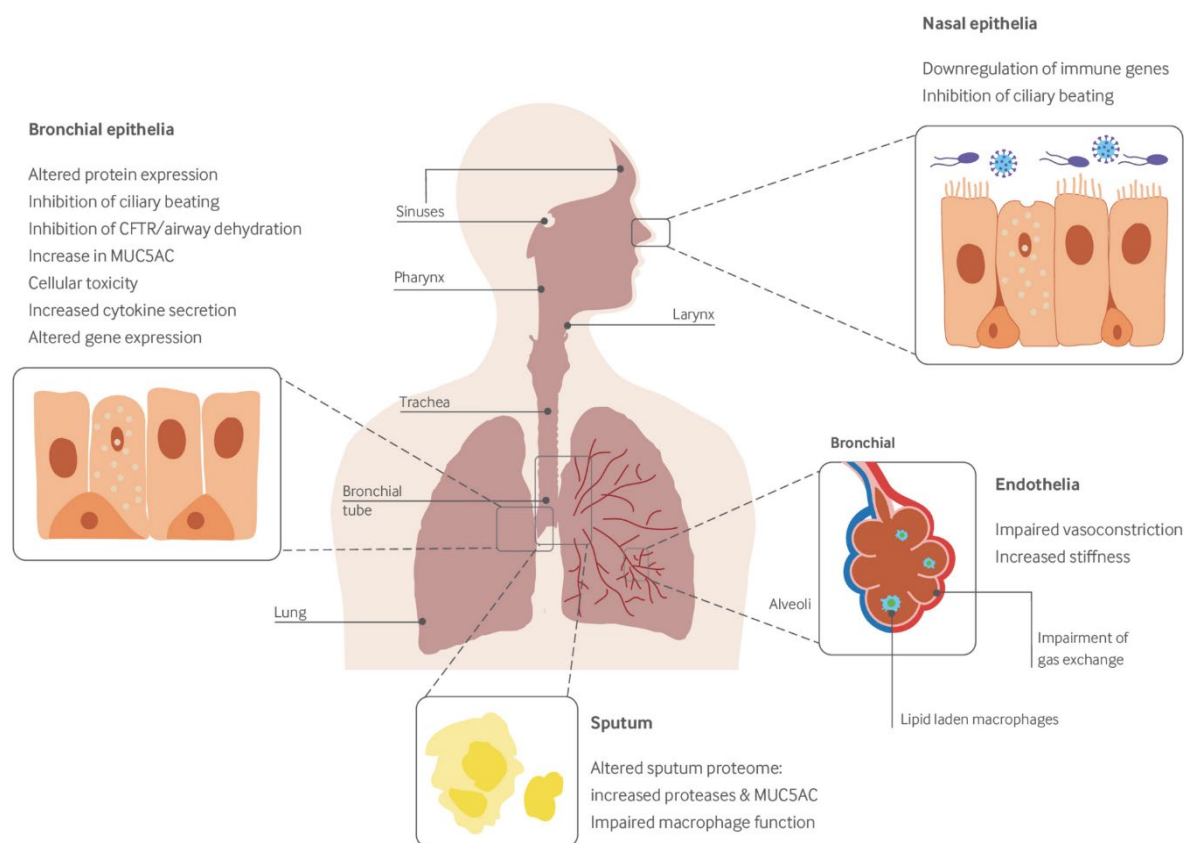


Figure 6. Depiction of multiple affected areas of respiratory tissue due to prolonged vape usage. (Gotts) Experimentation:

There is much known on the respiratory effects of vaping. However, little research has been focused on neurological effects, whether stimulated directly from vape smoke itself, or as a

byproduct of physiological respiratory responses. This experiment aims to perform a differential chemical analysis on various vaping products and correlating chemicals found within the vape liquids to implications on neurophysiological activity and pathways.

Vapes tested for the experiment were anonymously collected from students around the SUNY Brockport campus, bagged, and labeled. A total of 10 different e-cigarettes were collected for testing. The listed vapes are below, with marketed flavors:

Table 1. Experimental Vape Samples Collected

Electronic Cigarette/Vape	Associated Flavor
1. <i>Hyde DUO Rechargeable</i>	<i>Pink Lemonade & Cherry Lemonade</i>
2. <i>Hyde Color Edition</i>	<i>Lush Ice</i>
3. <i>Hyde Edge Rechargeable</i>	<i>Cherry Peach Lemonade</i>
4. <i>Juul</i>	<i>Virginia Tobacco (5%)</i>
5. <i>Vuse Original (Alto Pods)</i>	<i>Golden Tobacco (5%)</i>
6. <i>Juul</i>	<i>Menthol (5%)</i>
7. <i>Hyde Edge Rechargeable</i>	<i>Pina Colada</i>
8. <i>Hyde Edge Rechargeable</i>	<i>Strawberry Banana</i>
9. <i>Hyde Edge Rechargeable</i>	<i>Energize</i>
10. <i>SMOK Scar-P3</i>	<i>“Bad Drip – Bad Blood” *</i>

* The vape is not disposable and is filled with a separately bought e-liquid

Of the gathered vapes, the majority (6/10) of the vapes have come from the company *Hyde*, a more recent producer of disposable vapes. Vapes from *Hyde* seem to have become the dominant product among adolescents and young adults, as they are easily concealable, contain fruity flavors, and require little maintenance, as they are made to be disposable. The company has recently produced rechargeable vapes, allowing the user to fully consume all the juice within the reservoir. Originally, disposable *Hyde* vapes were built with non-rechargeable batteries that would die before all the juice within the reservoir had been consumed. This has been illustrated as an increase in internet videos showing the device being forcefully opened to access the battery. Holding frayed wires from basic charging cords (micro-USB, USB-C, USB-A, etc...) on

the positive and negative terminals of the vape battery for several minutes to charge the battery, the user is able to completely consume the remainder of the vape juice. *Hyde* no longer features the purely disposable vape on their website. However, the rechargeable form of the *Edge* has a listed battery capacity of 600mAh, providing approximately 3300 “puffs”, or hits (*Hyde*). Currently, *Hyde* markets a total of 30 different flavors within their products. Flavor categories include Breakfasts, Fruity flavors, Menthols, Desserts, Coolers, Candies, and Energy Drinks (*Hyde*). The other four collected vapes included two different flavored *Juul* pods, another pod-system (similar to *Juul*) device named *Vuse*, and e-liquid found in a *SMOK Scar-P3* from the company *Bad Drip*, a producer of nicotine salt vape juice for refillable vape systems. These other samples are interesting in their own however, due to the dominance of *Hyde* vapes within the SUNY Brockport community, as well as most college and high school campuses and communities around the northeast, and potentially the country. There is much concern over what ingredients are within vaping products and how these products interact with overall human physiology.

To perform a differential chemical analysis via GC-MS on the residual liquid found within each vape, an extraction procedure was established to efficiently remove all constituents into solution for testing. Vapes were taken apart and e-liquid reservoirs were located and accessed for extraction. Kimtech wipes were placed under vape contents to absorb any residual leakage. For the cases of the pod systems, such as *Vuse*, *Juul*, and the *SMOK Scar-P3*, juice was able to be clearly poured, or extracted via syringe, into a standard 20 mL glass scintillation vial. For the cases of the *Hyde* vapes, all samples contained a space within each device that was stored with a highly absorbent cotton-like product that was fully saturated with the e-liquid. The cotton reservoirs were removed and compressed using a micro spatula to the point where the saturated

liquid would be released into the scintillation vial. From there, 1mL of methanol (MeOH) was transferred via syringe into each vial containing vape juice. The vials were then swirled, stirred, and set to rest for several minutes before transferring into a GC-MS vial. After adequate mixing, 200 μ L of each liquid was pipetted, via P-1000, into a GC-MS vial for analysis. A total of 3 samples from each liquid were produced, totaling 30 samples.

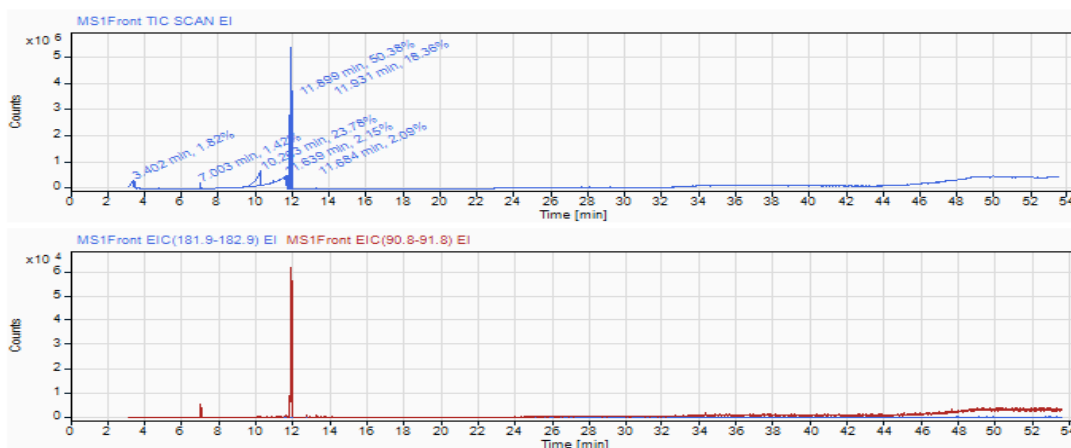
Chemical constituents within the vape juices were determined by GC-MS. E-cigarette liquids were diluted 100x into spectral grade methanol and injected into the GCMS system, an Agilent 7890A gas chromatograph with 5975 MSD detector (Agilent Technologies, Santa Clara, CA, USA). The system used helium as the carrier gas, flowing 1.2mL/min through an Agilent Technologies column (HP-5MS, 30 m x 0.250 mm, 0.25 μ m, 19091S-433). The oven program initiated at 45°C for one minute, ramped to 285C over seven minutes at 10C/min, ramped to 300C for ten minutes at 10C/min, and finally ramped a third time to 325C over five minutes at 5C/min. The total run time was 53.5 minutes, and the injection volume was 1 μ L from a 10 μ L syringe. The samples were analyzed by electron impact ionization in positive ion mode with mass range of 50-500m/z, with the source temperature at 230C and the quadrupole at 150C. Data analysis was performed using the Agilent ChemStation software (MSD ChemStation, E.02.02.1421, Agilent Technologies, Santa Clara, CA, USA), with ion scans searched against The NIST Mass Spectral Search Program database (Version 2.1.2.7, National Institute of Standard and Technology, Gaithersburg, MD, USA) for identification.

Results:

Each sample contains a separate TIC (Total Ion Chromatogram) and EIC (Extracted Ion Chromatogram) with respective peak results list. The TIC is colored blue while the EIC is colored red, and a larger TIC is depicted below the blue and red graphs. The processing method

used within the Agilent software compares the tested/unknown spectra to a database of known spectra based on mass to charge ratio and relevant abundance (Agilent). This Mass Spectrometry (MS) Library Search was able to produce injection reports, with identified peaks labelled with the respective chemical constituents within each e-liquid sample. Below are several example single-injection reports of different vape samples and a cross-peak analysis indicating constituents found within tested samples extracted using MeOH.

1. Hyde DUO Rechargeable – Pink Lemonade & Cherry Lemonade



Peak Results (Area Percent at least 1%)

RT (min)	Signal Description	Width (min)	Area	Height	Area%
3.402	MS1Front TIC SCAN EI	0.059	693665.1	236524.1	1.82
7.003	MS1Front TIC SCAN EI	0.269	543028.0	216328.5	1.42
10.263	MS1Front TIC SCAN EI	0.726	9067596.1	552518.1	23.78
11.639	MS1Front TIC SCAN EI	0.089	820336.5	272930.6	2.15
11.684	MS1Front TIC SCAN EI	0.040	796988.4	387909.8	2.09
11.899	MS1Front TIC SCAN EI	0.157	19211269.1	5415157.2	50.38
11.931	MS1Front TIC SCAN EI	0.096	7002604.0	4614178.7	18.36

Sum MS1Front TIC SCAN EI 38135487.2

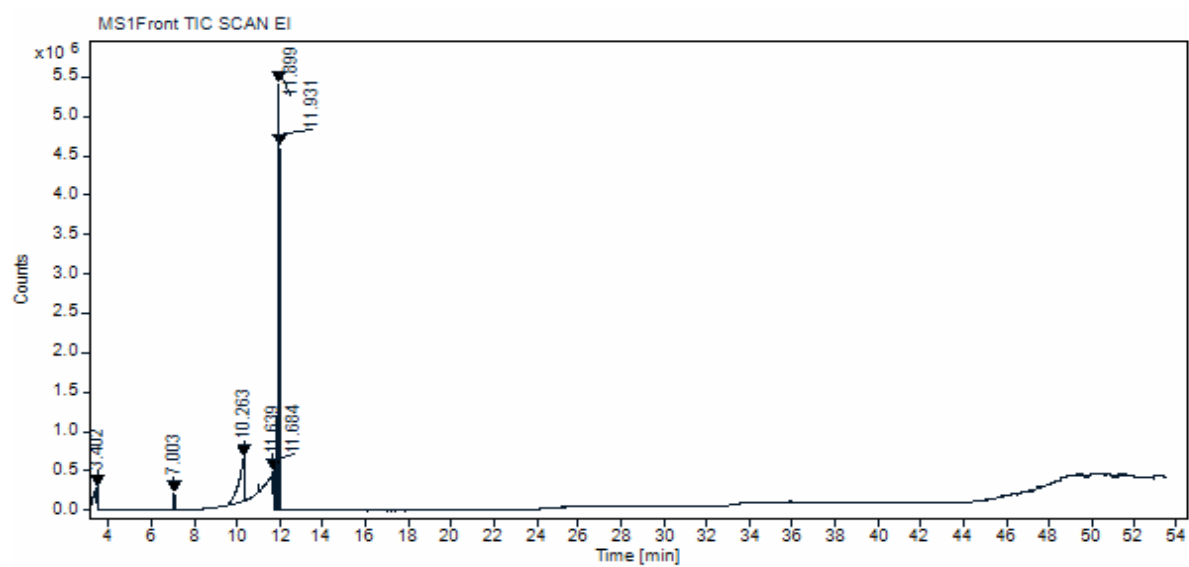
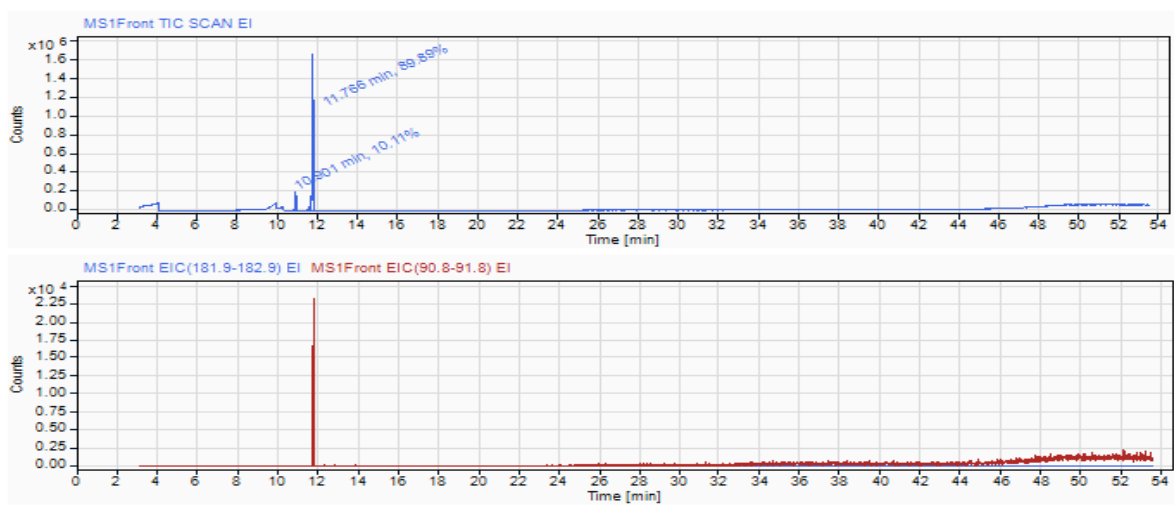


Table 2. Peak Results of *Hyde DUO Rechargeable* – Pink Lemonade & Cherry Lemonade

RT	Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS # Library Id
11.899	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)-	940	941	94.58	replib	54-11-5 15217
11.931	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)-	939	940	94.77	replib	54-11-5 15217
11.899	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)-	934	937	94.58	replib	54-11-5 15219
11.931	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)- pyrrolidinyl)-, (S)-	933	935	94.77	replib	54-11-5 15219
11.899	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)-	931	933	94.58	replib	54-11-5 15216
7.003	Benzyl alcohol	865	890	56.55	replib	100-51-6 14025
11.684	Glycerin	861	888	75.8	replib	56-81-5 9300
7.003	Benzyl alcohol	858	887	56.55	replib	100-51-6 14026
11.684	Glycerin	848	875	75.8	replib	56-81-5 9299
11.639	Glycerin	846	901	83.93	replib	56-81-5 9300
10.263	Benzoic acid	800	825	68.61	replib	65-85-0 20398
11.684	1,2,3,4-Butanetetrol, [S- (R*, R*)]-	786	809	9.85	replib	2319-57-5 9316

2. *Hyde Color Edition* – Lush Ice**Peak Results (Area Percent at least 1%)**

RT (min)	Signal Description	Width (min)	Area	Height	Area%
10.901	MS1Front TIC SCAN EI	0.153	538609.5	214472.5	10.11
11.766	MS1Front TIC SCAN EI	0.200	4786446.8	1823768.6	89.89
Sum MS1Front TIC SCAN EI			5325056.3		

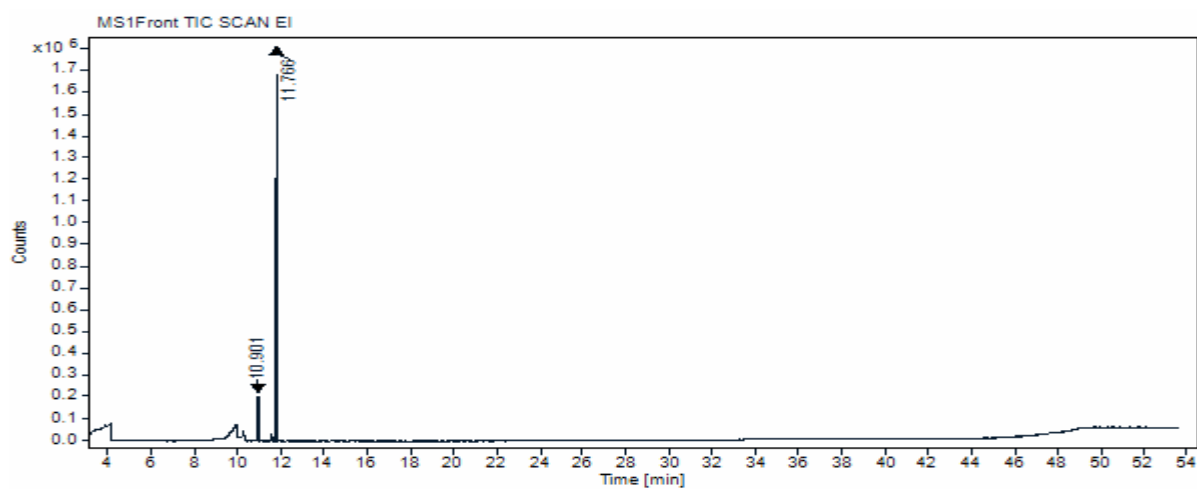
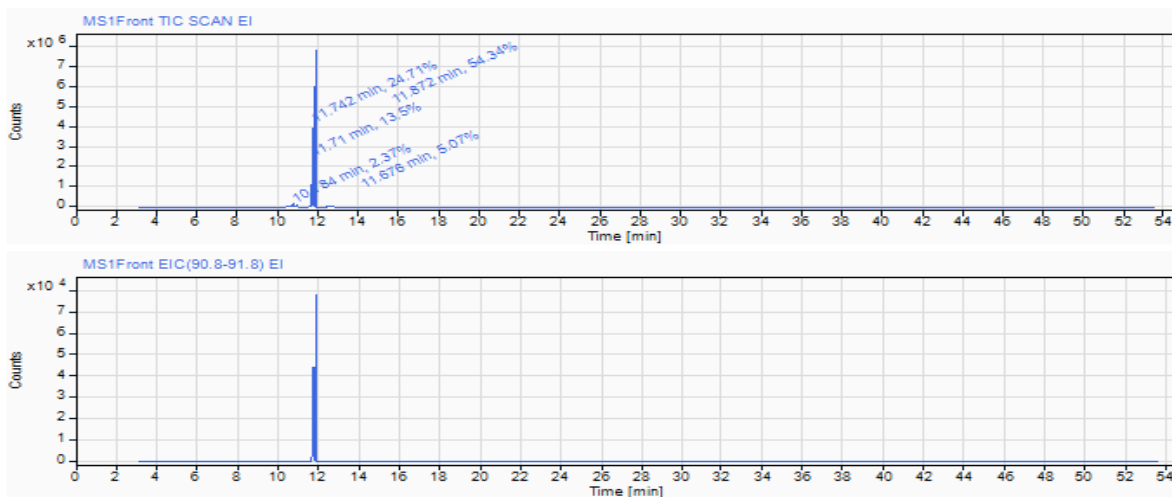


Table 3. Peak Results of *Hyde Color Edition* – Lush Ice

RT	Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS # LibraryId
11.766	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)-	917	918	93.52	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)-	904	906	93.52	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2- pyrrolidinyl)-, (S)- pyrrolidinyl)-, (S)-	902	909	93.52	replib	54-11-5 15215
10.901	2H-Pyran, 3,4-dihydro-2- methoxy-	674	759	31.74	replib	4454-05-1 8228
	2H-Pyran, 3,4-dihydro-2- methoxy-	670	751	31.74	replib	4454-05-1 8590
	3-Piperidinol, 1-ethyl-	658	671	18.28	replib	13444-24-1 22484

3. Hyde Edge Rechargeable - Cherry Peach Lemonade



Peak Results (Area Percent at least 1%)

RT (min)	Signal Description	Width (min)	Area	Height	Area%
10.784	MS1Front TIC SCAN EI	0.123	626958.0	169460.2	2.37
11.676	MS1Front TIC SCAN EI	0.151	1340635.9	819395.2	5.07
11.710	MS1Front TIC SCAN EI	0.037	3568717.7	2415787.5	13.50
11.742	MS1Front TIC SCAN EI	0.048	6530972.5	4148202.1	24.71
11.872	MS1Front TIC SCAN EI	0.123	14360848.6	8585544.5	54.34
Sum MS1Front TIC SCAN EI			26428132.7		

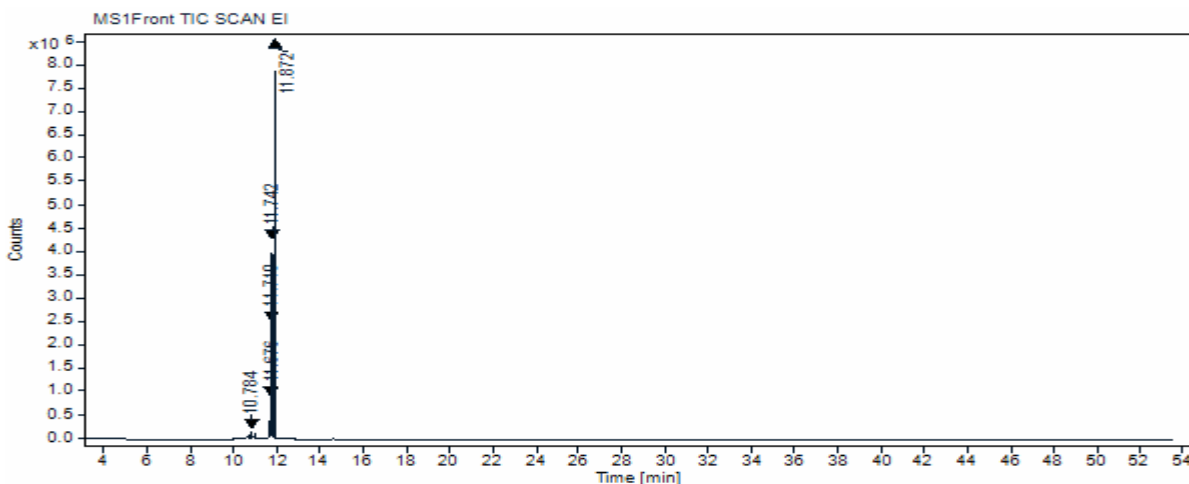


Table 4. Peak Results *Hyde Edge Rechargeable*

RT	Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS # Library Id
11.872	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	901	902	93.61	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	888	889	93.61	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	883	889	93.61	replib	54-11-5 15215
11.742	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	855	856	90.12	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	846	847	90.12	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	841	846	90.12	replib	54-11-5 15215
11.71	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	826	828	88.4	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	814	820	88.4	replib	54-11-5 15215
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	814	815	88.4	replib	54-11-5 15216
11.676	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	713	714	76.8	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	712	716	76.8	replib	54-11-5 15215
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	698	700	76.8	replib	54-11-5 15217
10.784	4-Pentyn-2-ol	657	746	31.04	replib	2117-11-5 4884
	2-Propanol, 1-chloro-	631	653	9.3	replib	127-00-4 4872
	Isopropyl Alcohol	628	856	8.21	replib	67-63-0 4907

4. *Juul* - Virginia Tobacco (5%)

*Inconclusive results

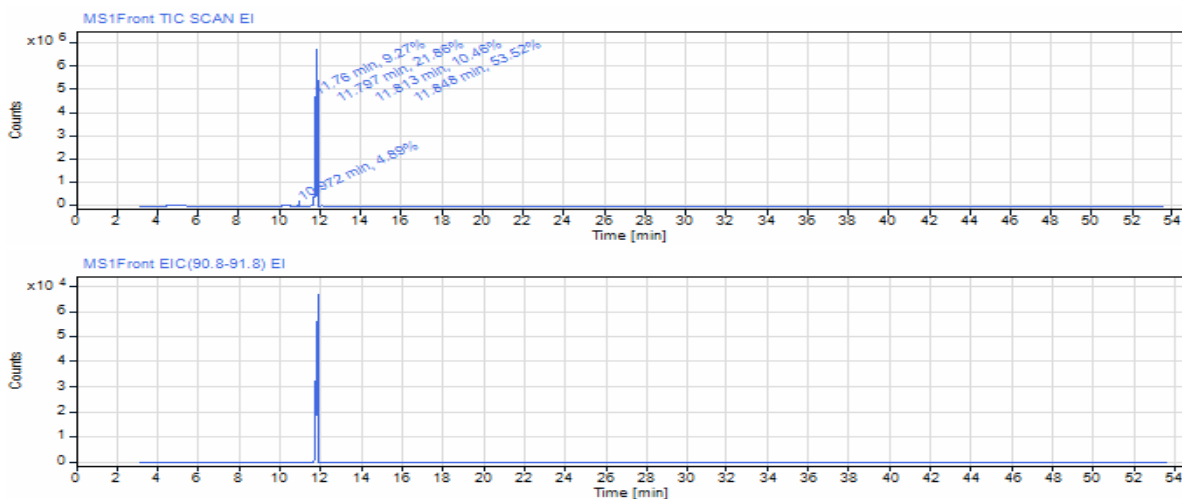
5. *Vuse Original (Alto Pods)* - Golden Tobacco (5%)

*Inconclusive results

6. *Juul* – Menthol (5%)

*Inconclusive results

7. Hyde Edge Rechargeable - Pina Colada



Peak Results (Area Percent at least 1%)

RT (min)	Signal Description	Width (min)	Area	Height	Area%
10.972	MS1Front TIC SCAN EI	0.235	895438.8	220825.4	4.89
11.760	MS1Front TIC SCAN EI	0.039	1696583.5	1577730.6	9.27
11.797	MS1Front TIC SCAN EI	0.038	4002533.8	2611030.3	21.86
11.813	MS1Front TIC SCAN EI	0.011	1914950.7	2825457.6	10.46
11.848	MS1Front TIC SCAN EI	0.041	9798992.9	6582138.4	53.52
Sum MS1Front TIC SCAN EI			18308499.7		

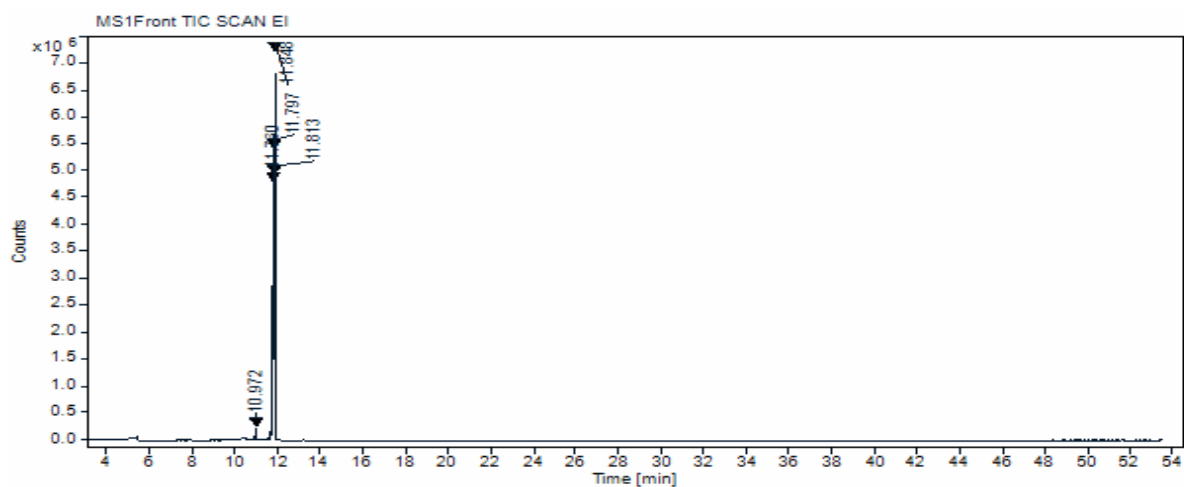
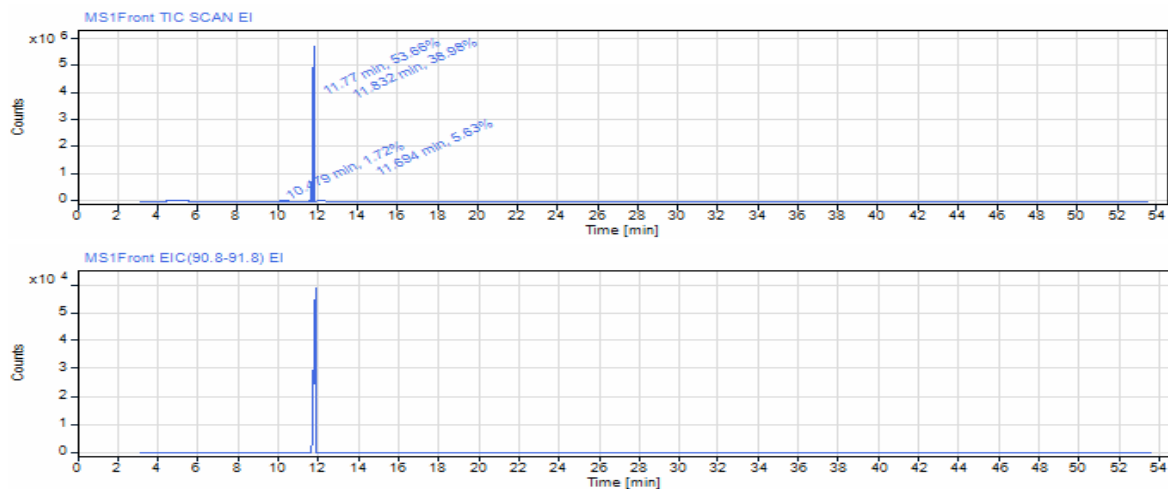


Table 5. Peak Results of *Hyde Edge Rechargeable* - Pina Colada

RT	Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS # Library Id
11.848	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	906	907	94.38	replib	54-11-5 15217
11.797	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	894	895	92.85	replib	54-11-5 15217
11.848	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	892	895	94.38	replib	54-11-5 15219
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	891	891	94.38	replib	54-11-5 15216
11.76	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	890	891	92.77	replib	54-11-5 15217
11.797	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	885	886	92.85	replib	54-11-5 15216
11.813	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	884	885	92.14	replib	54-11-5 15217
11.797	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	882	888	92.85	replib	54-11-5 15215
11.76	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	881	882	92.77	replib	54-11-5 15216
11.813	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	878	881	92.14	replib	54-11-5 15219
11.76	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	877	883	92.77	replib	54-11-5 15215
11.813	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	876	881	92.14	replib	54-11-5 15215
10.972	Butane	727	848	21.52	replib	106-97-8 2031
	Butane	727	818	21.52	replib	106-97-8 2029
	Butane	724	820	21.52	replib	106-97-8 2030

8. Hyde Edge Rechargeable - Strawberry Banana



Peak Results (Area Percent at least 1%)

RT (min)	Signal Description	Width (min)	Area	Height	Area%
10.479	MS1Front TIC SCAN EI	0.530	527798.5	43072.2	1.72
11.694	MS1Front TIC SCAN EI	0.162	1725267.3	907373.8	5.63
11.770	MS1Front TIC SCAN EI	0.101	16435130.1	5035526.1	53.66
11.832	MS1Front TIC SCAN EI	0.057	11938547.5	5752173.9	38.98
Sum MS1Front TIC SCAN EI			30626743.4		

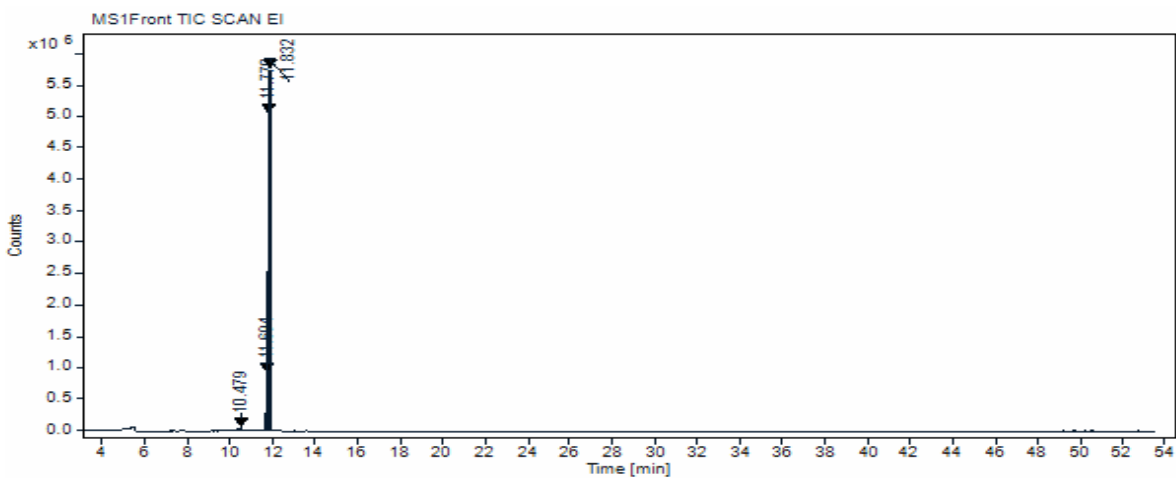
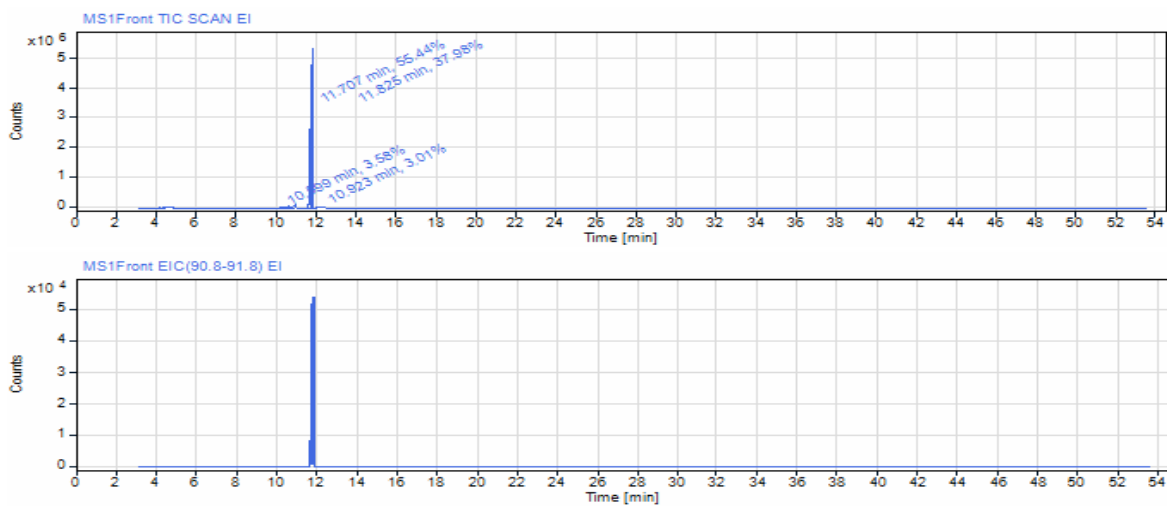


Table 6. Peak Results of *Hyde Edge Rechargeable* – Strawberry Banana

RT	Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS # Library Id
11.832	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	888	890	91.77	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	884	885	91.77	replib	54-11-5 15216
11.77	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	882	883	92.39	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	877	878	92.39	replib	54-11-5 15216
11.832	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	877	884	91.77	replib	54-11-5 15215
11.77	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	876	882	92.39	replib	54-11-5 15215
11.694	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	735	738	73.77	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	728	729	73.77	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	728	733	73.77	replib	54-11-5 15215
10.479	Isopropyl Alcohol	682	821	34.96	replib	67-63-0 4831
	2-Hydrazinoethanol	659	792	12.76	replib	109-84-2 4965
	4-Pentyn-2-ol	655	700	10.78	replib	2117-11-5 4884

9. Hyde Edge Rechargeable - Energize

**Peak Results (Area Percent at least 1%)**

RT (min)	Signal Description	Width (min)	Area	Height	Area%
10.599	MS1Front TIC SCAN EI	0.505	783846.2	68402.1	3.58
10.923	MS1Front TIC SCAN EI	0.261	658579.3	119421.6	3.01
11.707	MS1Front TIC SCAN EI	0.216	12146358.3	4835423.9	55.44
11.825	MS1Front TIC SCAN EI	0.131	8322119.6	5506243.1	37.98
Sum MS1Front TIC SCAN EI			21910903.4		

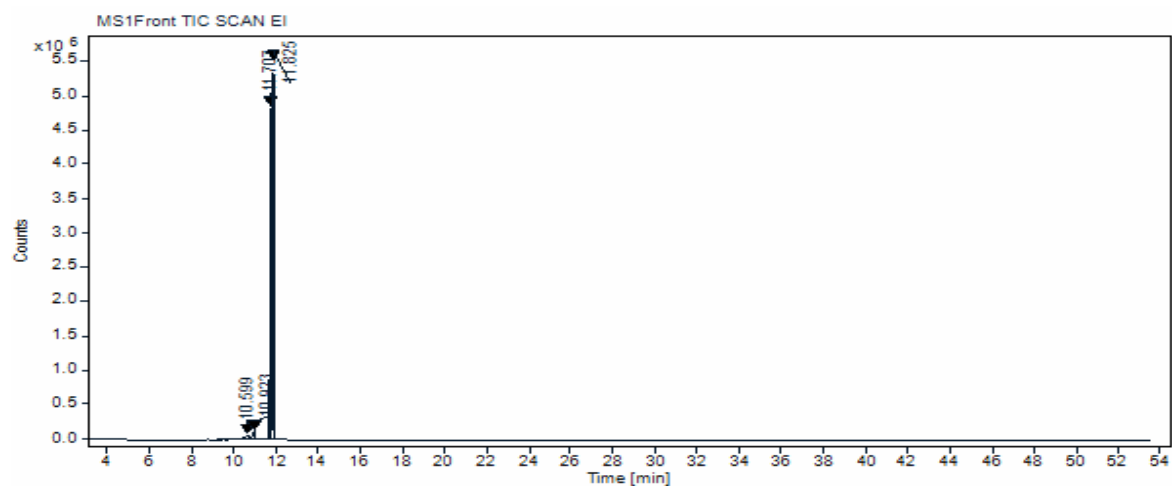


Table 7. Peak Results of *Hyde Edge Rechargeable - Energize*

RT	Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS # Library Id
11.825	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	878	879	91.06	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	877	879	91.06	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	872	877	91.06	replib	54-11-5 15215
11.707	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	870	871	90.69	replib	54-11-5 15217
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	864	864	90.69	replib	54-11-5 15216
	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	861	867	90.69	replib	54-11-5 15215
10.923	Acetone	785	886	36.79	replib	67-64-1 2856
	Butane	783	836	33.94	replib	106-97-8 2031
	Butane	777	816	33.94	replib	106-97-8 2030
10.599	Isopropyl Alcohol	713	868	30.08	replib	67-63-0 4830
	4-Penten-2-ol	701	752	20.03	replib	625-31-0 4935
	Isopropyl Alcohol	695	824	30.08	replib	67-63-0 4829

10. SMOK Scar-P3 – Bad Drip’s Bad Blood

*Inconclusive results

Inconclusive results were determined through observation of no signals, or significant peaks, within all the replicates of the same sample. This is likely due to a reduced ratio of e-liquid: MeOH, causing the concentration of e-liquid to be too low for the GC-MS to detect any significant peaks. The pod system vapes posed a slight problem, as liquids encased within plastic reservoirs were prone to leakage and evaporation if left unused for too long. This may have been the case with the collected pod system vapes, as there was less collected sample due to evaporation.

Future Analysis and Experimentation:

A multitude of compounds were found within each vape sample, including butane, isopropyl alcohol, glycerin, acetone, and benzyl alcohol. These commercially available compounds have previously established physiological health impacts. Interestingly, there were three compounds that were unexpected within the tested e-liquids. These compounds were 4-Pentyn-2-ol, 2-Hydrazinoethanol, and 3-(1-Methylpyrrolidin-2-yl) pyridine; each of which has some interesting and potentially profound effects on the body.

The first compound, 4-Pentyn-2-ol, found on Tables 4, 6, & 7, has a molecular formula of C_5H_8O , having a 5-carbon backbone structure with an alcohol substituent in the second position (along the second carbon) and a triple carbon-to-carbon bond in the fourth position within the structure. The structure is depicted below in Figure 7. It is a flammable substance and cited to be an eye, skin, and respiratory irritant (PubChem). The compound was found in samples 3,8, & 9.

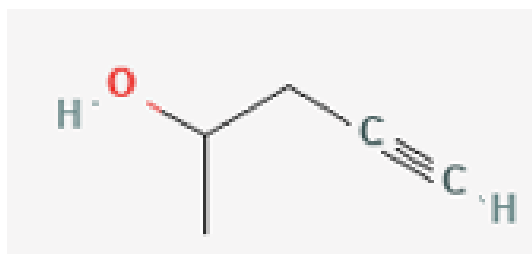


Figure 7. Structure of 4-Pentyn-2-ol

There have been multiple patents made using 4-Pentyn-2-ol, one of which offers is as an inhibitor of human ATGL (NIH). Human ATGL (hATGL) is known as adipose triglyceride lipase, the protein is essential in the breakdown of lipids (lipolysis), specifically triglycerides found in adipose tissues throughout the body, including skeletal muscle. An experiment studying the effects of ATGL expression on skeletal muscle found that increased triglyceride storage within the muscle has been linked to playing an “important role in the etiology of insulin resistance and type 2 diabetes mellitus” (Peterson and Shulman). One reason for that may be from ATGL deficiencies throughout the muscle (Jocken). Another experiment studying the effects of cerebral ischemia (insufficient blood flow to the brain) and microglia-derived adiposomes found that blocking adiposome (organelle in the form of a lipid droplet with protein in its membrane) formation with NS-398, an adiposome inhibitor, there was a significantly reduced “inflammatory activity and death rate of GOSD-treated (glucose-oxygen serum deprivation) microglia but also the brain infarct volume and motor function deficit of ischemic rats” (Lin). Given that 4-Pentyn-2-ol has been patented as an inhibitor for ATGL, which is known to aid in lipid metabolism (seen in Figure 8), there may be some effect from the inhalation of the chemical from vapes on inducing lipid buildup throughout various regions of the body and brain. The buildup may be linked to a higher risk of individuals developing

ischemic strokes. Further analysis may be done on triglyceride buildup within the brain and vape usage to determine how much of a correlation there is.

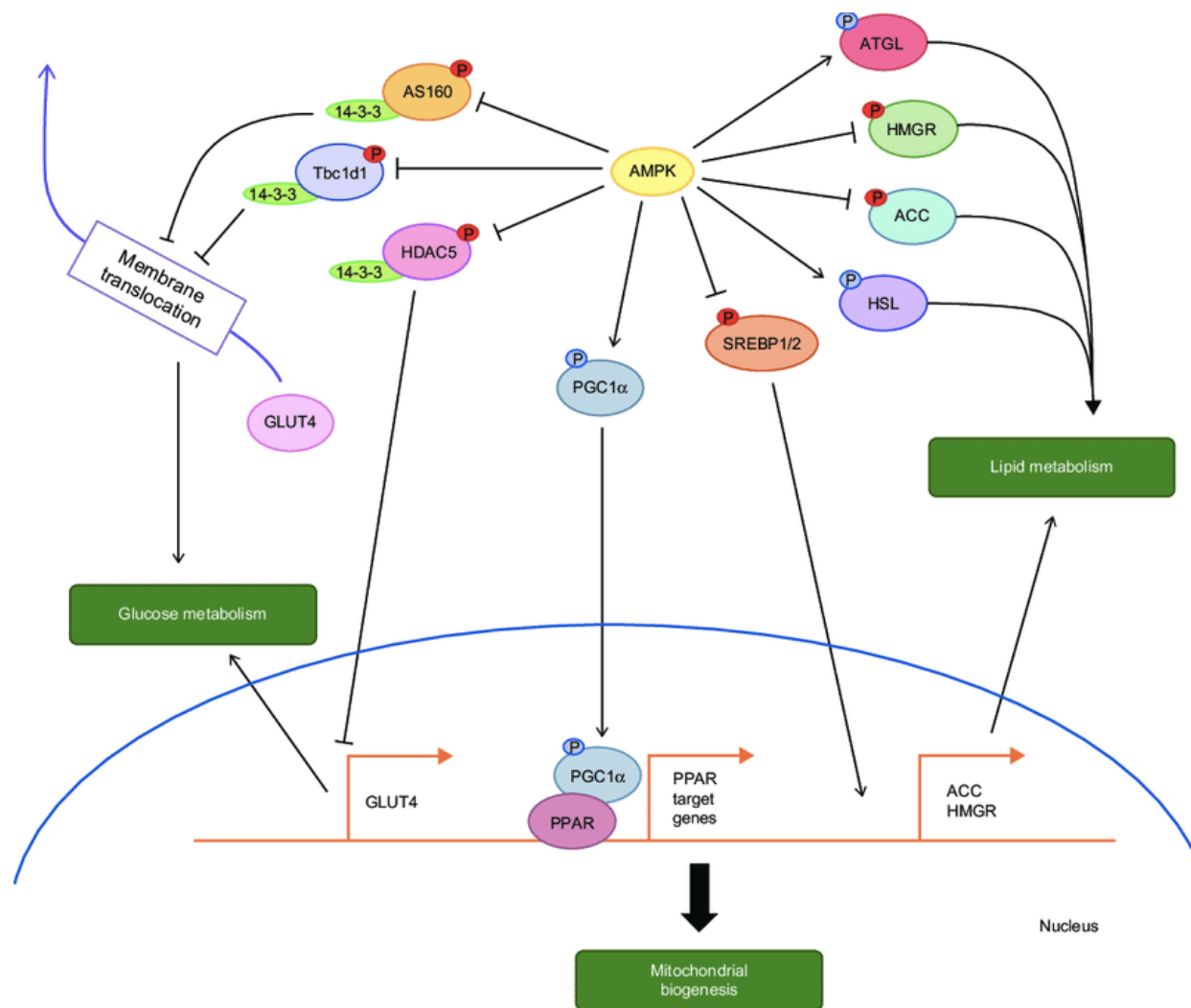


Figure 8. Pathway involving AGTL lipid metabolism. (Segatto)

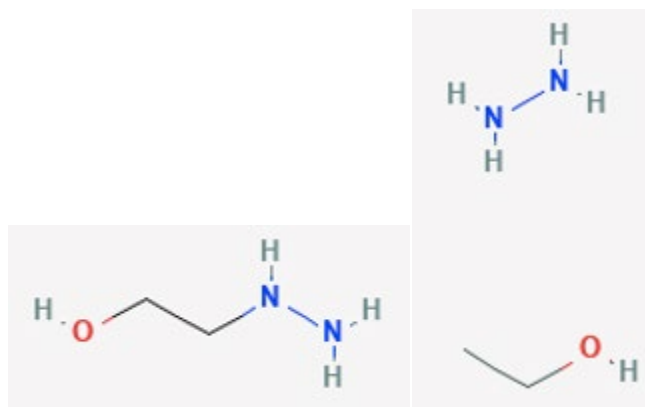


Figure 9. Structures of 2-hydrazinoethanol (left, PubChem 2) and hydrazine ethanol (right, PubChem 3)

Despite having little research in human physiology, 2-hydrazinoethanol is part of the hydrazine family, where certain compounds within this family have had research done on them, as shown in Figure 9. One compound with incredible similarity to 2-hydrazinoethanol is hydrazine ethanol, which is a mixture between separated compounds of hydrazine and ethanol. Their similarities in structure are shown above in Figure 9. In the human body, metabolism of 2-hydrazinoethanol may include hydrazine ethanol intermediates within the breakdown pathway. Hydrolytic and oxidative lyases within the brain may be responsible for aiding in the metabolism of 2-hydrazinoethanol. When inhaled, however, little is known about the metabolic processes. Interestingly, there is a patent for hydrazine ethanol being used as a compound in inhibiting poly (ADP-ribose) polymerase (PARP), an enzyme crucial in damaged DNA repair, regulation of transcription, and apoptosis (Rose). PARP1, is a key enzyme in base excision repair, helping recognize damaged DNA and activating to induce DNA repair through recruitment of histone, topoisomerases, and helicases. However, when DNA damage has exceeded the “threshold of repair”, excessive PARP1 activation may induce cell death, via the process of parthanatos. Excessive PARP1 stimulation has been linked to stroke, trauma, and neurodegenerative diseases such as, Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, and Amyotrophic lateral sclerosis (ALS) (Thapa). Hydrazine ethanol has been used in patents making PARP inhibitors, which have been effective at targeting BRCA deficient ovarian and breast cancers using synthetic lethality (Ke). Synthetic lethality is a type of genetic interaction where the co-occurrence, or co-expression, of two genes results in cellular death. Why 2-Hydrazinoethanol is found within vapes, how its metabolized, and what effects it has on the body are mainly unknown. Potential future studies pertaining to vape usage may find importance in determining PARP expression compared with vape use from vapes that have 2-hydrazinoethanol found within

its e-liquid. Considering vapes generally are known to damage lung epithelia and disrupt brain functioning, it is notable to identify a chemical remarkably similar to a chemical patented for use in cancer treatment.

The final compound of interest found in all tested vape samples was 3-(1-Methylpyrrolidin-2-yl) pyridine, commonly known as nicotine. Depicted below in Figure 10, the compound has a molecular formula of $C_{10}H_{14}N_2$ consisting of a 6-carbon ring structure (benzene), containing one nitrogen within the ring, bonded to a 5-carbon ring structure (cyclopentane), containing one nitrogen in place for one of the five carbons within the ring. The nitrogen within the 5-carbon ring is bonded to a protruding methyl substituent.

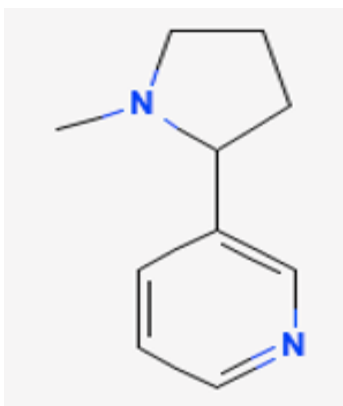


Figure 10. Structure of 3-(1-Methylpyrrolidin-2-yl) pyridine, also known as nicotine. (PubChem 4)

With a half-life of approximately 2 hours, nicotine is readily metabolized into cotinine by CYP2A6, a subfamily of cytochrome P450, within the liver. It has also been proven to be expressed in some other tissues, including nasal mucosa (Raunio). As seen in Figure 11, neurological effects of nicotine shows that it acts as a direct agonist of nicotinic acetylcholine receptors (nAChR) found within dopamine neurons in the ventral tegmental area (VTA) in the brain. Excitation from nicotine binding to the receptor induces sodium influxes into the axon terminals of the neuron and EPSPs (excitatory post-synaptic potentials). Mesolimbic activity is

stimulated from nicotine binding to nAChR's along the dopamine neuron, inducing a sense of positive emotion and reward. This is where and how nicotine addiction can occur as the user's mesolimbic, or "reward", pathway is stimulated from nicotine inhalation as a result from vaping. A study showing the effects of nAChR-mediated dopamine release in the *Drosophila melanogaster* highlights that nicotine-stimulated dopamine release had outlasted natural acetylcholine-stimulated release of dopamine (Pyakurel). Implications of the study may relate to the competitiveness of nicotine and acetylcholine at nicotinic receptors and the overall amount of time each compound spends interacting with the receptor.

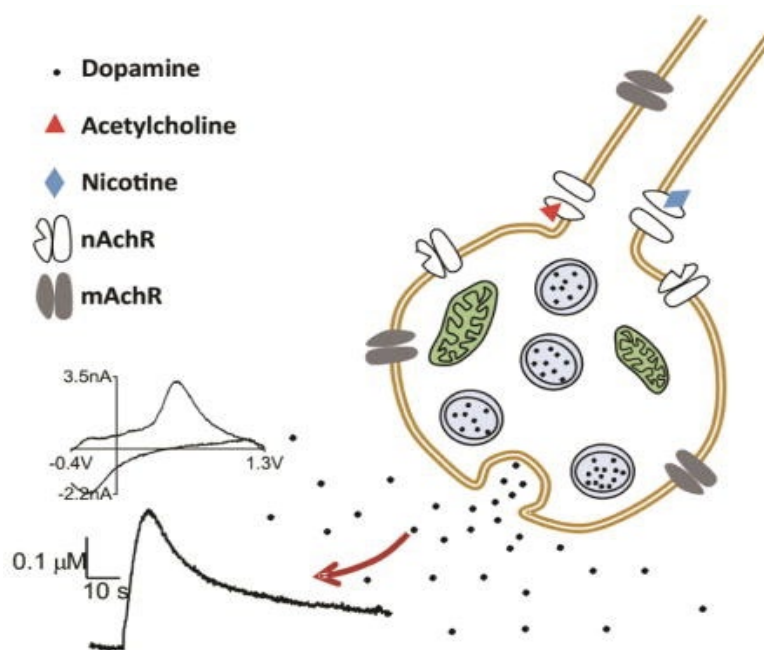


Figure 11. Nicotine acting on nAChR within a dopamine neuron. (Pyakurel)

Nicotine acts as a stimulant, boosting heart rate, attention span, and memory. Given that smokeable forms are paired with other carcinogenic chemicals, the addictiveness of nicotine allows for further damage to the body through ingestion of these various other harmful and carcinogenic chemicals.

Conclusion:

The rise of electronic cigarette use in the U.S., and throughout the world, has become increasingly impactful amongst adolescents and adults. During the late summer of 2019, the emergence of EVALI, a diagnosis for the adverse pulmonary symptoms brought on as a result of continued vape use, had only sparked further interest, funding, and research into vaping and their effects on human physiology as many scientists were perplexed on how a “safer” alternative to smoking cigarettes was causing severe pulmonary damage within relatively new users. Early research intended on determining the chemicals found within the vapes and finding direct connections to the symptoms brought on from EVALI. This research aimed to provide information on various chemicals found within e-liquids of currently, and popularly, used vapes on the market today, as well as draw connections and possible linkages to these chemicals and negative effects on various biological pathways within the human body. Overall, the tested compounds yielded some surprising results, with some chemicals, such as 4-Pentyn-2-ol and 2-Hydrazinoethanol posing some potentially serious health risks. Extended research may include increasing sample size and differing extraction solutions for GC-MS analysis in order to increase the library of chemicals that have been discovered in vaping devices. Increasing the chemical library may also pose new linkages to affected pathways within the human body. While direct testing of vapors on live epithelia may be difficult, *in vitro* studies using various cell cultures paired with proteomic, genomic, and secretomic studies may be beneficial for defining various effects brought on by the chemicals.

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