AMERICAN UNIVERSITY OF BEIRUT

ENHANCEMENT OF BENZENE EMISSIONS IN SPECIAL COMBINATIONS OF ELECTRONIC LIQUID MIXTURES

by
FATIMA HASSAN ELHAJJ MOUSSA

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FATIMA HASSAN ELHAJJ MOUSSA

Approved by:

Signature	
Dr. Digambara Patra, Professor Department of Chemistry	Advisor
Signature	
Dr. Mazen Ghoul, Professor Department of Chemistry	Member of Committee
Signature	
Dr. Joseph Zeaiter, Associate Professor Department of Chemical Engineering and Advanced Energy	Member of Committee

Date of thesis defense: November 10, 2022

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ABSTRACT
OF THE THESIS OF

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Introduction—
Electronic nicotine delivery systems (ENDS) are battery-powered smoking devices introduced to the market as safer alternatives to combustible cigarettes. Upon heating the electronic liquid (e-liquid), aerosols are released, including several toxicants such as volatile organic compounds (VOCs). Benzene has been given great attention as a major component of the VOCs group as it increases cancer risk upon inhalation. In this study, several basic e-liquids were tested for benzene emissions.

Methods—
Aerosol Lab Vaping Instrument was used to generate aerosols from ENDS comprised of different e-liquid combinations: vegetable glycerin (VG), propylene glycol (PG), nicotine (nic), and benzoic acid (BA). The tested mixtures were PG, PG+nic+BA, VG, VG+nic+BA, 30/70 PG/VG, 30/70PG/VG+nic+BA.
A carboxen polydimethylsiloxane fiber of a solid phase micro-extraction was placed in a gas cell to trap benzene emitted from ENDS. Collection was done using an online dynamic/static mode; benzene was adsorbed by the fiber during the puffing process and for an extra 15 min until reaching equilibrium. Then, using gas chromatography-mass spectrometry, benzene was quantified.

Results—
Benzene was quantified in VG, but not in PG or PG/VG mixture. However, benzene concentration increased in all tested mixtures upon the addition of nicotine benzoate salt. Interestingly, benzene was emitted at the highest concentration when BA was added to PG. However, lower concentrations were found in the PG/VG and VG mixtures with BA.

Conclusions—
Both VG and BA are sources of benzene. Enhanced emission, however, is mostly noticed when BA is mixed with PG and not VG.

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ABBREVIATIONS

AT	Average time
ALVIN	Aerosol lab vaping instrument
BA	Benzoic acid
BTEX	Benzene, toluene, ethylbenzene, xylene
BW	Body weight
CA	Benzene concentration
CAR-DVB	Carboxen divinylbenzene
CAR-PDMS	Carboxen polydimethylsiloxane
CDI	Chronic daily intake
CFP	Cambridge filter pad
CR	Cancer Risk
CSFi	Cancer slope factor
e-liquid	Electronic liquid
ED	Exposure duration
ET	Exposure time
ENDS	Electronic nicotine delivery system
FDA	Food and Drug Administration
GC	Gas Chromatography
GRAS	Generally Recognized As Safe
HTPs	Heated tobacco products
IARC	International Agency for Research on Cancer
JUUL	Juice USB Lighting

LC	Liquid Chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MFC	Mass flow controller
MS	Mass Spectrometry
NTA	Non targeted analysis
nic	Nicotine
P	Pressure
PG	Propylene glycol
ppb	Parts per billion
ppm	Parts per million
QA	Quality assurance
QC	Quality control
R	Resistance
ReaxFF	Reactive force filed
RT	Retention time
SBS	Sick building syndrome
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
SVOCs	Semi-volatile organic compounds
V	Voltage
VG	Vegetable glycerin
VOCs	Volatile organic compounds
WHO	World Health Organization

CHAPTER I

INTRODUCTION

A. Electronic Nicotine Delivery Systems

In 2004, Electronic Nicotine Delivery Systems (ENDS) were the first to be introduced to the market. Since then, they have been advertised as better or "safer" alternatives to tobacco cigarettes. [1] ENDS are battery-powered smoking devices that heat electronic liquid (e-liquid) to produce an aerosol containing nicotine and flavorings. ENDS use is currently common among teenagers and young people especially students in middle and high schools.[2] Popularity of ENDS is due to various perceptions: (1) ENDS are safer compared to conventional cigarettes because e-liquids are composed of different proportions of propylene glycol (PG), glycerol (VG), and flavoring substances that are classified as "Generally Recognized As Safe" (GRAS) by the U.S. Food and Drug Administration (FDA)[3]; (2) smoke-related chemicals and nicotine are delivered less by ENDS[4]; (3) e-liquids come in a variety of tastes that appeal to young people[5]; and (4) using ENDS may help people quit smoking[6]. Consequently, ENDS use has now become the most commonly used form of tobacco smoking in the U.S.[7] However, the available research data on ENDS are still considered less comprehensive in comparison to that on conventional tobacco cigarette smoke.[8] Effectively, several toxicants are produced in the mainstream of ENDS vapor including nicotine, glycols, carbonyls, including dicarbonyls and hydroxycarbonyls, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), tobacco-specific nitrosamines (TSNAs), and metals.[9-12] Many of these compounds are known toxicants including carcinogens such as formaldehyde and acetaldehyde.[13]

In fact, the release of these compounds of different concentrations is dependent on the formulation and flavor of the e-liquids, as well as the voltage used.[13, 14] Since tobacco products are evolving rapidly researchers have been determinedly developing suitable techniques to assess toxic emissions and public health impact of these products.

B. ENDS: Operation and Compartments

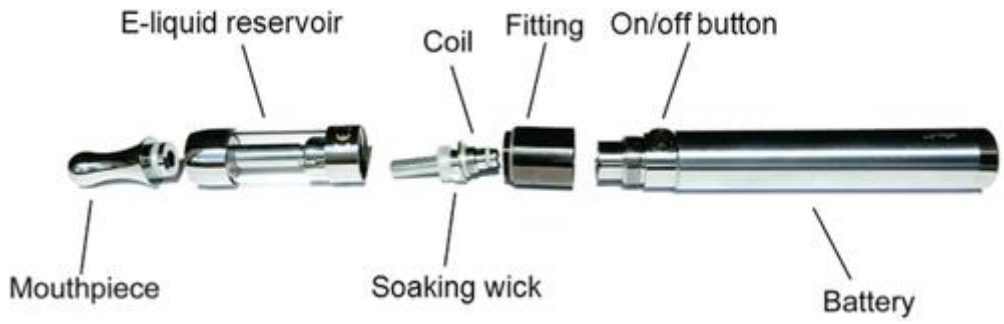

Figure 1. ENDS Compartments. [15]

ENDS of different designs work in the same way and have similar compartments.[15] The basic structure of ENDS is illustrated in Figure 1. It consists of a battery made of lithium that activates the device, an automizer consisting of a coil and a wick, a cartridge that holds the e-liquid, and a mouthpiece that delivers the aerosols to the user.[16] The liquid found around the coil heats up and undergoes vaporization once the heater coil gets activated during a puff. Activation of the coil is the result of either pushing a button or sparking an air-flow sensor by inhaling through the mouthpiece. Vapors formed are driven by the air passing through the ENDS device away from the coil, then the vapor undergoes recondensation to create aerosols that are similar to tobacco smoke as shown in Figure 2. [17]

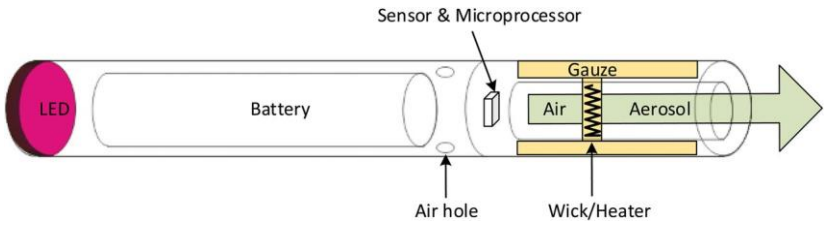

Figure 2. A scheme showing aerosol generation from ENDS device. [17]

C. Generations of ENDS

As the ENDS market grew significantly, other generations of these electronic powered devices arose, all of which operating on the same principles. The earliest generation of ENDS devices was known as Cig-a-like. Being disposable or rechargeable, these types of devices resembled traditional tobacco cigarettes. When inhaled, the device's LED light, which resembles the tip of a lighted tobacco cigarette, goes on, allowing one to keep an eye on how it is being used.[18] However, it has been proven that the effectiveness of these devices in delivering nicotine is minimal.[19, 20]

Figure 3. ENDS devices evolution showing the different generations in the market

"Personal vaporizer" is the name given to the second generation of ENDS. It has the appearance of a pen or laser pointer. To turn on the heating coil with such devices, push the "on/off" button. The second generation of gadgets also includes a rechargeable battery and a refilled cartomizer. Therefore, the customer has the option to adjust the battery's power output and liquid composition to his preferences.[18]

"Mods" are the third-generation devices. They function at very high power levels that reach up to 200W. Ohm's law states that the power (P), measured in watts in an electrical circuit, is equivalent to the square of voltage (V), divided by resistance (R), measured in ohms: $P = V^2 /R$.[21] A coil with a resistance lower than unity can produce a high power output because the relationship between battery power and coil resistance is inverse. Due to the fact that these devices operate at lower resistance than other ENDS devices, they are called "sub-ohm". As a result, decreasing resistance causes the atomizer coil's temperature to rise quickly. This causes the coil to evaporate more liquid, producing more taste, and huge clouds.[22] This clarifies why young people found these devices to be appealing and popular.

The fourth generation is distinguished by a temperature sensor that enables the user to manually regulate the temperature in addition to the functions of the third generation. One of the most important factors influencing the amount and composition of aerosols generated by ENDS is coil heat temperature.[23] Additionally, studies have shown that greater temperatures result in higher nicotine delivery rates as well as higher formaldehyde and other toxicant emissions into the aerosol.[13]

Juice USB Lighting (JUUL), a new high-tech generation, was introduced to the US market in June 2015 by Pax Labs in response to dramatic increase of ENDS among youth.[24] In contrary to prior ENDS versions, JUUL has just two parts, a USB-

rechargeable battery and JUUL pods that contain flavored prefilled liquid and a heating coil. JUUL pods are recognized as an aerosol inhalation mouthpiece.[24, 25]
The pre-filled liquid was discovered to have a high nicotine content of 60 mg/mL and a PG/VG ratio of 30/70.[25, 26] According to Talih et al., JUUL is an electronic device with a supra-ohm resistance of 1.6 and a maximum battery power output of 8.1 W.[25] Compared to combustible cigarettes, it produces less carbonyls when operating at low power maintaining its temperature at 215°C.[27]

The latest generation of ENDS are the disposable pod-style devices (such as Puff Bar, Ignite, and Lush) which are small, stylish, ready-to-use vaping devices prefilled with nicotine salt.[28] They come in different flavors such as mango, mint, strawberry, etc. and are sold at low prices making it appeal to youth and young adults.[28]

D. E-liquid Composition

E-liquids existing in the market show high variability in their composition. The two major components of an e-liquid are usually propylene glycol (PG) and vegetable glycerin (VG) (>90%).[29] It is possible for users to customize their e-liquid composition by some ENDS devices. Thus, they can vary the ratio of the two major constituents (PG and VG).

Some flavorings and nicotine of variable concentrations might be added, and they comprise no more than 10% of the total e-liquid composition.[30] In addition, six distinct acids might be added to e-liquids to make nicotine salts. The use of nicotine salt in e-liquids is crucial due to three factors. First, the liquids become more acidic, the resulting aerosols are no longer alkaline, perhaps causing less irritation to the upper

respiratory tract and throat.[31] Second, regardless of the salt type in tobacco or e-cigarette liquids, nicotine has been shown to transfer similarly from product to aerosols. However, some salts (such as nicotine citrate) produce aerosols with varying proportions of free-base to mono-protonated nicotine salts, indicating that the kind of nicotine salt may have an impact on how much nicotine is delivered.[32] Third, studies of thermal stability and the identification of decomposition products are required because the acid component or thermal breakdown products of these formulations may result in toxicity.[33] However, pharmacological studies have proved that the aerosol released by ENDS using e-liquids containing nicotine salts resembles cigarette smoke more accurately while simultaneously having a higher nicotine concentration than liquids without nicotine salts.

As PG and VG are present in high quantities, they are considered as the carriers of the addictive component and flavorings starting from the user's mouth, passing through the throat, and reaching the lungs after getting vaporized. [34]

Tackling some physical properties of the two major components of e-liquid, PG is considered as a flavorless carrier with minimal viscosity compared to VG which naturally has a sweet flavor and higher viscosity. As a result of these properties, larger vapors and sweet taste are offered by e-liquids with higher ratio of VG. Whilst more PG composition in the e-liquid makes it more possible to strongly carry flavors and other additives but it can also cause a feeling of hit in the throat of the user.[35]

E. Toxicity Assessment of ENDS

The absolute predominance of ENDS attracted the full attention of scientists and researchers. Their focus has been and remains on evaluating the toxicity and safety of

ENDS. While vaping, chemical processes including oxidation and thermal deterioration may occur. Therefore, ENDS aerosol toxicity is evaluated biochemically on cell lines and animal experiments, or chemically by establishing the chemical profile of ENDS aerosols.[36]

The FDA states that the two primary solvents of e-liquid, PG and VG, are typically acknowledged as safe food additives but are only permitted in certain standardized meals.[37] As a result, some reports concentrated on finding toxicant pollutants in ENDS solutions. For instance, using liquid chromatography-mass spectroscopy (LC-MS), TSNAs which are strong carcinogens were detected and quantified in ENDS refill solutions.[38] In addition, some flavored e-liquids contained aldehydes including benzaldehyde and vanillin, which may cause respiratory irritation.[39] According to the U.S. Environmental Protection Agency (EPA), other aldehydes such as acetaldehyde and formaldehyde are categorized as potential human carcinogens. [13]

Moreover, numerous studies have discovered the existence of harmful and cancer-causing substances in ENDS aerosols such metals, reactive oxygen species (ROS), and carbonyls as a result of chemical changes brought on by ENDS activation.[13, 40, 41]

Furthermore, a variety of metals including Cadmium (Cd), Nickel (Ni), and Lead (Pb) were found in ENDS aerosols. [11] Studies showed that long exposure to these metals from ENDS aerosols may cause pulmonary diseases.[42]

Most importantly, an amount of benzene, a known human carcinogen, was discovered in e-liquid samples that was greater than the amount permitted in pharmaceutical products.[43] By using gas chromatography-mass spectrometry (GC-MS) and headspace solid-phase micro extraction (SPME), other VOCs such toluene, xylenes, and ethyl benzene were also found in the liquids. [43] Researchers believe that

the use of hydrocarbon solvents in the extraction of nicotine or tastes from natural plants is the cause of the release of these VOCs. [43]

F. Volatile Organic Compounds

1. Background

Volatile organics are a wide variety of compounds with boiling points ranging from 0°C to about 400°C. According to WHO these compounds have been classified into four categories, as shown in table 1. This classification is based on different volatilities of these compounds. For instance, VOCs are those compounds of boiling points varying from 50- l 00°C to 240-260°C. Regarding the molecular weight of these compounds, it ranges from 50-70 to about 300 Dalton. [44]

Table 1. Classification of volatile organics [44]

Classification	Abbreviation	Boiling point range	Vapor pressure
Very volatile organic compounds	VVOCs	<0, 50-100	
Volatile organic compounds	VOCs	50-100, 240-260	$>10^{-2}$
Semi volatile organic compounds	SVOCs	240-260, 380-400	10^{-2} _ 10^{-8}

2. Human Exposure to VOCs

Consumer goods (such as room fragrances), construction products (such as paints and adhesives), and daily activities (smoking, driving, using detergents) are the main sources of exposure to VOCs. Traditional outdoor sources of exposure, such as chemical plants, oil refineries, vehicles, and hazardous waste sites, tend to be modest, typically making up little more than 25% of individual exposure.[44] **Table 2** summarizes the most common VOCs and their corresponding sources.

Table 2. Common Volatile Organic Compounds and their sources [44]

Chemical	Major sources of exposure
Acetone	Cosmetics
Alcohols (ethanol, isopropanol)	Spirits, cleansers
Aromatic hydrocarbons (toluene, xylenes, ethyl benzene, trimethylbenzenes)	Paints, adhesives, gasoline, combustion sources
Aliphatic hydrocarbons (octane, decane, undecane)	Paints, adhesives, gasoline, combustion sources
Benzene	Smoking, auto exhaust, passive smoking, driving, pumping gas
Carbon tetrachloride	Fungicides, global background
Chloroform	Showering (10 min average), washing clothes, dishes
p-Dichlorobenzene	Room deodorizers, moth cakes
Formaldehyde	Pressed wood products
Methylene chloride	Paint stripping, solvent use
Styrene	Smoking
Tetrachloroethylene	Wearing or storing dry-cleaned clothes, visiting dry cleaners
I, I, I-Trichloroethane	Wearing or storing dry-cleaned clothes, aerosol sprays
Trichloroethylene	Unknown (cosmetics, electronic parts, correction fluid)
Terpenes (limonene, ex-pinene)	Scented deodorizers, polishes, fabrics, fabric softeners, cigarettes, food, beverages

3. Human Health Effects

a. Acute effects

High VOCs levels lead to acute effects on human health including “eye, nose, and throat irritation, and central nervous system responses such as dizziness, headaches and loss of short-term memory”. These mentioned symptoms are often observed in people living in new buildings or those undergoing renovation. In such new or renovated

buildings VOCs are detected and they cause sick building syndrome (SBS). Studies showed that the Levels of VOCs are often elevated in indoor air of such buildings. [45]

b. Chronic effects (cancer)

Several VOCs such as benzene and vinyl chloride are considered human carcinogens. However, other VOCs such as methylene chloride, trichloroethylene, tetrachloroethylene, chloroform, p-dichlorobenzene are mostly animal carcinogens and may be considered as human carcinogens. A-pinene is a VOC that is known to be a mutagen. To assess the carcinogenic risk of the mentioned VOCs several methods have been employed in toxicology-related research which are beyond the scope of this thesis. However, the identification and quantification of VOCs have been achieved using different analytical techniques which are explained in the following section.

G. Methods for VOCs Collection and Quantification from Tobacco Products

To assess the impact of smoking on human health, it is essential to identify and measure some chemical compounds present in cigarette smoke and Heated Tobacco Products (HTPs) vapors such as VOCs, carbonyls, and tars.

Few methods have been established in literature to collect and quantify VOCS from mainstream cigarette smoke and HTPs. One method is based on passing mainstream cigarette smoke through a Cambridge filter pad (CFP); then an impinger containing 10 mL of methanol is used to cryogenically capture the vapor phase, which is formerly cooled to a temperature of less than 70 °C in a dry ice/isopropanol bath. After that, the solution of the impinger is subjected to gas chromatography−mass spectrometry (GC/MS) analysis for identification and quantification.[46] Another method utilizes quartz filters to trap particulate matter produced by the mainstream

cigarette smoke. Then, sampling bags are placed to collect the vapor phase. The samples containing VOCs are then stored in a freezer at −20°C before being analyzed using GC/MS.[47] A third method that has been optimized to analyze VOCs generated from cigarettes and HTPs uses sorbent cartridge packed with a glass fiber filter. In this method a Carboxen 572 (CX572) is used to isolate gaseous compounds without the need for traditional impingers. After collecting the mainstream cigarette smoke with the cartridge, VOCs are eluted using a pre-determined volume of the non-polar solvent carbon disulfide. Then the compounds are allowed to settle for some time before being spiked with an internal standard and getting analyzed using GC/MS. [48]

H. Importance of Establishing a New Method to Collect and Quantify VOCs

Despite the robustness of the aforementioned methods that have been established to trap mainstream cigarette smoke and to quantify VOCs, they still have several limitations where researchers encounter difficulties during sampling. These limitations include the requirement of multiple, sometimes large, sampling devices such as an impinger with a cryogenic bath. In addition, high amount of solvent is consumed in case of using an impinger which increases the time needed to complete the sampling. Moreover, using Sampling (Tedlar) bags for collection requires huge amount of bags or the need of several clean-up steps in between samples. This might cause contamination due to the difficulty of cleaning these sampling bags. Moreover, none of the mentioned techniques have performed similar methods for setting the calibration curve and the samples collected. This indicates that there is no compensation achieved for any losses of VOCs on the filter and the tubes during sampling. These limitations call for the need to develop an in-situ collection and sampling method to identify VOCs. In an attempt to

complement the literature, this thesis focuses on quantifying the carcinogenic volatile compound “Benzene” emitted by ENDS using a novel method. The influence of e-liquid composition (PG and VG ratios) and the addition of nicotine and benzoic acid are also assessed.

CHAPTER II

BENZENE SAMPLING, ANALYTICAL METHOD OPTIMIZATION AND VALIDATION

A. Introduction

Electronic nicotine delivery systems (ENDS) are popular devices advertised as better alternatives than traditional tobacco products.[49] The main solvent system in the liquid reservoir is glycerol (VG), propylene glycol (PG), or a mixture of VG and PG in addition to nicotine (nic). Other additives may include benzoic acid (BA) and flavoring chemicals.[50] Due to the high demand and popularity of ENDS, public health authorities put efforts to investigate their harmful effects on users.[51] Specifically, several studies have compared the aerosol composition profiles of cigarettes and ENDS, constituted of particulate matter, metals, carbonyls, polycyclic aromatic hydrocarbons, and volatile organic compounds (VOCs).[52-54] In general, it was found that the coil resistance, type of coil, the applied power (which is directly related to the temperature), solvent constituents, and the puff topography affect the aerosol chemical composition in ENDS.[55, 56]

Gas-phase aerosol constituents, especially VOCs including benzene are highly important due to their toxicity and carcinogenicity as defined by the International Agency for Research on Cancer (IARC).[57-59] Benzene is a volatile compound mainly analyzed with gas chromatography coupled with mass spectrometry (GC-MS). [14] Different methods were reported in the literature to trap and quantify VOCs from conventional cigarettes and ENDS smoke. For example, the most used methods include solvent-based impingers and solid sorbent tubes with thermal desorption. [11, 60] Other methods involved collecting aerosols in a gas sampling bag while utilizing solid phase

micro-extraction (SPME) for extracting the compounds of interest. [61] Despite the robustness of the methods presented, limitations such as: the long sampling time, high solvent consumption, several clean-up steps, and background contamination issues call for the need to develop an in-situ collection and sampling method to identify benzene and VOCs.

In the present study, quantification of benzene emissions by e-liquids of different compositions of VG and PG was assessed using an optimized in situ online dynamic/static SPME-GC-MS method. Moreover, the effect of adding nic and BA on benzene emissions was also studied.

B. Experimental Work

1. Optimization of the Experimental Setup

The optimal experimental conditions of the sampling procedure were determined so that any error in the quantification of benzene is minimized. This included optimizing several parameters such as the number of puffs, the power applied, pressure applied by Aerosol Lab Vaping Instrument (ALVIN), preparation of the calibration curve, the container used for gas collection, conditions of the gas cell, SPME conditioning time, SPME exposure time to the gas phase, and the position of the SPME fiber in the gas cell which are detailed in Table 3.

Table 3. Optimization of the experimental parameters and setup

Variables	Adopted parameters	Disregarded trials	Justification
Number of puffs	3	5, 10, and 15	to prevent oversaturation of the glass fiber filter at 45 W
Power	45 W	15 and 30 W	to account for the detection limit of the GC-MS at low power
Pressure applied by ALVIN	negative pressure	positive pressure	to prevent the contamination of the pump upon evaporating e-liquids
Preparation of the calibration curve	Tedlar bag connected to the sampling set-up	direct exposure of the SPME to standards prepared in Tedlar bag	to compensate for any losses of VOCs on the filter/tubes during sampling
Container for gas collection	Gas cell	Tedlar Bag	to allow for both dynamic and static extraction of VOCs rather than only static in the case of using a Tedlar bag
Gas cell conditions	clean in between sampling, allow for drying using an oven, and cool before next use	not cleaning and not allowing the cell to cool down	to allow for allow for proper trapping and quantification of VOCs using a clean and cold gas cell
SPME conditioning time	5 min	10 and 20 min	to desorb the SPME fiber exposing the SPME fiber to the conditioning temperature for 5min is enough to release any leftovers from previous extraction
SPME exposure time to gas phase	during puffing +15 min	10, 15, and 20 min	to reach equilibrium of VOCs concentrations in the sample matrix and the fiber coating
Position of the SPME fiber in the gas cell	fixed	variable	to have a consistent position of the fiber in the gas cell through all samples as the extraction is taking place dynamically

2. Standards, Solvents and SPME Fiber

Propylene glycol (PG;99.5%; CAS no. 57-55-6), vegetable glycerin (VG; 99%−101%; CAS no. 56-81-5), nicotine (nic, ≥99%), and benzoic acid (BA, ≥99.5%) were purchased from Sigma-Aldrich. An ENDS device (the Sub-Ohm Minibox C), stainless steel organic cotton coils, and a nichrome coil (0.50 Ω resistance) were purchased from online vendors. BTEX (Benzene, toluene, ethylbenzene, xylene) standard tank (1ppm, Restek) was used to prepare different concentrations of benzene for the calibration curve. Nitrogen gas (>99.995%) was used for dilution purposes for the preparation of the calibration curve. SPME fiber used were 85μm carboxen polydimethylsiloxane (CAR-PDMS) and 65μm carboxen divinylbenzene (CAR-DVB). One and five liters Tedlar bags made up of polyvinyl fluoride film were purchased from Cel-Sci Corp.

3. Preparation of Calibration Samples

The calibration curve was prepared using a standard tank of BTEX (1ppm). 5L Tedlar bags were filled with 1L nitrogen gas (dilution gas) using a mass flow controller (500ml/min). Then by applying the proper dilution calculations, and using a gas tight syringe, five different concentrations of benzene were prepared (2, 5, 10, 20, and 40ppb).

4. Parameters of GC–MS Analyses

For GC-MS analysis, a Trace GC Ultra system coupled with a triple Quadrupole spectrometer was used, equipped with Xcalibur software. Compounds were separated using a DB-5MS:5%-phenyl-methylpolysiloxane capillary column (30m, 0.25mm, 0.25μm) with helium as carrier gas in a constant flow rate of 1 mL/min. The temperature program was set at 40℃ and held for 1 min, increased to 50 ℃ at a rate of

5 ℃/min, then increased to 100℃ at a rate of 15℃/min and held for 0.5 min, then the temperature increased at a rate of 20℃/min until reaching 250℃ which was held for 1min. The total run time was 14.83min.

5. Aerosol Generation

Aerosols were generated using ALVIN.[62] ALVIN is a digital puffing machine that replicates the puffing behavior of ENDS users. A total of three 4s puffs were produced by ALVIN. To set the calibration curve, five liters Tedlar bags filled with benzene standards (2 to 40ppb) were connected to the instrument as a source of aerosols. In the sampling line, a glass fiber filter pad was placed upstream to trap particulate matter and to allow the passage of gas phase into the gas cell. VOCs were adsorbed by a CAR-PDMS SPME fiber previously placed in the cell. The SPME fiber was exposed to a total of three running puffs (dynamic), then it was subjected to a total of 15min exposure until reaching equilibrium (static) before its injection into the GC inlet. Similarly, the ENDS device fitted with a coil head replaced the Tedlar bag and was connected to ALVIN to generate aerosols. E-liquids tested in this experiment were PG, PG+nic+BA, VG, VG+nic+BA, PG/VG, PG/VG+nic+BA. Figure 4 is an illustration of the sampling setup used to trap benzene in this experiment.

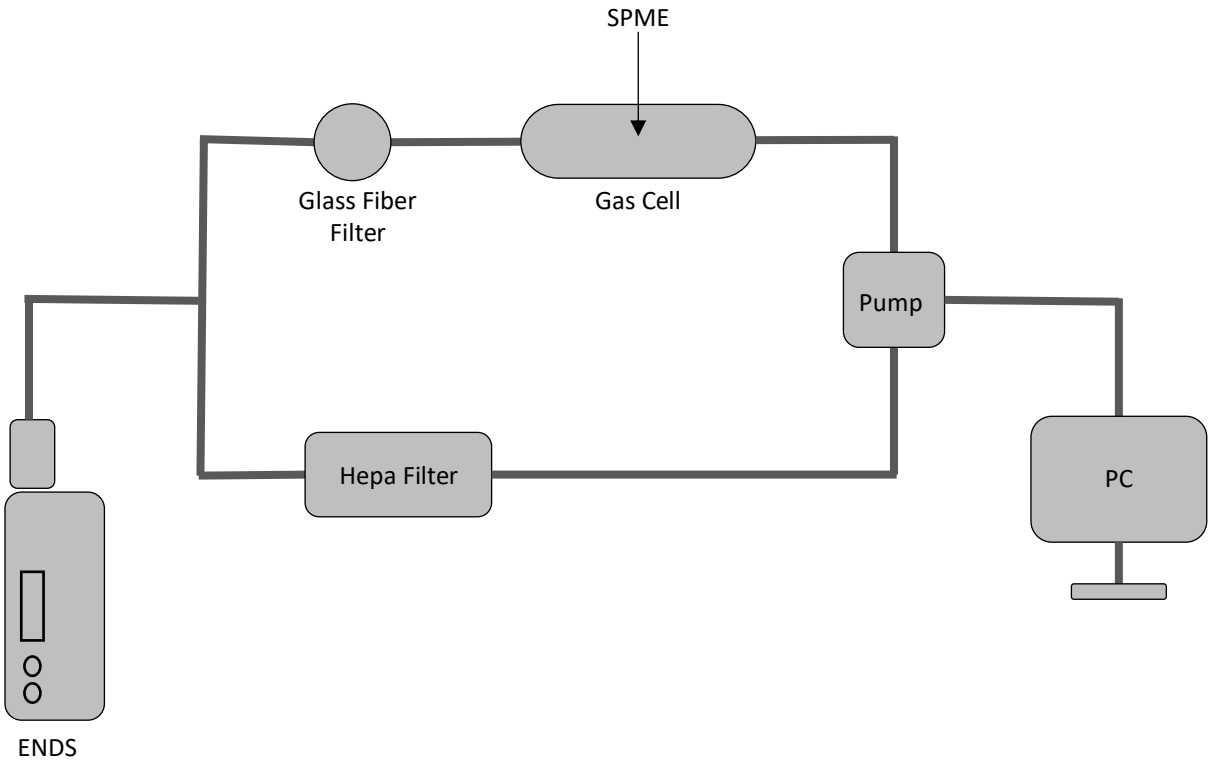

Figure 4. A scheme showing aerosols generation from ENDS using ALVIN.

6. Method Validation

In order to assess the reliability, consistency, and accuracy of the developed method, validation is an essential aspect of the quality control (QC) and quality assurance (QA) practices. Linearity, limit of detection, limit of quantification, and repeatability are the main evaluation criteria.

a. Linearity

The linearity test confirms that Benzene is present in a range where its chromatographic response is linearly correlated with their concentration. Examining the calibration curve's correlation coefficient is a popular method for determining its validity. A direct calibration curve is used in this investigation and benzene standards ranging between 2 and 40 ppb are prepared. The correlation coefficient is found to be 0.9943. (Figure 5)

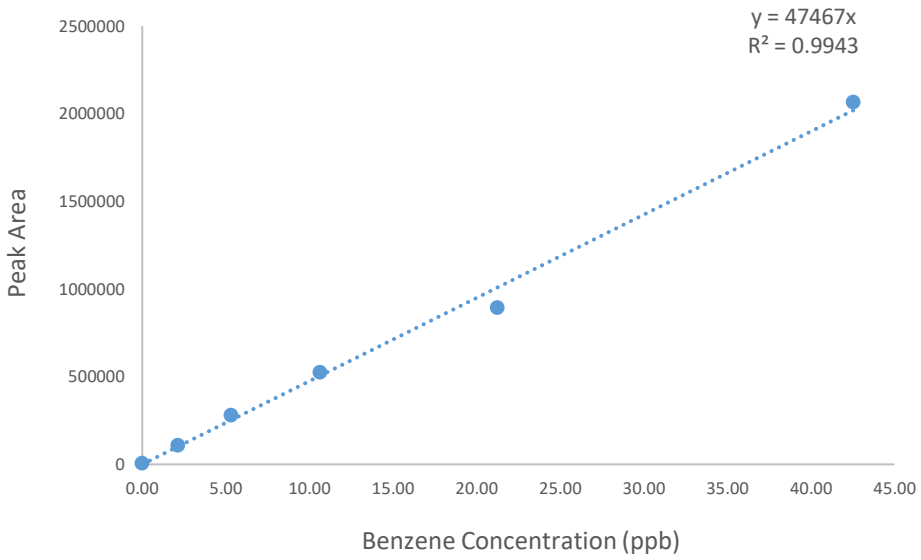

Figure 5. Calibration Curve in quantifying the e-cigarette aerosol benzene concentrations. Peak area: area under the peak.

b. Limit of detection

The limit of detection (LOD) is the lowest concentration of analyte which is possible to be detected but not necessarily quantified. LOD is calculated by utilizing the standard deviation response as well as the slope based on the following equation:

$$LOD = 3\sigma/s$$

σ is the standard deviation of seven replicates of the analyte "benzene" prepared at low concentration and s is the slope of the calibration curve. Seven replicates of the blank were used to evaluate LOD. The results have shown that the detection limit of benzene is 0.22ppb.

c. Limit of quantification

The lowest concentration of an analyte that can be detected with an acceptable level of accuracy and precision is known as the limit of quantitation (LOQ). LOQ is

evaluated by utilizing the standard deviation response as well as the slope based on the following equation:

$$LOQ = 10\sigma/s.$$

LOQ was measured using seven blank replicates. The results have shown that quantification limit of benzene is 0.37ppb.

d. Repeatability

The term "repeatability" refers to the degree of consistency in measurements made with the same equipment, by the same operator, and on the same day.
It is measured using analytical data from at least five measurements at three distinct concentrations (low, medium and high), and is represented as the percent relative standard deviation (%RSD) of those values. The degree of tested concentration, the complexity of the matrix, and the type of analysis are the basis for the acceptance criteria. The results show that %RSD ranges between 2.79% and 11.45% which is within the acceptable range.

Table 4. LOD and LOQ of benzene reported by different studies and the current study

Quantification Method	**LOD[a]**	**LOQ[b]**	**Year**	**Reference**
SPE-GC/MS	0.15 µg/150 puffs	0.45 µg/150 puffs	2014	[11]
Sampling bag- GC/MS	0.00171 µg	-	2012	[47]
Pad/impinger- GC/MS	0.168 µg/collection	0.560 µg/collection	2016	[54]
SPME- GC/MS	0.00015 µg/3 puffs	0.00026 µg/3 puffs	2022	Current study

[a] Limit of detection. [b] Limit of quantification

7. Non-Targeted Analysis

Non-targeted analysis (NTA) uses screening chromatography and high-resolution mass spectrometry in the process of characterizing complicated materials without knowing the chemical composition of the mixture beforehand.[63] For the purpose of finding a likely match, this technique makes advantage of molecular characteristics that are suggestive of unknown particular chemicals, such as accurate mass, retention time (RT), and molecular fragmentation.[64, 65] In the present study, NIST library was used to test for the presence of VOCs other than the targeted benzene in the obtained chromatograms. Since CAR-PDMS SPME fiber cannot trap all types of VOCs, a carboxen divinylbenzene (CAR-DVB) SPME fiber was used for this purpose. Each of the six E-liquids (PG, VG, PG/VG, PG+nic+BA, VG+nic+BA, and PG/VG+nic+BA) was examined in triplicates while extracting VOCs using CAR-DVB.

8. Statistical Analysis

t-test was used to assess the difference in benzene emissions among the tested e-liquids. P-values are reported within the results section. A p-value ≤ 0.05 represents a statistically significant difference between two sets of measurements.

CHAPTER III

RESULTS AND DISCUSSION

In this chapter, results of the experimental examinations performed along with the explanation of each outcome obtained are represented.

A. Results

1. Gas-Phase Benzene Levels

Results in figure 6 show that VG e-liquid emitted the highest level of benzene (3.96±0.49 µg/m^3) compared to the mixture PG/VG ($P<0.004$) and to the e-liquid comprised of only PG only ($p<0.002$). Levels of benzene in the PG and PG/VG mixtures were below the detection limit.

Emission of benzene from VG increased by 69% (6.68± 0.567 µg/m^3) after the addition of nicotine benzoate salt at15 mg/g. This increase in emission was also observed in PG and PG/VG mixture where the benzene concentration went from being not detected in the absence of nicotine benzoate to 11.64±0.190 µg/m^3 and 62.85± 33.4 µg/m^3 in PG+nic+BA, and 30/70 PG/VG+nic+BA, respectively. Statistical analysis reflected a significant difference in the measured benzene levels (after adding benzoic acid) with the increase of PG fraction. For instance, the quantity of benzene produced by VG+nic+BA (6.28 µg/m^3) was less than that produced by PG/VG+nic+BA ($p<0.003$), and that latter being less than the quantity produced by PG+nic+BA ($p<0.05$).

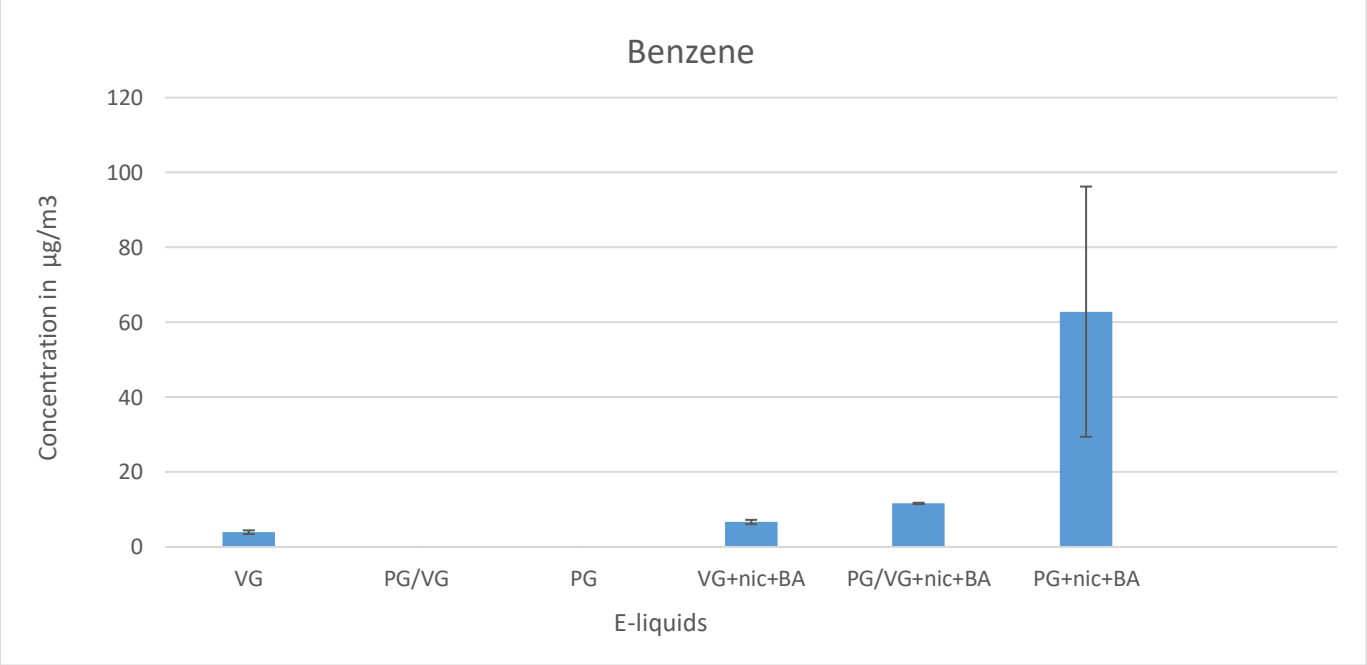

Figure 6. Benzene concentrations in pure e-liquids of different proportions of PG and VG and in the presence of nicotine (nic) and benzoic acid (BA).VG: 100% vegetable glycerin, PG: 100% propylene glycol, PG/VG: 30% propylene glycol, 70% vegetable glycerin.

2. Results of the Non-Targeted Analysis

Results of the non-targeted analysis obtained by CAR-PDMS and CAR-DVB fibers are shown in table 5 and table 6 respectively. Various organic compounds have been detected including acetaldehyde, benzaldehyde and the alkanes: cyclodecane and tetradecane. One important observation about all VOCs identified with both types of fibers is that they have been all detected in the e-liquid with the highest fraction of PG and in the presence of nicotine and benzoic acid (PG+nic+BA).

Table 5. Non-targeted Analysis of the vapor phase trapped by CAR-PDMS SPME fiber

Compound	Retention Time	m/z	SI	RSI	PG	PG+nic+ BA	VG	VG+nic+BA	PV	PG/VG+nic+BA
Acetaldehyde	1.43	29	933	941	+	+		+		+
Propylene oxide	1.59	58	912	917		+				+
Allyl alcohol	1.76	57	784	899		+	+			+
biacetyl	1.96	43	848	945		+	+			
2-Butanone	2.01	43	871	913		+				
α-Methylfuran	2.06	82	827	854		+	+			
1,3-Cyclohexadiene	2.34	79	745	846		+				
3-Hexen-1-yne	2.38	79	648	857		+				
2,3-Dihydrofuran	2.44	70	791	839		+	+			
Benzene	2.55	78	928	931		+		+		+
2,3-Pentanedione	2.88	57	666	823		+				
2,5-Dimethylfuran	3.06	96	753	826		+				
Methyl methacrylate	3.13	69	748	816	+	+	+	+	+	+
Ethyl glycolate	3.41	105	721	974	+	+	+	+	+	
Toluene	3.92	91	754	835	+	+	+	+	+	+
Furfural	4.92	96	898	905		+	+			
Ethylbenzene	5.36	91	744	888		+				
o-Xylene	5.49	91	622	814	+	+		+	+	+
m,p-Xylene	5.8	91	845	889		+			+	
Benzaldehyde	6.71	105	693	820		+				
cyclodecane	7.05	255	829	899	+	+				

Table 6. Non-targeted Analysis of the vapor phase trapped by CAR-DVB SPME fiber

Compound	Retention Time	m/z	SI	RSI	PG	PG+nic+ BA	VG	VG+nic+ BA	PV	PG/VG+nic+ BA
Silanediol, dimethyl-	2.55	77	712	780		+	+	+	+	+
Benzene	2.79	78	823	931	+	+	+	+	+	+
3-Piperidinol	3.31	101	681	865		+				
Ethyl glycolate	3.39	105	789	875		+	+	+		
Chlorogenic acid	3.79	117	755	867	+	+			+	+
Toluene	3.92	91	891	952	+	+	+	+	+	+
Ethyl benzene	5.35	91	855	892	+	+	+	+	+	+
Ethylene acetate	5.43	88	789	879		+		+		+
m,p-xylene	5.47	91	866	925	+	+	+	+	+	+
Styrene	5.78	104	745	782	+	+	+	+	+	+
o-xyelen	5.81	91	811	878	+	+		+	+	+
Oxime-, methoxy-phenyl	5.85	133	672	811	+	+	+	+	+	+
Benzaldehyde	6.73	105	725	830	+	+	+	+	+	+
Cyclodecane	7.07	255	655	790	+	+			+	+
2-Propanol, 1-(2-methoxy-1-methylethoxy)-	7.35	93	722	780	+	+			+	+
Sylvestrene	7.55	93	780	885	+	+	+	+	+	+
3, dodecene	9.05	83	675	825	+	+			+	+
Tetradecane	9.12	71	715	780	+	+		+	+	+
2,4,6-Trimethyldecane	9.91	71	695	795		+				
Nicotine	10.38	145	798	921		+		+		+
Pyridine, 4-(1-pyrrolidinyl)-	10.37	147	724	788		+				+

B. Discussion

In this study, a novel method is presented to quantify toxicants emitted by ENDS. This method is validated to be precise and repeatable. It can also be used to quantify emissions at low concentrations regardless of the low number of puffs adopted.

E-liquids of different constituents were tested. VG was found to produce benzene at concentrations much higher than PG and PG/VG mixtures in which the concentrations were below the detection limit. These results are in agreement with the mechanisms proposed by Ooi et al. in 2019 where pure PG is not able to produce benzene **Figure** due to the absence of the precursor "acrolein" which is produced by VG (**Figure**). [66]

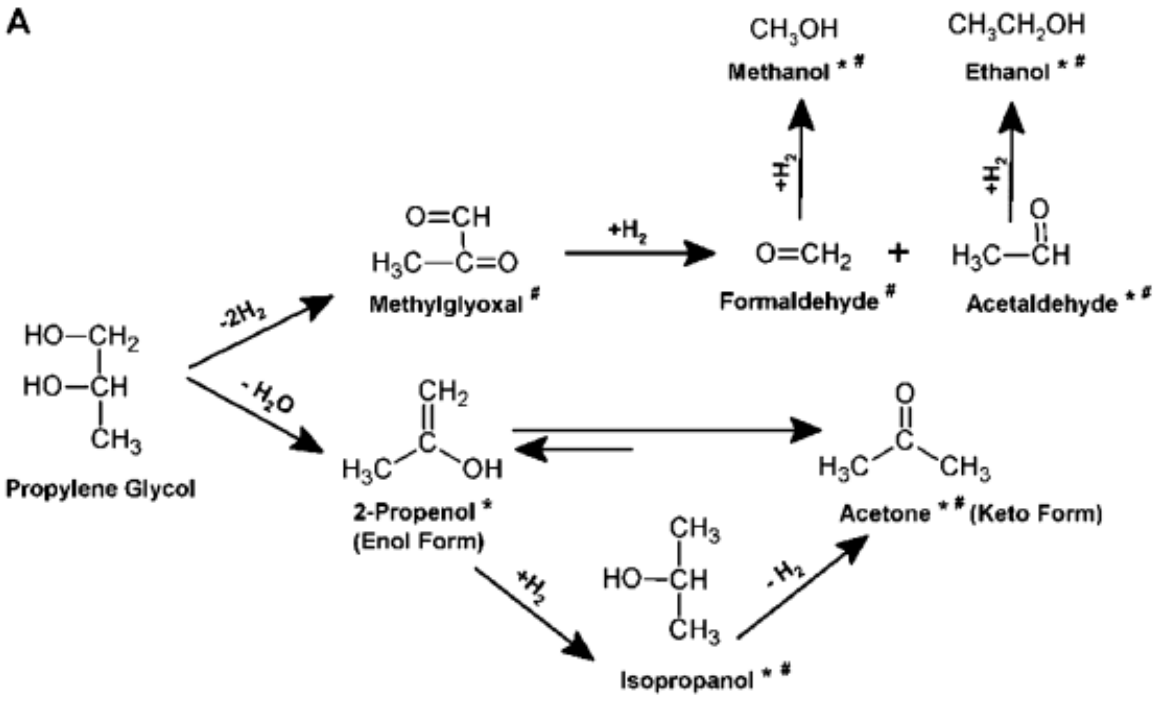

Figure 7. Thermal Decomposition of Propylene Glycol (PG). Retracted from Ooi et al., 2019 [66]

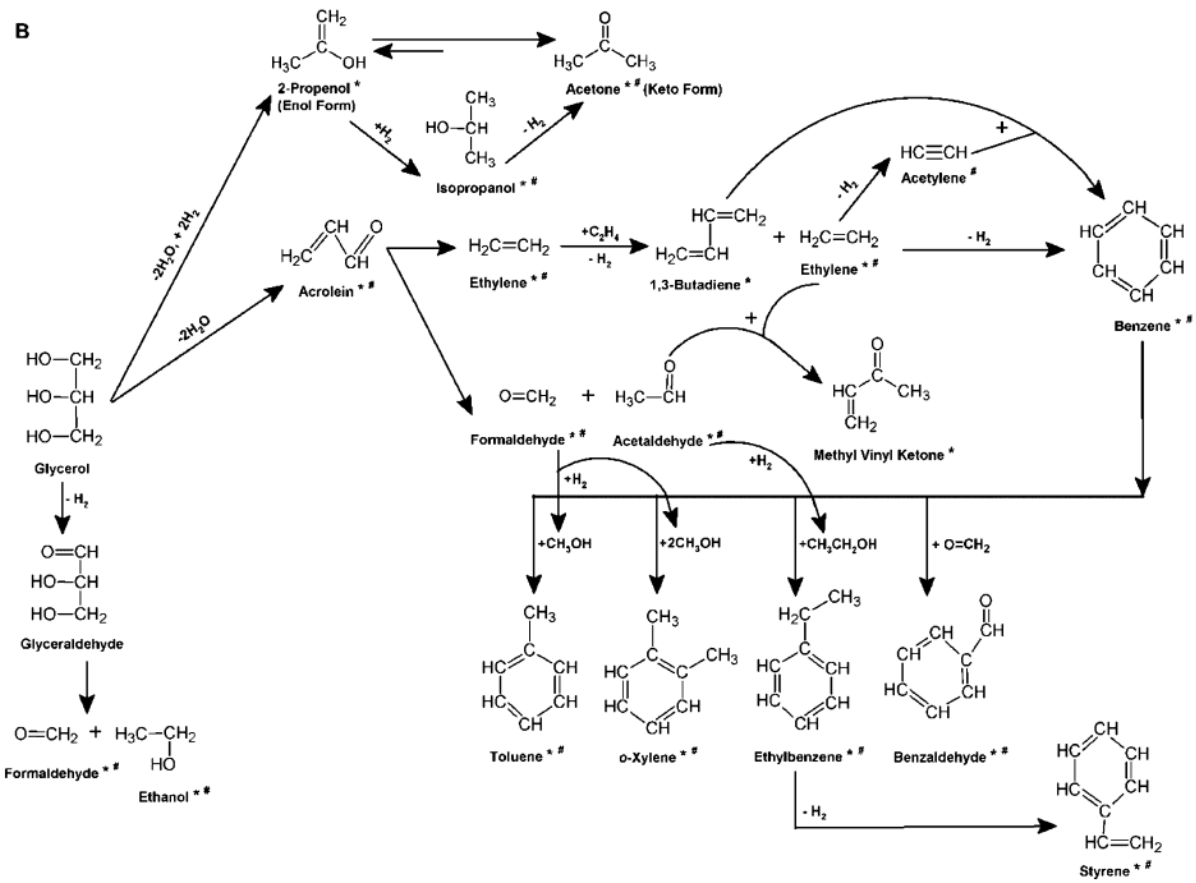

Figure 8. Thermal Decomposition of Vegetable Glycerin (VG). Retracted from Ooi et al., 2019 [74]

The quantity of benzene emitted by the tested PG/VG mixture was expected to be quantified since benzene emissions had been positively correlated with the increase of VG fraction in the e-liquid mixture [66]. However, benzene level was below the detection limit in the tested PG/VG e-liquid. This could be attributed to the low number of puffs performed in our experiment (3 puffs).

Upon adding nicotine and benzoic acid to each of the tested e-liquids, benzene levels increased in PG+nic+BA, VG+nic+BA, and 30/70 PG/VG+nic+BA mixtures. This indicates that the decarboxylation reaction of BA is an additional source of benzene in these solutions as it was reported in the literature (Figure 9)[67].

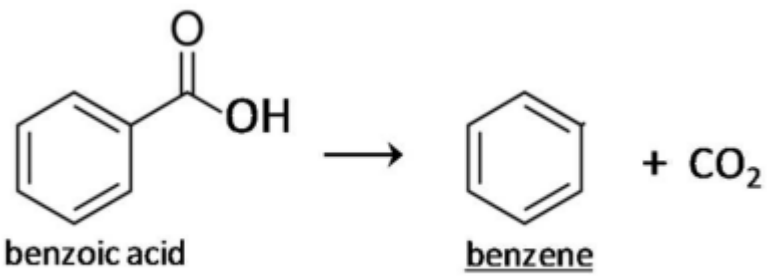

Figure 9. Decarboxylation of Benzoic Acid. Retracted from Pankow et al.,2017 [67].

More importantly, analysis of the results showed that an increase of PG fraction in the e-liquid, in the presence of nicotine and BA, led to an increase of benzene emissions as well as other VOCs detected through non-targeted analysis. This is attributed to the difference in the volatility of the tested mixtures.

According to Raoult's law, the total vapor pressure "P_T" of the e-liquid mixtures depends on the solvents (S): PG, VG, or PG/VG, as well as on the solutes: BA, and nic. (Equation 1)

$$P_T = P^0_S X_{S+} P^0_{BA} X_{BA+} P^0_{nic} X_{nic} \qquad \text{Equation 1}$$

Since the mole fractions "X_S, X_{BA} and X_{nic}" and the partial pressures "P^0_{BA} and P^0_{nic}" are equal in all tested solutions, then the total vapor pressure of the mixture will be totally dependent on the partial vapor pressure of the solvent "P^0_S". The vapor pressure of PG is reported in the literature to be higher than that of VG (1.01 bar and 3.32×10^{-2} bar respectively at 188°C: the boiling point of PG at standard atmospheric pressure). [68] Thus, a larger fraction of PG in the solvent mixture compared to VG strengthens the volatility of the e-liquid and leads to a higher consumption of the e-liquid and accordingly, higher aerosols emission.[35] This is confirmed by the obtained

results of the mass consumption of each of the tested e-liquids. The consumed mass of the e-liquid PG+nic+BA is 0.24±0.026g which is significantly higher than that of the e-liquid PG/VG+nic+BA (0.17±0.007g, $p<0.05$) and the e-liquid VG+nic+BA (0.16±0.013, $p<0.04$). Figure 10 shows the mass consumed (g) by each of the tested e-liquids.

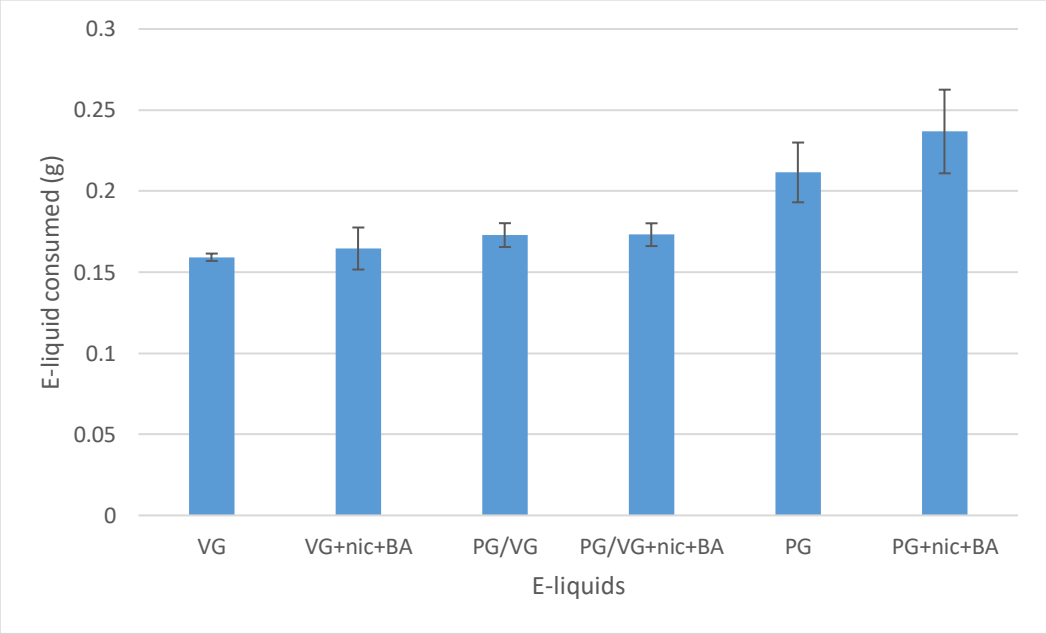

Figure 10. E-liquid Consumption (g)

Furthermore, studies by Talih and coworkers have shown similar behavior of nicotine through formulating a mathematical model and experimental investigation. It has been confirmed that the greater volatility of PG allows PG-rich e-liquids to evaporate more rapidly than VG-rich e-liquids. This resulted in higher nicotine flux in the e-liquid of higher PG ratio.[69]

These results were also validated using molecular dynamics simulations via the reactive force field (ReaxFF) provided by the Amsterdam Density Functional. ReaxFF

works on the molecular level to predict system behavior by computing total energies at every time increment based on theoretical quantum chemistry and empirical data.

Preliminary runs were conducted on a box containing three benzoic acid molecules and 10 molecules of either PG or VG. The system was first geometrically optimized, then subjected to a temperature regime from 1000K to 3000K to obtain real-time results. As seen in **Figure** and **Figure**, benzene was formed more readily and in abundance in the system containing PG over that of VG. To elaborate; benzene radical is formed from benzoic acid directly in both environments PG and VG. Later on, benzene intermediate (C_6H_5) forms benzene. The rate constant "k" for benzene intermediate formation in VG environment is 5.17×10^{-5} lower than that in PG environment (2.05×10^{-4}). This means that the rate of elementary reaction is lower in VG than that in PG. Extracted molecular number profiles confirmed that benzene was formed more readily and in abundance in the system containing PG over that of VG.

Hence, it is suggested that the evaporation of an e-liquid of higher PG fraction in the presence of nic and BA led to a higher level of benzene in the gas phase.

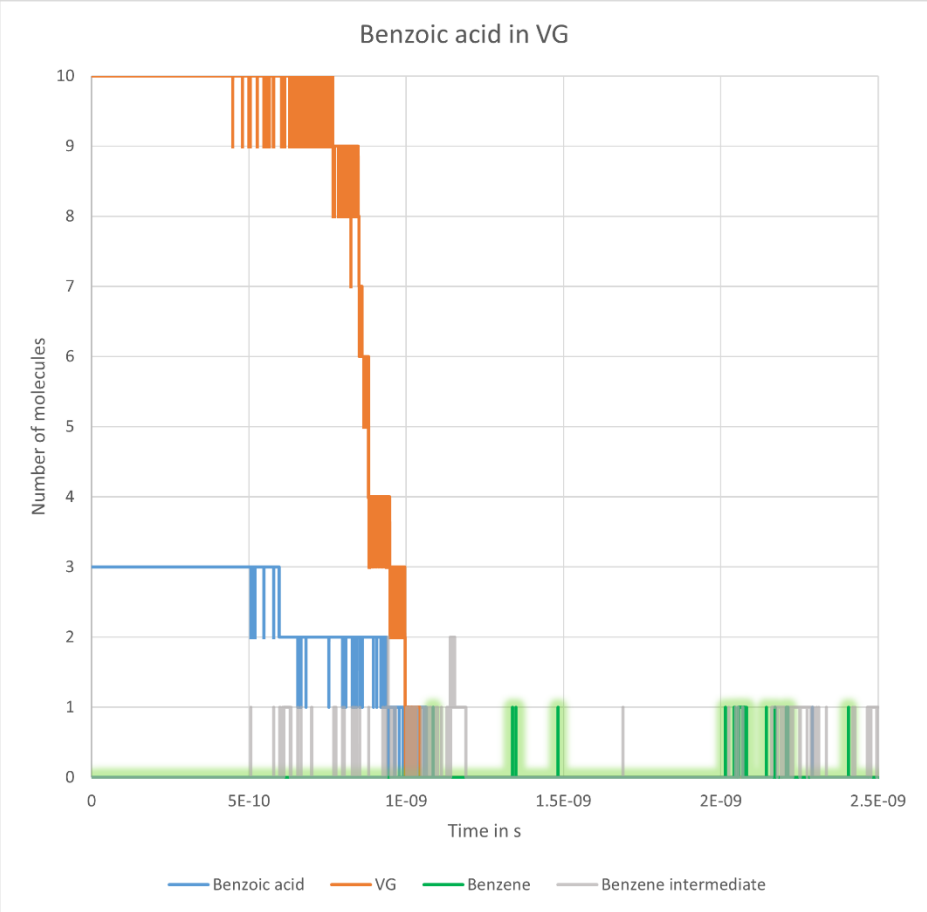

Figure 11. Benzene emissions by benzoic acid in VG environment.

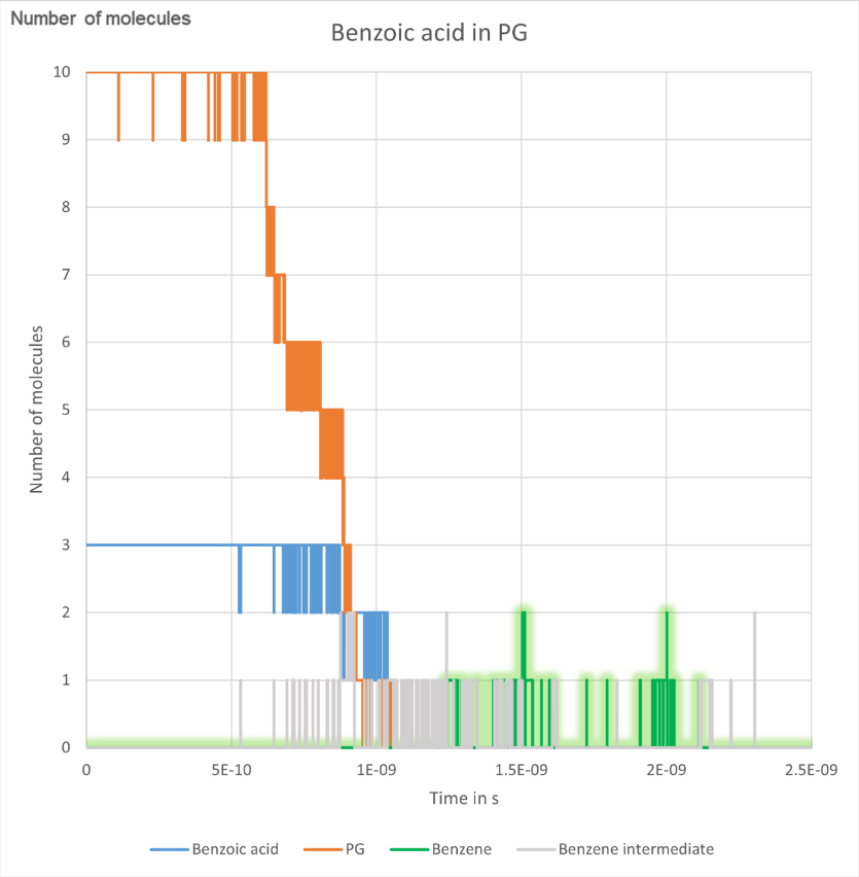

Figure 12. Benzene emissions by benzoic acid in PG environment.

C. Assessment of Benzene Toxicity

To assess the toxicity of benzene concentrations obtained, the corresponding cancer risk (CR) is calculated based on the following equations: [70]

$$CR = CDI \times CSFi \quad \text{(Eq. 2)}$$

And,

$$CDI = (CA \times IR \times ET \times ED)/(BW \times AT) \quad \text{(Eq. 3)}$$

Where,

CR: Cancer Risk

CDI: Chronic Daily Intake

CSFi: Cancer Slope factor (2.73×10^{-2} mg kg^{-1} d^{-1}, RAIS, U.S. EPA, 2007)

CA: benzene concentration (μg/m^3)

IR: Inhalation Rate (0.875 m^3/h, U.S. EPA, 2011)

ET: Exposure Time (h/d)

EF: Exposure Frequency (365 d/y)

ED: Exposure Duration (y)

BW: Body Weight (Kg)

AT: Average Time for cancer estimation (70y×365d/y=25,550d)

Suggest a 60 year old smoker of an average body weight 80 Kg started vaping at the age of 16 an average of 140 puffs/day, each puff lasting for an average of 4 sec. Working in the extreme case where the smoker is vaping an e-liquid composed of 100 PG+nic+BA and benzene is being emitted at its highest level= 62.85+33.4= 96.25μg/m^3 in a total of 3puffs.

Thus, CDI=(4491.67×0.875×0.156×44)/(80×25550)= 0.01319

And, CR= 0.0071× 2.73×10^{-2} = 3.6×10^{-4}

The overall cancer risk is about 3.6×10^{-4}, which is higher than the upper limit of the acceptable range (10^{-4} to 10^{-6})[71] indicating a cancer risk for ENDS consumers on the long term.

CHAPTER IV

CONCLUSION

As a generic assessment of ENDS toxicity, benzene emissions were quantified using a novel semi-online dynamic method. First, we compared these emissions from e-liquids of different PG/VG proportions. Results showed that VG is the component responsible of emitting benzene from the e-liquid. Then, we studied the effect of adding nicotine benzoate salt on the initially tested e-liquids. Accordingly, benzene levels elevated in all solvents. Thus, we confirmed that benzoic acid is a source of benzene emissions due to decarboxylation reaction. In addition, benzene emissions resulting from benzoic acid increased upon increasing PG fraction in the solvent mixture. This was attributed to the difference in the volatility between PG and VG. Since PG has a higher vapor pressure than VG, thus more PG content in the e-liquid increases the ability to strongly convey flavors and other additives such as BA. This result was also validated by ReaxFF where the extracted molecular number profiles confirmed that benzene was formed more readily and in abundance in the system containing PG over that of VG.

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