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Heavy Metals found in JUUL's Electronic Cigarettes

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Dedication

I dedicate this to my parents who gave me an incredible support system throughout the entire duration of this research, my sister who gave me the drive and inspiration to pursue this topic, and to Erin Ridge who gave me nothing but encouragement and support.

Acknowledgements

I would like to thank both Professor Dutcher and Professor Raymond for they have expertly guided my research, while giving me the freedom to drive the research in the direction I wanted.

I would like to thank Monica Hoover for her help in perfecting the methodology of the standards and samples along with her assistance with the use of the ICP-MS. She helped me understand how the ICP-MS works and without her help none of the samples could have been analyzed.

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I would also like to thank Thomas Thul for his help with the design of the modified JUUL, without his help this research would've never been able to compare before and after vaping.

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Table of Contents

Abstract	1
Introduction	2
Background	7
Heating Element Composition	7
JUUL Modified Design	8
ICP-MS	10
Experimental Procedure	11
Standard Preparation	11
Sample Preparation	14
ICP-MS Conditions	16
Results & Discussion	18
Survey Scan Results	18
Initial Expectations	19
Before and After Vaping Results	20
Quality Control Change Discovery	29
Early and Late Lot Number Results	30
Conclusions	39
References	40

Table of Figures

Figure 1: JUUL e-cigarette in hand	3
Figure 2: JUUL pod with e-liquid, heating element, and wick	5
Figure 3: Modified JUUL e-cigarette in hand	9
Figure 4: Magnesium concentration before and after vaping	23
Figure 5: Chromium concentration before and after vaping	24
Figure 6: Iron concentration before and after vaping	25
Figure 7: Nickel concentration before and after vaping	26
Figure 8: Copper concentration before and after vaping	27
Figure 9: Zinc concentration before and after vaping	28
Figure 10: Magnesium concentration of early and late lot	32
Figure 11: Chromium concentration of early and late lot	33
Figure 12: Iron concentration of early and late lot	34
Figure 13: Nickel concentration of early and late lot	35
Figure 14: Copper concentration of early and late lot	36
Figure 15: Zinc concentration of early and late lot	37
Figure 16: Lead concentration of early and late lot	38

Abstract

Electronic cigarettes better known as e-cigarettes have been consistently rising in popularity with new products entering the market daily. E-cigarettes function by vaporizing a liquid composed of propylene glycol, glycerin, benzoic acid, nicotine, and flavor additives with the use of a metallic heating element. This vapor produced is then inhaled by the user. The composition of this liquid is critical to discovering the possible health effects.

This study investigates the presence and concentrations of heavy metals in the e-liquid before and after vaping. An ICP-MS was used because of its ability to scan several metals rapidly and its high sensitivity. In the analysis, it was found that there was a statistically significant increase in the concentration of chromium and nickel. The chromium concentration for JUUL's Virginia Tobacco flavor increased slightly from 122 to 128 ppb, while the nickel concentration increased from 54 to 97 ppb both of which are statistically significant increases.

With very little regulation or oversight of the e-cigarette industry as a whole, and with no reporting requirements for labels of e-liquid content, manufacturers are frequently changing their formulations, even for the same product. JUUL has historically had issues controlling the quality of their product so it was not surprising to discover that while running experiments there was a statistically significant difference between batches of e-liquid purchased almost one year apart. Specifically, there was a decrease in concentrations of magnesium, iron, nickel, copper, zinc, and lead analyzed from the experiments run on November 04, 2020 and December 09, 2019. This is likely due to the fact that Altria, a large tobacco company, bought 30% of JUUL's stocks, which gave them the power and know how to get their product's quality under control. For example, the zinc concentration on December 09, 2019 was 195,000 ppb and on November 04, 2020 the concentration dropped to 9,690 ppb.

Introduction

Electronic cigarettes, better known as e-cigarettes, were introduced to the United States in 2006 [1]. Since then, they have been trending as an alternative to cigarettes. Unfortunately, with various fruity and sweet flavors available they have been becoming more popular with a younger audience. Even though there are age restrictions for nicotine products, they are still getting into the hands of minors. Studies show that approximately 4.1 million high school students and 1.2 million middle school students vape as of November 2019 [2]. An image of a JUUL's e-cigarette, which is commonly and simply referred to as a JUUL, can be seen in **Figure 1**. As seen in the figure, the e-cigarette is small and has a close resemblance to a large USB drive. This allows it to go unnoticed in public areas and even schools. These products can be hazardous in the hands of anyone, let alone developing minors. Minors also breathe in more air relative to their size than adults do, which would result in more of any hazardous materials present entering their system [3]. This research will be delving further into what metals can be found inside of JUUL's products, and more specifically what concentration can they be found at before and after vaping.



Figure 1. A JUUL e-cigarette in person's hand to represent its size [4].

These hazards can be better understood by learning how e-cigarettes work. They typically work by using a battery to resistively heat the metallic heating element, which will begin to vaporize a liquid inside the e-cigarette. Specifically, for JUUL e-cigarettes the heating element is activated simply by the user breathing in which will activate a pressure sensor inside the device, this is unique to most e-cigarettes because most other devices require a button to activate. This vapor will then come out of the mouthpiece and into the user's mouth and eventually it will be inhaled into the lungs. By now understanding the process of how they work, we can tell that the main way that using e-cigarettes can become dangerous is dependent on the composition of the liquid for the e-cigarette, which is more commonly known as e-liquid. Since JUUL is the largest e-cigarette company, this report will focus on their e-liquid. Their e-liquid is composed of propylene glycol, glycerin, benzoic acid, nicotine, and flavor additives, where the exact chemistry and make-up is not disclosed on labels, packaging, nor online. Although the major components by themselves show no reported significant or obvious hazards, the heating element is made of chromium, nickel, and iron and can reach temperatures as high as 250°C [5]. It is expected that temperatures this high will likely cause the chromium, nickel, and iron in the heating element to leech into the e-liquid. JUUL submerges their heating element directly in the e-liquid as can be seen in **Figure 2**. This could become a hazard because the metals that leech out of the heating element will go directly into the e-liquid that will be vaporized and inhaled.



Figure 2. A JUUL pod that contains the e-liquid, heating element, and wick (left). A heating element and wick for better visual (right). This shows visually how the heating element and wick are directly in contact with the e-liquid.

This research involved a comparison of the e-liquid before and after vaping in order to determine if heavy metals, leached from the coil, are entering the liquid and ultimately being inhaled into the lungs which could have significant and permanent consequences. Lead is a well understood and dangerous element because it can interfere with physical and mental development [6]. These effects can be especially dangerous to minors because their bodies and brains have not yet fully formed. Nickel has also been found to be hazardous, particularly when introduced into the lungs directly. In extreme cases, nickel can cause reduced lung function, chronic bronchitis, and even cancer [7]. Chromium does not have many direct hazards reported, although if the chromium is hexavalent it is found to cause nosebleeds, nasal congestion, erosion of the teeth, perforation of the nasal septum, chest pain and bronchitis along with increasing the risk of several cancers such as lung, nasal, and sinus [8, 9]. Iron, copper, and zinc have very similar health effects. All have been shown to cause a flu-like illness that has symptoms such as tight chest, cough, and metallic taste. This is known as metal fume fever [10, 11, and 12]. These potential health risks are all for high concentration exposure, and these were stated because there is very little known about the long-term exposure to low concentration. This research will further advance our understanding of the potential health effects from using e-cigarettes by determining what heavy metals and in what concentrations are produced from vaping e-cigarettes. This information could ultimately allow the consumer of these nicotine products to be adequately educated on the potential consequences of vaping.

There is very little research done on the long-term health effects of e-cigarettes because not enough time has passed for those effects to become apparent. Quantifying these heavy metals in the liquid could further link the long-term side effects of heavy metals to the long-term side effects of e-cigarettes. With new products constantly entering the market, adequate research surrounding these are necessary to ensure the users are well educated on all possible side effects or hazards that are currently unknown. This kind of research is essential to ensure that, as a society, we do not repeat history, since these drug delivery devices mimic cigarettes.

Background

Heating Element Composition

The company JUUL stated on their website that the heating element was made of a mixture of chromium and nickel, better known as nichrome. To verify that, an environmental scanning electron microscope (ESEM) with an Energy Dispersive X-Ray (EDX) was used. The EDX can scan a point of the material and record the composition of the point analyzed. The EDX works by focusing an electron beam on the inner shell of an atom knocking out this electron, and with this electron missing the outer electrons will shift down or relax to replace this missing electron. When this occurs, the atom shifts down an energy level and emits an x-ray that is then analyzed by the EDX. The energy of these x-rays are unique to each element [13].

Two heating elements were scanned using the EDX. One was a new heating element taken directly out of the JUUL pod and the other was a heating element that was used to vape off all of the e-liquid inside a JUUL pod. Once these samples were removed from the JUUL pod, they were rinsed several times with ultrapure water before entering the ESEM. It was then simply put into the It was found that the brand-new heating element from a JUUL pod is made up of approximately 56.3% nickel, 23.3% iron, and 16.2% chromium by weight with the remaining 4.2% containing trace amounts of carbon and silicon. The used heating element was made up of 53.2% nickel, 21.7% iron, and 15.2% chromium by weight with the remaining 9.9% containing trace amounts of carbon, silicon, oxygen, sodium, and aluminum. Both of these trace amounts of various elements are small enough to be ignored and the slight changes from a brand-new heating element to a used heating element results is likely due to the additional oxygen, sodium, and aluminum.

JUUL Modified Design

The JUUL was modified for this study in order to produce a consistent and repeatable activation of the e-cigarette without needing a person to physically vape the device. The final design of the modified JUUL can be seen in **Figure 3**. The piece labeled 1 in **Figure 3** is the pressure sensor. To expose it a small hole was drilled through the aluminum casing. Tubing was attached to this pressure sensor and that tubing led to a peristaltic pump that was pulling a vacuum on this pressure sensor to activate it. This is to imitate the user creating suction with their mouth, which is the normal way of activating the e-cigarette. The piece labeled 2 in **Figure 3** is the airflow intake. First, a small hole was drilled into the side of the aluminum case. This piece was then attached to that hole and this was done to replace to the suction that a user would apply to create the airflow necessary to draw the vapor out of the device. To create this airflow, tubing was put over this attachment and dry air was run through this tubing to mimic the user inhaling the vapor.

To activate this e-cigarette the flowrate was set to 2.4 liters per minute, which is the approximate flowrate for a human when inhaling, and the peristaltic pump was turned on without the tubing being attached to the pressure sensor. The only time that the tubing was attached to the pressure sensor is when the device is ready to be activated. When the e-cigarette is activated, the vapor produced will exit through the black mouthpiece on the top of the device. The e-cigarette was only activated under a well-ventilated hood to ensure the experimenter was not exposed to the vapors.

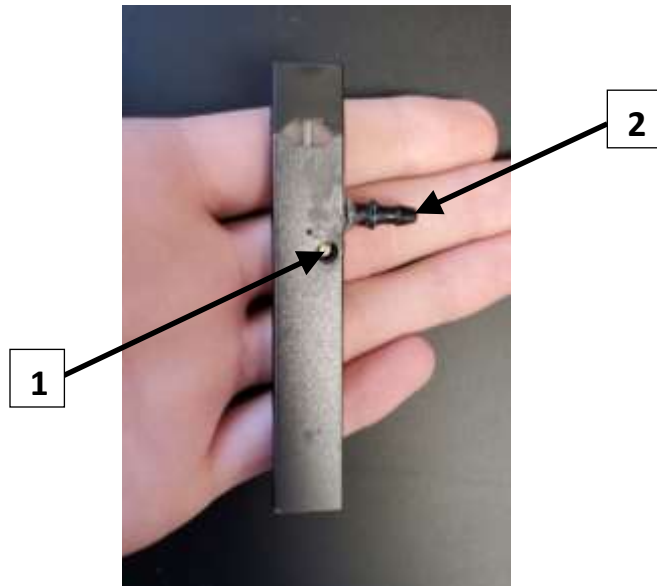


Figure 3. A visual of the two modifications made to the JUUL to enable activation without a user.

ICP-MS

The Inductively Coupled Plasma Mass Spectrometer (ICP-MS) that was used for this study works by the sample liquid being pulled into the nebulizer by the autosampler [14]. This source was used for the rest of the explanation of the ICP-MS. A nebulizer turns the sample liquid into an aerosol that is then mixed with Argon gas. The aerosol then passes through to the plasma ion source, which is at an approximate temperature of 7,000°C. This turns the liquid droplets of the sample into positively charged ions. It then proceeds into the ion lens, which uses a small voltage to deflect the positively charged ions ultimately removing any negative or neutral ions. Kinetic energy discrimination (KED) flows helium gas against the flow of the sample ions. This will cause the polyatomic ions to collide with the helium gas making it unable to pass through to the mass spectrometer. These two processes remove a lot of background noise and is how the ICP-MS system's high sensitivity is achieved. The analyte ions are then analyzed by the mass spectrometer and the recorded intensities are displayed on the computer program. The ICP-MS gave intensities of the blank, standards, and the various samples. Through the standard calibration curve, concentrations were calculated from those intensities. This high sensitivity allows the instrument to measure concentrations as low as its limit of detection (LOD), which with the elements analyzed in this research can be as high as 2 ppt. Therefore, it becomes incredibly important to use ultrapure water and to rinse several times.

Experimental Procedure

Before we analyzed the heavy metals in JUUL's e-liquid, there were several steps that needed to be taken before we were ready to perform the experiment. This consisted of creating a nitric acid rinse, creation of the diluted standard stock, creation of the standards, preparation of the e-liquid, and creation of the samples.

Standard Preparation

The first step to prepare the standards for the experiment was creating 1 liter of Nitric acid rinse. A glass 1-liter volumetric flask was used for this. Since this experiment is analyzing the concentration of heavy metals in a solution as low as 1 ppb, ultrapure water (UPW) was used. This water was measured to have a minimum resistivity of 17.8 M Ω where absolute pure water has a resistivity of 18.2 M Ω . Approximately 100 milliliters of UPW was added to the volumetric flask. 100.0 milliliters of Macron Fine Chemicals' 10% nitric acid were added to the same volumetric flask. The contents were swirled until the contents were thoroughly mixed. The volumetric flask was filled with UPW up to the 1-liter line.

The next step of preparing the standards was rinsing the 50 milliliter centrifuge tubes with the 1% nitric acid rinse that was previously created. 50 and 10 milliliter centrifuge tubes were used throughout this experiment because the ICP-MS autosampler can only hold centrifuge tubes of these two sizes. The 1-liter volumetric flask that contains the 1% nitric acid rinse was overturned several times to assure the contents were mixed thoroughly. Approximately 5 milliliters were added to the centrifuge tubes. The cap was then screwed on and the contents were shaken vigorously. The remaining contents were emptied into a waste container and these steps were repeated three times for each centrifuge tube necessary.

One of the recently rinsed centrifuge tubes was filled with 10.0 milliliters of UPW. 4.0 milliliters of the 10-ppm standard were added to the centrifuge tube. This standard stock purchased from Inorganic

Ventures which contains a mixture of metals of known concentrations. This mixture contains all of the metals that will be focused on in this thesis, which consists of magnesium, chromium, iron, nickel, copper, zinc, and lead. The contents of the centrifuge tube were shaken vigorously before remaining 26.0 milliliters of UPW was added. The contents were once again shaken vigorously. This resulted in a diluted standard stock solution with a concentration of 1 ppm.

The remaining centrifuge tubes were used for the standards. 40 milliliters of UPW were added to the 50 milliliter centrifuge tubes. 4.00 milliliters of UPW were removed then 4.00 milliliters of 10% nitric acid was added. The contents of the centrifuge at this time were shaken vigorously. UPW was removed from the centrifuge tube and the specific volume removed can be found using **Table 1**, shown below. The volume removed for the respective standard being prepared can be found in the row labeled "stock solution volume". Once the UPW was removed the same volume of the 1 ppm standard stock was added to the centrifuge tube. The contents were shaken vigorously to assure the standard created was well mixed.

Table 1. Volumes used to prepare the desired Standards

Standard (ppb)	0	1	10	100	250
Final Volume (mL)	40.00	40.00	40.00	40.00	40.00
Stock Soln. Volume (mL)	0.00	0.04	0.40	4.00	10.00
Nitric Acid 10% (mL)	4.00	4.00	4.00	4.00	4.00
UP Water (mL)	36.00	35.96	35.60	32.00	26.00
% Nitric Acid Soln.	1.00	1.00	1.00	1.00	1.00

Sample Preparation

The first step of preparing the samples was rinsing the 10 and 50 milliliter centrifuge tubes with the 1% nitric acid rinse that was previously created. The 1-liter volumetric flask that contains the 1% nitric acid rinse was overturned several times to assure the contents were mixed thoroughly. Approximately 2 and 5 milliliters were added to the 10 and 50 milliliter centrifuge tubes respectively. The cap was then screwed on and the contents were shaken vigorously. The remaining contents were emptied into a waste bin and these steps were repeated three times for each centrifuge tube necessary.

The next step of preparing samples was to vape approximately a third of the e-liquid's mass. To begin this the mass of the pod and the contents was measured, which on average came out to be between 2.1 and 2.2 grams. Then the pod without any e-liquid in it was measured, which lead to an average around 1.275 grams. These values were subtracted to calculate the e-liquid's mass which is on average around 0.875 grams. Then a third of the mass being vaped was targeted resulting in a mass around 1.850 grams including the mass of the pod.

The preparation of the new and vaped e-liquid is the same, therefore they were done in the same step. All values can be easily seen in **Table 2**, shown below. 0.25 milliliters of the e-liquid were added to a recently rinsed 50 milliliter centrifuge tube. Then 2.00 milliliters of 10% nitric acid were added and the contents were shaken vigorously to assure the e-liquid and nitric acid were thoroughly mixed. This step is critical because if the acid does not have proper contact with the e-liquid then the heavy metals inside will not dissolve or ionize which will result in the ICP-MS not being able to pick up their signal. Once the contents were mixed, 17.75 milliliters of UPW were added to properly dilute the sample. These contents were once again shaken vigorously. The sample was then allowed to sit for a minimum of 24 hours to digest. Finally, approximately 2 milliliters of the sample were filtered into a recently rinsed 10 milliliter centrifuge tube through a 0.45 micrometer syringe filter.

Table 2. Volumes used to prepare all Samples

Flavor (New and Vaped)	Menthol	Virginia Tobacco
Final Volume (mL)	20.00	20.00
E-Liquid Volume (mL)	0.25	0.25
Nitric Acid 10% (mL)	2.00	2.00
UP Water (mL)	17.75	17.75
% Nitric Soln.	1.00	1.00

ICP-MS Conditions

These samples were then given to the ICP-MS with the conditions outlined in **Table 3**, shown below. This instrument then correlated the following intensities for each standard and sample for all of the heavy metals being analyzed.

Table 3. ICP-MS Operating Conditions

Official Instrument Name	Thermo Scientific iCAP RQ ICP-MS
Sample Uptake Time (sec)	60
Wash Time (sec)	20
Wash Solution	1% Nitric Acid Solution
Number of Readings per Scan	1
Number of Scans per Sample	15
Peristaltic Pump Rate (rpm)	ISTD: 20 and Sample: 40
Tubing (mm)	ID: 0.508 and L: 460
Nebulizer Gas Flow Rate (L/min)	1.09
Internal Standards	6Li, 45Sc, 89Y, 159Tb, and 209Bi
Isotopes Scanned	Mg24, Cr52, Fe56, Ni60, Cu65, Zn66, and Pb208
Measurement Mode	KED
Dwell Time (sec)	0.1
Channels	1
Channel Spacing (u)	0.1
Sample Cone	Ni-102737 P/N 3600812, Ni-96127 P/N 1311870
Skimmer Cone	Skimmer Cone Insert 2.8

Results & Discussion

Survey Scan Results

Using the ICP-MS that was described previously in the methods section a survey scan was done analyzing a sample of JUUL's e-liquid. A survey scan analyzes a sample for nearly all metals on the periodic table simultaneously and will produce a rough estimate of its concentration. This survey scan showed that there were significant concentrations of magnesium, chromium, iron, nickel, copper, zinc, and lead. These metals were determined to be the metals further analyzed in this study. These metals are similar to metals found in other e-liquids [15].

Initial Expectations

The concentrations of all metals were expected to increase by 50% when comparing the before and after vaping. This increase was expected because it was believed that the heavy metals would stay behind in the remaining e-liquid as the rest of the e-liquid would be turned into vapor leaving the device. This decrease in volume while the mass stayed constant would result in that 50% increase. This was not seen in the results and will be discussed in further detail in the before and after vaping results section. An increase in concentration of metal regardless of the flavor was seen for chromium, nickel, and iron. For this reason, it was believed that the heating element was acting as the source of this increase.

Before and After Vaping Results

Table 4 shows the concentrations with standard error for both flavors before and after vaping, along with p-values to show statistical significance. P-values show the probability that the averaged concentrations are actually the same. For example, if the p-value is 0.002, then that means that there is a 0.2% chance that those averages are statistically insignificant. **Figure 4-9** below show each of the metal concentrations for both flavors before and after vaping. The figure for lead is not shown below because all of the concentrations were below the limit of detection (LOD) for the ICP-MS. Magnesium was shown to significantly increase in concentration after vaping for Menthol by increasing by 9% with a p-value of <0.0001, but with Virginia Tobacco it showed the opposite although it was a small difference of 1% with a p-value of 0.0092. These concentrations can be seen in further detail in **Table 4** and **Figure 4**. In **Table 4** and **Figure 5**, which displays the concentrations of chromium before and after vaping. For Menthol flavor an increase of 4% was observed with a p-value of 0.0014, and for Virginia Tobacco an increase of 5% was observed with a p-value of 0.0002. Iron was shown to increase in concentration after vaping for Menthol with an increase of 17% with a p-value of 0.2246, while the Virginia Tobacco flavor showed an 8% increase with a p-value of 0.4847. This can be seen in further detail in **Table 4** and **Figure 6**. In **Table 4** and **Figure 7**, which displays the concentration of nickel before and after vaping. For Menthol an increase of 267% was observed with a p-value of 0.1352, while the Virginia Tobacco an increase of 80% was observed with a p-value of <0.0001. Copper was shown to have a decrease in concentration for Menthol of 53% with a p-value of 0.1350, while Virginia Tobacco decreased by 89% with a p-value of 0.0151. This can be seen in further detail in **Table 4** and **Figure 8**. Zinc showed an increase in concentration after vaping for Menthol with an increase of 87% with a p-value of 0.0404, but Virginia Tobacco showed a decrease of 50% with a p-value of 0.0915. This can be seen in further detail in **Table 4** and **Figure 9**. The change in concentration of chromium, nickel, and iron is likely because the heating element used in JUUL's device has chromium, nickel, and iron in it. The heating element is raised to a temperature range of 200 to 250 °C which potentially encourages the leeching of the metal [5].

An increase in concentration shows that there must be a source emitting this metal into the e-liquid and it is now unlikely that the metal would stay inside of the pod as the e-liquid is vaped off because if the metal preferentially stays in the e-liquid then it would result in a 50% increase in concentration since a third of the e-liquid's mass was vaped off. A decrease in concentration is believed to show that the metal preferentially leaves the e-liquid. It was confirmed that the metal was not plating on the heating element, this was done by comparing the heating element of a new JUUL pod and a completely vaped one. As previously discussed, an EDX in an ESEM was used to analyze the elemental components of each and the results showed no addition of magnesium, copper, zinc, and lead. All Virginia Tobacco and Menthol samples discussed in the before and after vaping section have lot numbers JG23SA10A and JG31NA16A respectively.

Table 4. Concentrations of Heavy Metals in ppb before and after vaping

	Menthol Before Vaping	Menthol After Vaping	Menthol P-values	Virginia Before Vaping	Virginia After Vaping	Virginia P-values
Magnesium - 24	2,672 ± 1	2,914 ± 7	<0.0001	2,064 ± 1	2,047 ± 6	0.0092
Chromium - 52	122 ± 1	127 ± 1	0.0014	122 ± 1	128 ± 1	0.0002
Iron - 56	468 ± 15	546 ± 61	0.2246	523 ± 52	567 ± 34	0.4847
Nickel - 60	45 ± 1	165 ± 78	0.1352	54 ± 1	97 ± 2	<0.0001
Copper - 65	32 ± 11	15 ± 1	0.1350	38 ± 13	4 ± 2	0.0151
Zinc - 66	4,756 ± 211	8,914 ± 1923	0.0404	9,690 ± 2526	4,811 ± 1189	0.0915
Lead - 208	Below LOD	Below LOD	N/A	Below LOD	Below LOD	N/A

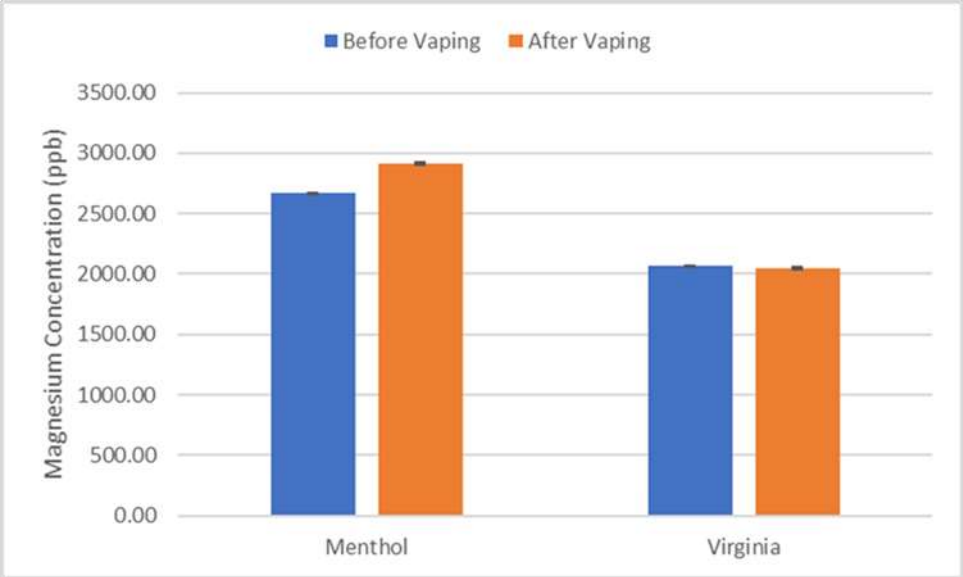


Figure 4. Magnesium shows an increase in concentration after vaping for Menthol by increasing by 9%, but with Virginia Tobacco it showed a small decrease of 1%.

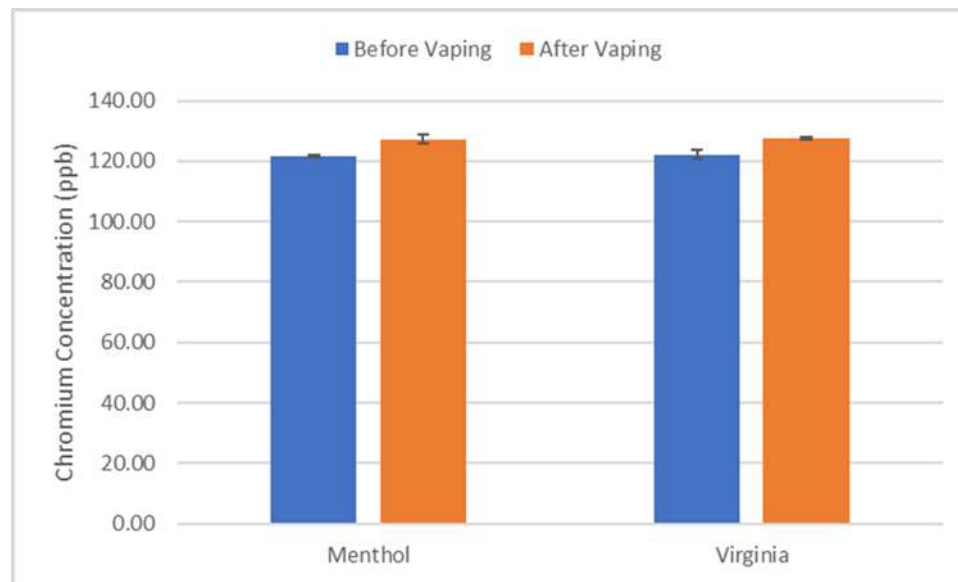


Figure 5. Chromium shows an increase in concentration before and after vaping with the largest being a 5% increase.

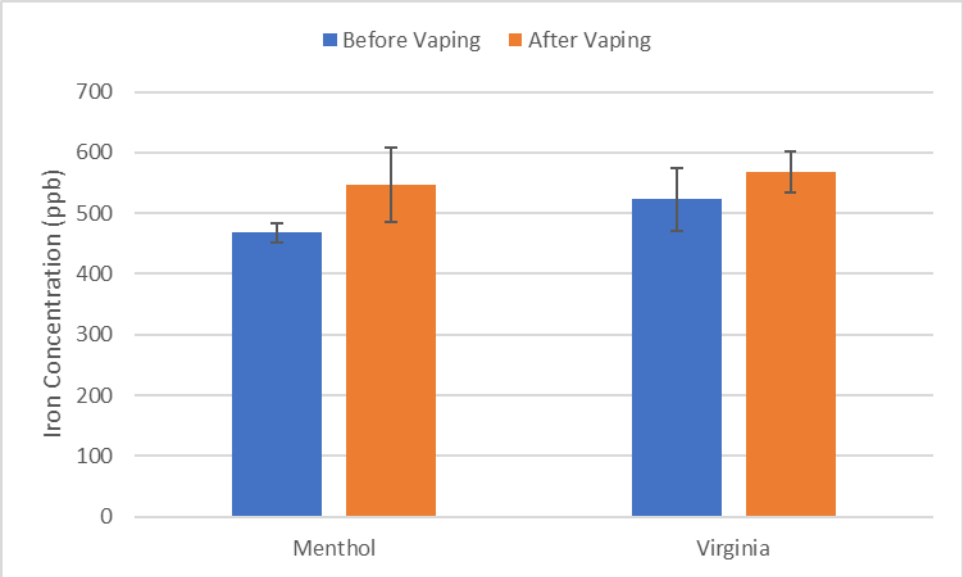


Figure 6. Iron shows an increase in concentration after vaping for Menthol with a small difference of 17%, while the Virginia Tobacco flavor did not show a statistically significant difference.

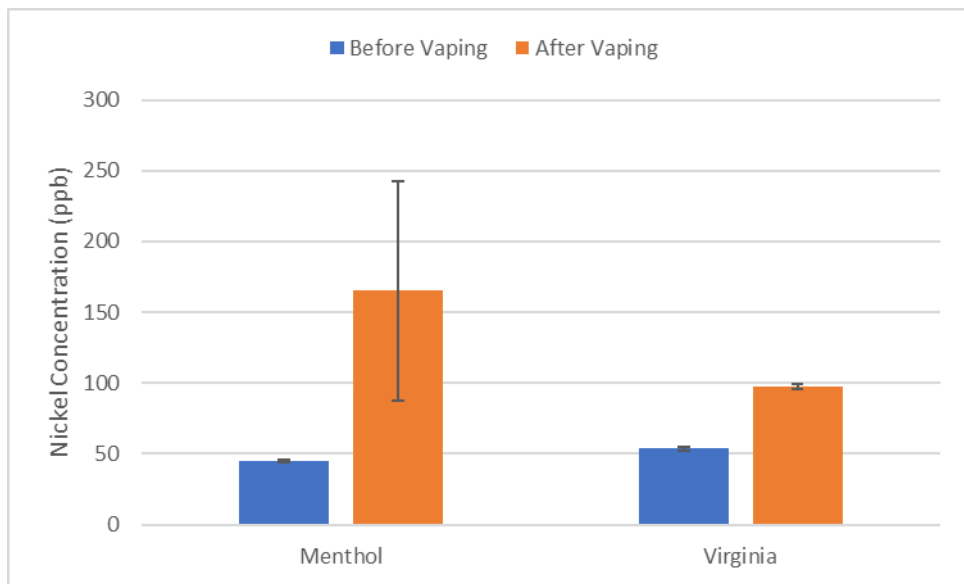


Figure 7. Nickel shows an increase in concentration before and after vaping with the largest being a 269% increase.

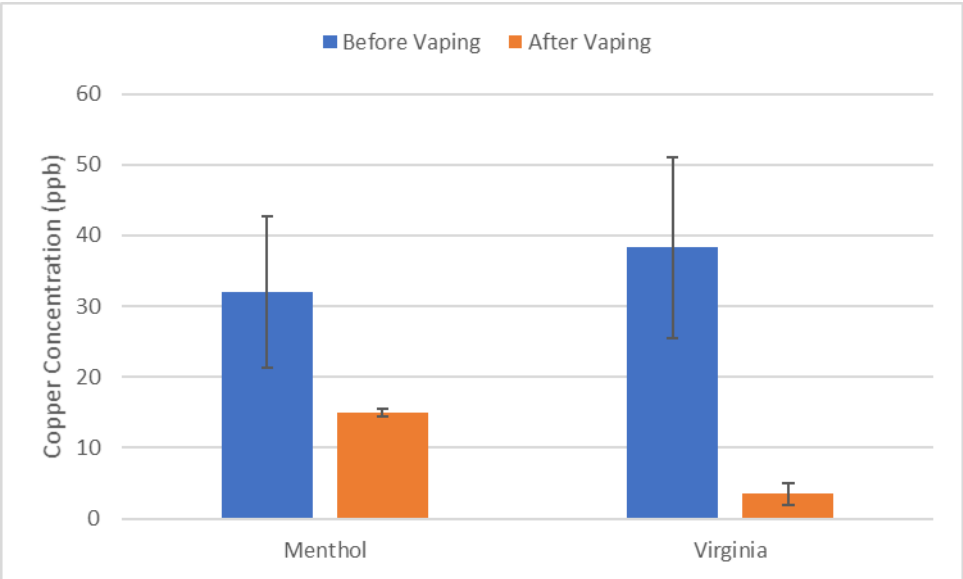


Figure 8. Copper shows a decrease in concentration after vaping for Menthol and Virginia Tobacco of 53% and 91% respectively.

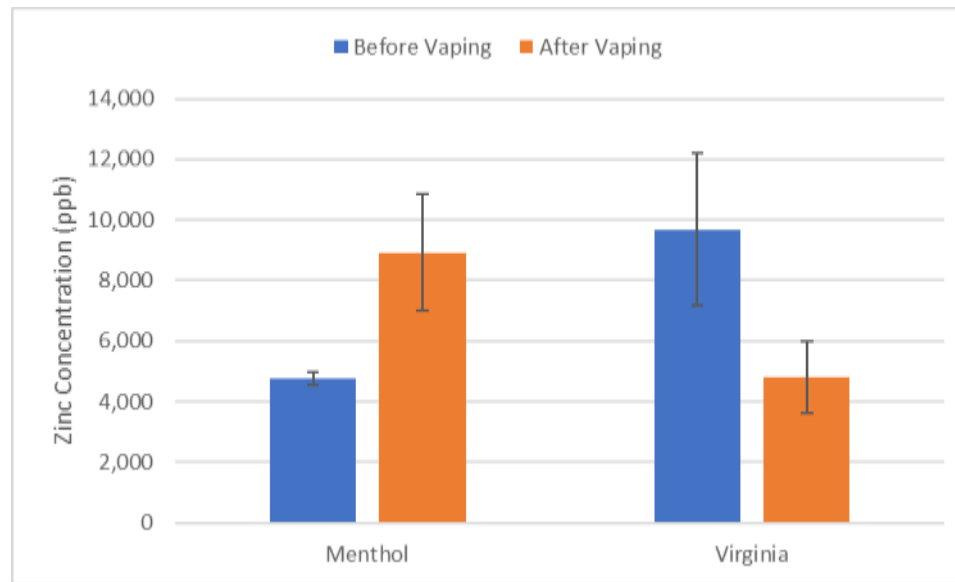


Figure 9. Zinc shows an increase in concentration after vaping for Menthol with an increase of 87%, but Virginia Tobacco shows a decrease in concentration of 50%.

Quality Control Change Discovery

After some preliminary analysis of the e-liquid, it was found that the sample had large amounts of metals in both Menthol and Virginia Tobacco flavors, which were from lot numbers HE10PA09A and HE07PA09A, respectively. These samples were bought around the experimental run date, which was December 09, 2019. Later another analysis of the e-liquid resulted in different concentrations for both Menthol and Virginia Tobacco, which were from lot numbers JG31NA16A and JG23SA10A, respectively. These samples were also bought around their experimental run date, which was November 04, 2020. The digestion protocol changed slightly by changing the nitric acid from Alfa Aesar's 65-70% to Macron Fine Chemicals' 10% and the only thing this changed from the method described previously is the dilution factor. This likely is not what changed the concentrations because the pH for 65-70% nitric acid is -1.20 and for the 10% nitric acid is -0.38. The pH for both nitric acids will be sufficient to dissolve metal into solution. To further support this there have been other research articles using 10% nitric acid to dissolve lead in other e-liquids [16]. This likely means that it is the difference in lot numbers resulting in the change of concentration, which also makes sense because it is around the time that JUUL was having significant quality control issues that were repeatedly documented by the media. For example, The New York Times published an article that talks about how one million contaminated mint flavored JUUL pods were knowingly sold and not recalled [17]. The issue with using this previous data was that the experiment was not run in a way to decrease the results uncertainty because the method was still being perfected. This led to only three data points being measured and as previously discussed later experiments discovered that the first 5 data points were variable. They were variable because the uptake of the ICP-MS was slower than initially thought. Although in the comparison that will be made it does not affect the data significantly because if the data were to change with more samples it would likely increase. It would likely increase because more of the sample would reach the ICP-MS before it began its analysis. This low sample size and variability is also reflected in the standard error bars on each figure as well as shown in the table below.

Early and Late Lot Number Results

Table 5 is displayed below to show exact concentrations with standard error of both flavors before vaping for both experiments along with their corresponding p-values. **Figures 10-16** shown below display each of the metal concentrations for both flavors before vaping for both experiments. This data shows that the concentration of magnesium, iron, nickel, copper, zinc, and lead for the experiment on November 04, 2020 being lower than the experiment run on December 09, 2019 is statistically significant. Magnesium was shown to have a decrease in concentration for Menthol of 56% with a p-value of <0.0001, while Virginia Tobacco decreased by 41% with a p-value of <0.0001. This is displayed specifically in **Table 5** and **Figure 10**. While all the other concentrations decreased significantly, for unknown reasons the concentration of chromium for Menthol with an increase of 1120% with a p-value of <0.0001, while Virginia Tobacco was showing a decrease in concentration from below LOD to 122 ppb, which is displayed in **Table 5** and **Figure 11**. In **Table 5** and **Figures 12**, iron was shown to have a decrease in concentration for Menthol of 34% with a p-value of 0.0001, while Virginia Tobacco showed a 21% decrease with a p-value of 0.2807. Nickel was shown to have a decrease in concentration for Menthol of 54% with a p-value of <0.0001, while Virginia Tobacco showed a 45% decrease with a p-value of <0.0001. This is displayed specifically in **Table 5** and **Figures 13**. In **Table 5** and **Figures 14**, copper was shown to have a decrease in concentration for Menthol of 91% with a p-value of <0.0001, while Virginia Tobacco showed an 84% decrease with a p-value of <0.0001. Zinc was shown to have a decrease in concentration for Menthol of 94% with a p-value of <0.0001, while Virginia Tobacco showed a 95% decrease with a p-value of <0.0001. This is displayed specifically in **Table 5** and **Figures 15**. In **Table 5** and **Figures 16**, lead was shown to have a decrease in concentration for Menthol of ~100%, while Virginia Tobacco also showed a ~100% decrease. This is likely because lead was the most criticized heavy metal they had and dropped below LOD.

	Menthol Early Lot	Menthol Late Lot	Menthol P-values	Virginia Early Lot	Virginia Late Lot	Virginia P-values
Magnesium - 24	6,066 ± 682	2,672 ± 1	<0.0001	3,491 ± 470	2,064 ± 1	<0.0001
Chromium - 52	10 ± 6	122 ± 1	<0.0001	Below LOD	122 ± 1	N/A
Iron - 56	706 ± 76	468 ± 15	0.0001	661 ± 85	523 ± 52	0.2807
Nickel - 60	97 ± 8	45 ± 1	<0.0001	98 ± 9	54 ± 1	<0.0001
Copper - 65	339 ± 40	32 ± 11	<0.0001	237 ± 40	38 ± 13	<0.0001
Zinc - 66	79,098 ± 2,282	4,756 ± 211	<0.0001	195,390 ± 2,768	9,690 ± 2526	<0.0001
Lead - 208	76 ± 7	Below LOD	N/A	66 ± 2	Below LOD	N/A

periments before vaping

Table 5. Concentrations of Heavy Metals in ppb of both experiments

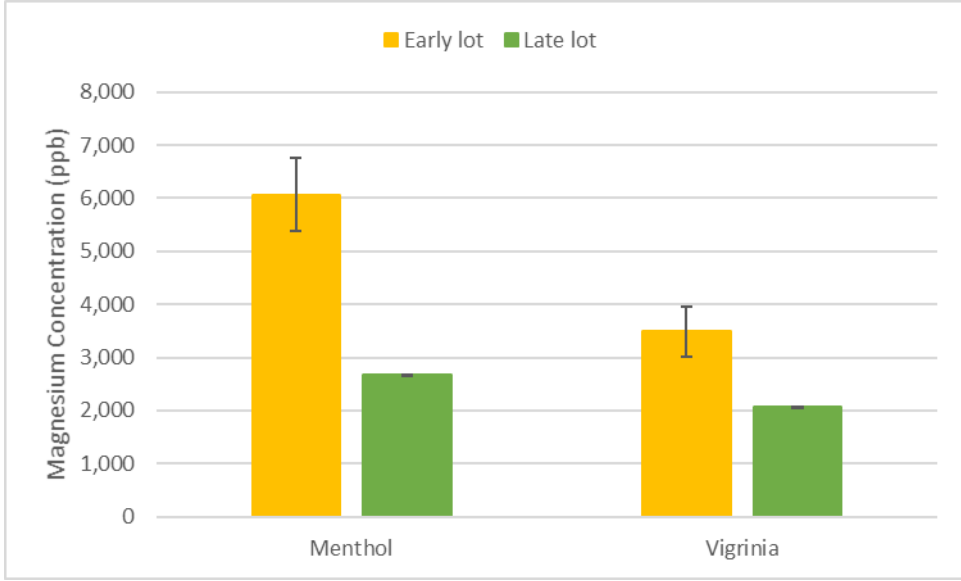


Figure 10. Magnesium shows a decrease in concentration with the largest being a 56% decrease.

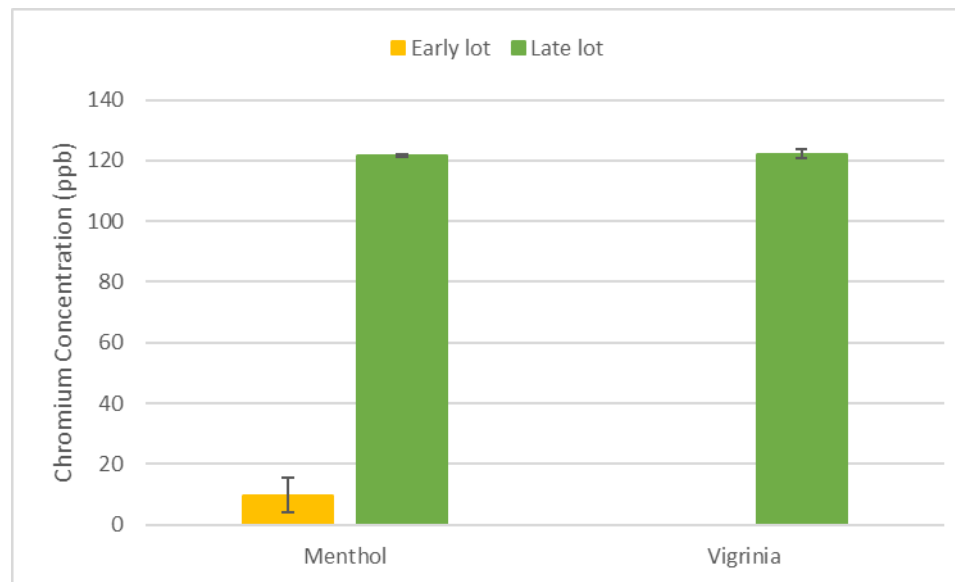


Figure 11. Chromium shows an increase in concentration with the largest increase going from below LOD to 122 ppb.

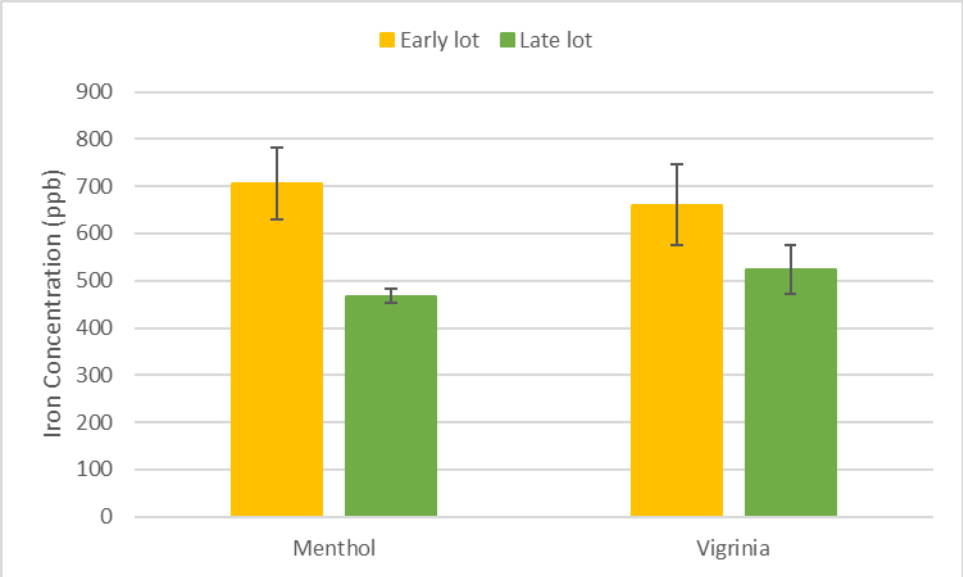


Figure 12. Iron shows a decrease in concentration with the largest being a 34% decrease.

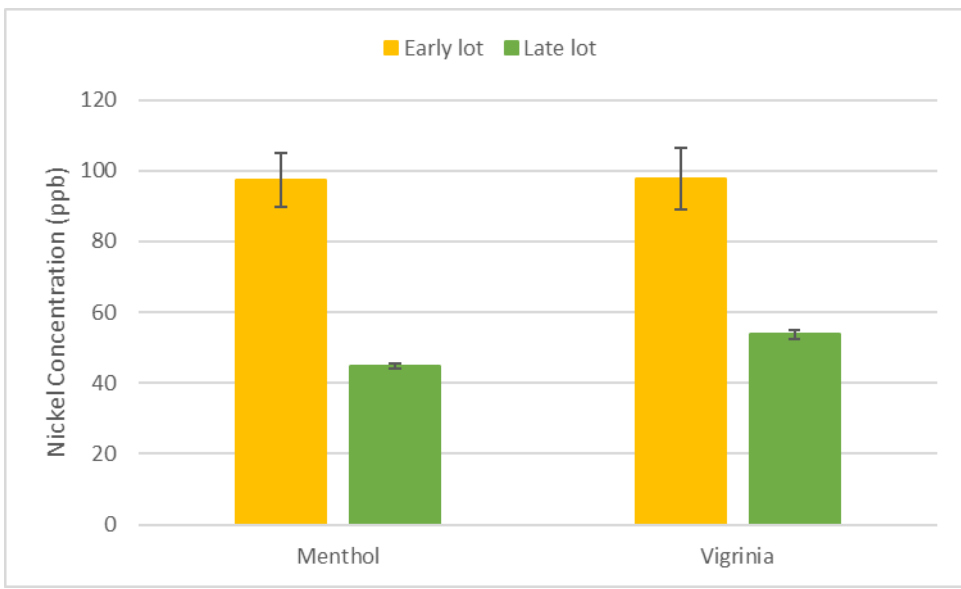


Figure 13. Nickel shows a decrease in concentration with the largest being a 54% decrease.

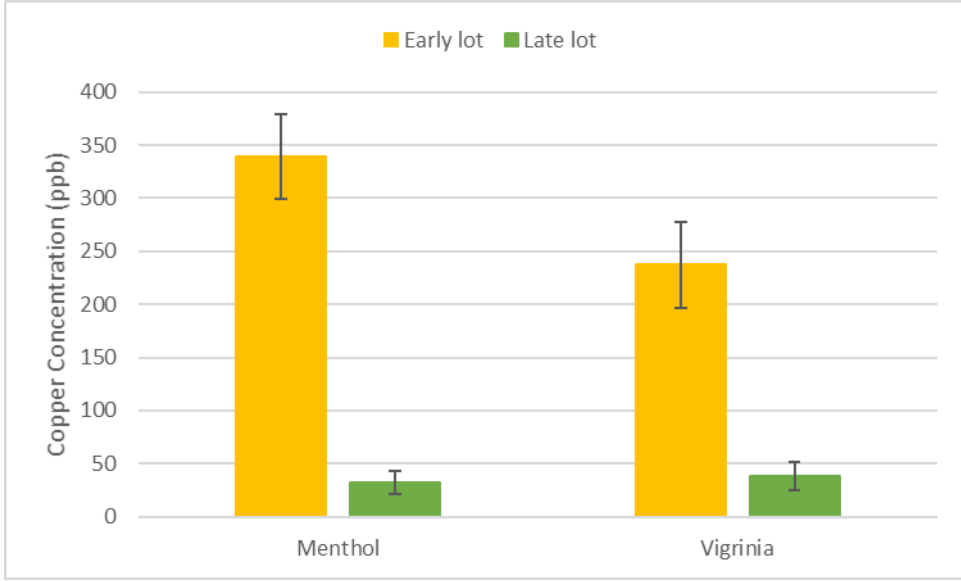


Figure 14. Copper shows a decrease in concentration with the largest being a 91% decrease.

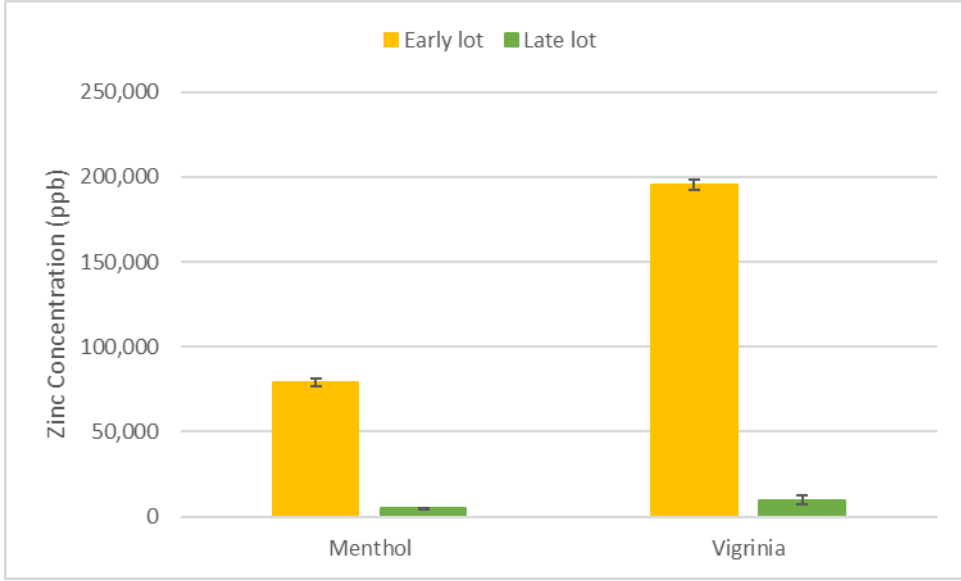


Figure 15. Zinc shows a decrease in concentration with the largest being a 95% decrease.

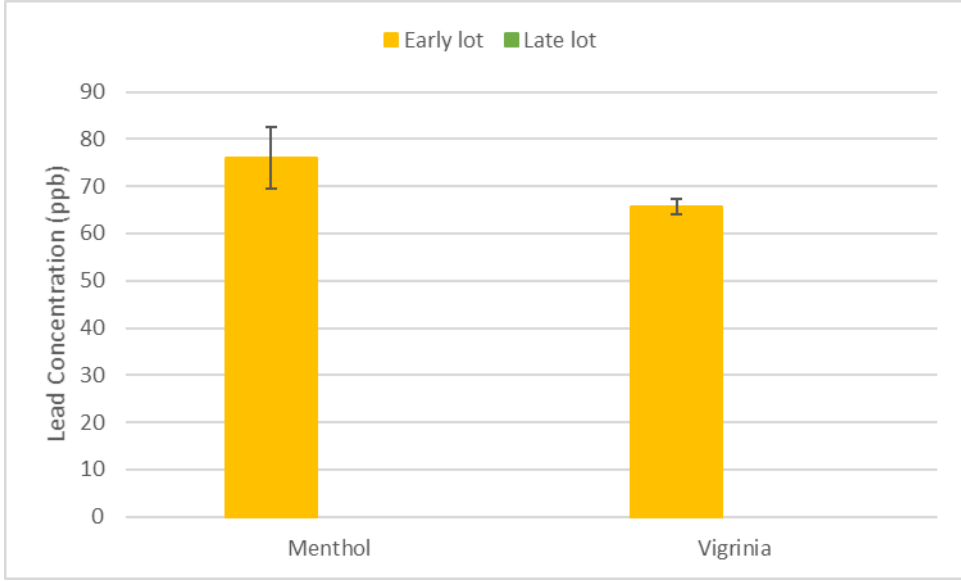


Figure 16. Lead shows a decrease in concentration with the largest decrease going from 76 ppb to below LOD

Conclusions

This study set out to determine what heavy metals were in JUUL's e-liquid as well as determining if using this device altered the amount of the heavy metals in the e-liquid. Through the use of the ICP-MS, we were able to conclude that there are heavy metals, more specifically, magnesium, chromium, iron, nickel, copper, and zinc inside JUUL's e-liquid. We were also able to show that the use of JUUL's e-cigarette significantly increased the amount of chromium and nickel in the e-liquid. This is because the heating element that the e-cigarette uses contains both chromium and nickel which are presumed to have leached from the metal into the liquid being vaped. While analyzing the e-liquid, there was a sudden change in the amount of heavy metals between e-liquid batches purchased almost one year apart. This change occurred shortly after a surge in quality control issues came to light through the media and after Altria bought 30% of JUUL's stock. Although it is promising to see such a large reduction in the amount of heavy metals, it should not be mistakenly understood as suggesting e-cigarettes are safe. There are still heavy metals inside of JUUL's products and heavy metals can easily accumulate in the body, and with no government oversight of e-liquid production, there is no guarantee from batch to batch or year to year what the exact chemical content of an e-liquid might be. This is also not mentioning any of the other major health concerns that come with the use of e-cigarettes. The results of this research is the first step in answering some of the bigger questions about heavy metals and e-cigarettes.

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