Electronic Cigarettes: Using Gas Chromatography Mass Spectrometry Analysis on Nicotine Free Electronic Cigarette Liquids

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Abstract

Background and Purpose: Electronic cigarettes are gaining vast popularity because the perceived impression about electronic cigarettes is they are a safer alternative to conventional smoking (Belluz, 2015). As a result, more teenagers are switching to electronic cigarettes either as a smoking cessation tool, or for recreational use. However, it is supported by the evidence review that there is nicotine mislabeling between what the manufacturer has labeled and the actual nicotine content in the liquids (Goniewicz et al., 2012). This is a critical health concern for teenagers and recreational users because they are exposed to nicotine, which is a neurotoxin that creates the addiction for smoking. As a result, over a period of time, recreational electronic cigarette users have a higher chance of switching to conventional smoking (Bach, 2015). Hence, the purpose of this research is to determine whether nicotine can be found in nicotine free electronic cigarette liquids

Methods: The nicotine content in the electronic cigarette liquids will be determined using Gas Chromatography - Mass Spectrometry. Inferential statistics such as a one tailed t-test will be done using Microsoft Excel and SAS to see if nicotine can be detected in nicotine-free electronic cigarette liquids and if there is a statistically significant difference.

Results: The two p-values from the parametric test were 0.2811 and 0.2953. The p-value to reject the null hypothesis was set at 0.05. Because the p-values from the inferential statistics were greater than 0.05, the null hypothesis was not rejected and the actual nicotine content is equal to what the manufacturer had labeled as nicotine free.

Discussion: Although the inferential statistics indicated that there was no statistical significance in nicotine concentration, two out of the ten nicotine free electronic cigarette liquids measured nicotine levels above 0 ppm.

Conclusion: There was not a significant difference in nicotine concentration found in the electronic cigarette liquids and the actual nicotine concentration is equal to the labeled concentration. However, because the sample size of only ten is too small, there is a potential for type 2 error. Also, the samples came from only two manufacturers. Therefore, the results from this research are not representative for all the electronic cigarette liquids. More research should be conducted to provide scientific evidence to stop recreational electronic cigarette users from the exposure of electronic cigarettes as these could act as a stepping-stone towards smoking conventional cigarettes. Teenagers who start smoking at an early age will be more probable to long term health effects such as cardiovascular diseases, and lung cancer (CDC, 2015).

Keywords: Electronic Cigarettes, Conventional Smoking, Nicotine, Gas Chromatography Mass Spectrometry, Nicotine-Free electronic cigarette liquids

Introduction

General Information

Electronic cigarettes are nicotine delivery devices, which are designed for smoking cessation. Instead of burning tobacco, a user draws air through the device and activates the atomizer to produce an aerosol (Electronic Cigarette, n.d.). Marketers claim that electronic cigarettes are safer than conventional cigarettes because the electronic cigarette does not burn tobacco and produce the chemicals found in conventional cigarette (Belluz, 2015). However, there are carcinogenic compounds such as formaldehydes as well as other heavy metals such as nickel and cadmium found in the aerosol generated by the electronic cigarette (Farsalinos et al., 2015). Studies have also shown that although propylene glycol, a carrier solvent in electronic cigarettes, is not a carcinogen, the oxidation of propylene glycol by the atomizer in an electronic cigarette creates carcinogenic byproducts such as formaldehyde (Cheng, 2014).

Decline in Tobacco Smoke

Ever since the *Tobacco Control Act* was introduced in 1997, there has been a decline in the tobacco use population. For example, Health Canada (2012) estimated that there is a 7% decline in tobacco users for Canadians who are aged 15 years and older. The underlying reason why there is a decline in the smoking population which consists of Canadians who are 15 years and older is because the *Tobacco Control Act* restricts advertisement of tobacco products as well as prohibit the use of tobacco in schools (Tobacco Control Act, 2015).

Despite that there is a decline in tobacco use, the introduction of electronic cigarettes which is portrayed as a healthier alternative has attracted many people for recreational use. In fact, in the United States, electronic cigarette use has tripled among middle and high school students within one year (CDC, 2013). This increase in electronic cigarette use amongst teenagers suggests that they might view that the use of electronic cigarette is acceptable in the social norm. In addition, youth who tried e-cigarettes will be more inclined to try

regular tobacco cigarettes the following year (Hynes, 2014). As a result, Dr. Tom Frieden stated that the increase of electronic cigarette uses "[can] re-glamorize smoking." (Partnership for Drug Free Kids, 2014, p. 1).

Teenagers and recreational users who are exposed to electronic cigarettes are more inclined to try conventional cigarettes because the electronic cigarette "juice" may contain nicotine. Nicotine is one of the main chemicals that cause addiction because the brain sends out signal of craving when the concentration of nicotine in the bloodstream is low (Plito, 2015). And, addiction is more severe for teenagers because their brain is still developing. Teenagers who are exposed to nicotine at a early stage are more likely to become daily smokers by the time they are in adulthood (Bach, 2015).

Motive for this study

As of 2015, the *Tobacco Control Act* in Canada does not have electronic cigarettes under the definition section (Tobacco Act, 2015). As a result, Environmental Health Officers (EHOs) do not enforce any legislation with regards to electronic cigarette's.

However, there has been some recent legislative changes with regards to electronic cigarettes. For example, Chen (2013) compiled some data from the FDA which demonstrated that electronic cigarette users were showing minor adverse human effects such as dizziness, nausea and shortness of breath and severe adverse effects such as second degree burns, lost of vision and disorientation. And, in Spring 2015, the government introduced the *Tobacco Control Amendment Act* which amends the *Tobacco Control Act* to control the sale of electronic cigarette's to minors (British Columbia, 2015).

Although electronic cigarette manufacturers claim that the use of electronic cigarettes is a safer alternative than conventional cigarette, there are some critical information gaps, which fails to prove electronic, cigarettes are completely safe to use. For instance, Goniewicz's et al. (2012) determined that the nicotine content in 9 out of 20 nicotine cartridges sampled differed by 20% from what the manufacturer had labeled. Also, Farsalinos et al. (2015) concluded that there is a -15% to 21% discrepancy in nicotine concentration.

This is an important issue for people who may want to try electronic cigarette recreationally because they can be potentially be exposed to inaccurate nicotine concentration mislabeling in the cartridges. The purpose of this research project is to determine if there is a discrepancy between what the manufacturer labeled in the nicotine-free electronic cigarette liquids and the actual nicotine content in a sample of liquids purchased locally. This will be done through chemical analysis using Gas Chromatography Mass Spectrometry (GC-MS). If there is a discrepancy in what the manufacturer labeled, it can support the need for better regulation of electronic cigarettes at the federal and provincial level.

Evidence Review

Nicotine Mislabelling

Goniewicz et al (2012) did an extensive study that determined the nicotine content in the refillable liquid for electronic cigarette. The level of nicotine in aerosol released from the electronic cigarette varied from 0.3 to 8.7 mg per 150 puffs and 0.5 to 15.4 mg per 300 puffs. It is important to note that some of the products vary in efficiency to aerosolize nicotine in the cartridges. For instance, most of Goniewicz's et al. (2012) samples were able to deliver the same amount of nicotine consistently per 300 puffs. However, one of the products was ineffective in nicotine vaporization, delivering only 31% of the nicotine present in the cartridge. The liquid cartridges were also tested and found that 9 out of the analyzed cartridges had nicotine content differing by 20% from values than what the manufacturer had labeled. Trehy et al. (2011) analyzed cartridges produced by manufacturers such as NJOY and CIXI and found nicotine content in cartridges varied significantly than what the manufacturer had labeled. This suggested that nicotine impurities were present in the refillable liquid. Nicotine mislabeling was also an issue when Hutzler et al. (2014) analyzed 28 different brands, which 10 of those brands were declared nicotine-free, from a German retailer. Results from the analysis indicated that sevenout of the ten brands were identified to have nicotine content from 0.1 – 15 ug/ml. The other eighteen brands, which did not declare nicotine-free, had nicotine levels

between 0.1- 324 ug/ml (Hutzler et al. 2014).

Carbonyl Compound Testing

Most of the refillable electronic cigarettes liquid contains carrier solvents such as propylene glycol and glycerin. When these solvents undergo thermal decomposition due to heating by the battery, carcinogenic chemicals such as formaldehyde and acetaldehyde are formed. The amount of carcinogens produced depends on the battery voltage. For instance, Jensen et al. (2015) found that at 3.0V, levels of formaldehyde-releasing agent were minimal. However, at a higher voltage such as 5.0, a mean of 380 ug per sample of formaldehyde was detected. Kosmider et al., (2014) did a similar study and found that highest levels of carbonyl generated were the refillable solutions using propylene glycol solvents. As the battery voltage increased from 3.2 V to 4.8V, there was a 200-time increase in formaldehyde and acetaldehyde and acetone levels. These carbonyl levels were comparable to carbonyl compounds emitted by conventional cigarettes. Bekki et al., (2014) measured carbonyl compounds in e-cigarettes aerosol using cartridges spiked with hydroquinone and 2-4-dinitrophenylhydrazine followed by High Performance Liquid Chromatography (HPLC). Thirteen Japanese brands were tested and four out of the thirteen brands did not generate any carbonyl compounds. However, the remaining nine brands generated a variety of carbonyl concentrations. The maximum formaldehyde concentration from these nine brands was 140 ug per 10 puffs. Other brands of refillable liquids were also tested for comparison. Varlet et al. (2015) analyzed refillable liquid brands in Canada found formaldehyde concentration in the refill liquids ranged from 0.1 to 9.0 ug/g.

Several conclusions can be drawn from this measurement. First, using propylene glycol as a carrier solvent generates the most carbonyl compounds (Bekki et al., 2014). Secondly, there should be an ideal voltage where emission of carbonyl compounds is at its minimum (Bekki et al., 2014). Also, it is hard to compare what the actual carbonyl concentration is because each researcher uses a different measurement unit. For example, Varlet et al., (2015) measured formaldehyde concentration in units of ug/g whereas

Bekki et al., (2014) used ug per 10 puffs. The adverse health effects which carbonyl compounds such as formaldehyde can create are irritation and burning sensation in eyes. At larger concentration, formaldehyde can cause cancer in the nasal cavity (Health Canada, 2012).

Heavy Metals analysis

Goniewicz et al. (2014) used Induced Coupled Plasma - Mass Spectrometry (ICP-MS) to analyze heavy metals such as cadmium, lead and mercury in the refillable liquids. In their study, all of the liquids except for one were tested positive for cadmium. The concentration range for cadmium is between 0.01 to 0.22 ug per 150 puffs. Nickel and lead were found in all of the 12 products and the concentration range is 0.11 to 0.29 ug and 0.03 to 0.57 ug per 150 puffs respectively (Goniewicz et al., 2014). Williams et al. (2013) conducted a more extensive research on the presence of nanoparticles in electronic cigarette fluid. The main objective of the research was to compare the nanoparticles found in electronic cigarette to nanoparticles found in tobacco smoke. The unit of measurement was described as concentration of the inorganic compound per 10 puffs of electronic cigarette aerosol. Interestingly, metals such as sodium, iron, aluminum and nickel were found to be higher than conventional smoking (Williams et al., 2013).

Toxicity studies about heavy metals have been well documented under Health Canada. Cadmium is listed as a carcinogen under the National Institute for Occupational Health and Safety (CDC, 2015). The US EPA has an oral reference dose of 0.5 ug/kg bw/day for cadmium (Health Canada, 2012). Other heavy metals such as sodium, iron, aluminum and nickel also have adverse health effects. Intake of high sodium, iron, aluminum and nickel amounts can cause respiratory illness such as shortness of breath and lung irritation (Williams et al., 2013).

Tobacco Specific Nitrosamines

Tobacco Specific Nitrosamines (TSNA) is one of the compounds, which is mostly associated with negative health effects in tobacco smoke. A component of Farsalinos, & Gillman et al. (2015) study examined TSNA in the refillable liquid samples. Results showed that

TSNA is present in ng/ml, which are 2-3 orders of magnitude lower than the TSNA that is found in tobacco smoke. Hence, from this analysis, electronic cigarettes are a safer alternative than conventional smoking because the level of TSNA that is produced by electronic cigarettes are minimal compared to conventional smoking.

Strengths and Limitations

Overall, these results provide insight on how electronic cigarette's can be harmful. All of the studies confirm that there are carcinogens and heavy metals present in the aerosol. However, there is insufficient information how these carcinogens may react in the body by inhaling these vapors. In addition, there is no uniformity in methods to obtain the data because each researcher uses their own method, resulting in a discrepancy in results. Most of the sampled liquids are from other parts of the world such as Germany or Japan. More refillable liquid research should be emphasized in the US or Canadian market. The effect of carrier solvents, flavor ingredients and aerosol generation products should be studied more in depth as their chemical compositions contain allergens or carcinogens. Electronic cigarette devices have different working voltages. The researcher could be using different voltages to generate these aerosols, which make the data biased. Analytical methods to obtain the data and sample preparation are different for all researchers. Thus, the data collected may not be compared between each researcher, as the data may be different in detection limit, resolution, and confidence intervals. Lastly, the researcher may have a conflict of interest because the electronic cigarette's manufacturer often funds some of the literature studies.

Conclusions from the Evidence Review

Farsalinos & Gillman et al., (2015) discovered that TSNA in the refillable liquid is far less than TSNA in tobacco smoke. However, carcinogenic compounds and heavy metals are still found in the refillable liquids. Nicotine content labeling maybe accurate for some brands; however, some brands which claim to be nicotine-free may contain nicotine impurities. There is a lack of research on carrier solvents because propylene glycol is the most susceptible compound to undergo

thermal decomposition to produce carcinogenic compounds such as formaldehyde and acetaldehyde (Bekki et al., 2014). Kosmider et al. (2014) suggested that using polyethylene glycol as a carrier solvent as this solvent may reduce toxicity and decomposition of products. Overall, this evidence review provides information about the chemical composition in electronic cigarette's and how the performance of electronic cigarette's can increase carbonyl generation such as formaldehyde which is classified as a carcinogen (Health Canada, 2012). The objective of this research is to determine if there is a discrepancy in nicotine concentration between what the manufacturer labeled and the actual nicotine concentration by chemical analysis a sample collected locally in BC. If there is a nicotine mislabeling issue, this would support the need to include electronic cigarette's in the *Tobacco Control Act*. Furthermore, there would be a need to reduce access of electronic cigarette's to youth because there is a potential that exposure to small amounts of nicotine can create an addiction to cigarettes. Having electronic cigarettes defined as apart of the *Tobacco Control Act* would allow the designated Tobacco Enforcement Officer to enforce the legislation.

Methodology

The focus of this research was to determine whether there was nicotine in nicotine free electronic cigarette liquids through chemical analysis by Gas Chromatography Mass Spectrometry (GCMS). Two hypothesis statements, null and hypothesis were used to describe the experiment. The null hypothesis stated the mean concentration of nicotine in nicotine free electronic cigarette liquids is 0 ppm. Whereas, the alternative hypothesis stated the mean concentration of nicotine in nicotine free electronic cigarette liquids is greater than 0 ppm.

Materials Used

The following equipment was necessary for data collection. Ten electronic cigarette liquids were purchased with a variety of flavours such as methanol, apple and peach. Gas Chromatography Mass Spectrometry (GCMS) is an instrument to analyze all the chemical components in the electronic cigarette liquids. The results from the GCMS

were displayed on the computer with the appropriate software. SAS and Microsoft excel software programs were used for inferential statistics analysis. In terms of sample preparation, methanol was used to dilute the electronic cigarette liquids into a range which GCMS can detect. And, quinoline, a chemical compound, was used as an internal standard for chemical analysis purposes.

Step by step method

Electronic cigarette nicotine free liquids were purchased on the week of January 25, 2016. Kevin Soulsbury performed the pilot study in the week of Feb 1, 2016 by testing one of the nicotine containing electronic cigarette liquids by using Gas Chromatography Mass Spectrometry (GCMS) to ensure that the nicotine retention peak showed up on the chromatogram. After analyzing the results from the chromatogram, Kevin Soulsbury determined that all parameters were working properly to conduct the experiment. Sample preparation consisted of preparing nicotine stock solution, quinoline stock solution and the actual electronic cigarette liquid samples. A measured amount of 0.0521g of nicotine from the stock was dissolved with 50ml of methanol in a volumetric flask to create a 1000mg/L concentrated standard solution. Also, a measured amount of 0.066g of quinoline from the stock was dissolved with 50ml of methanol in a volumetric flask to create a 1000mg/L concentrated standard solution. For the purpose of generating a five-point calibration curve, different volumes of the nicotine stock solution were pipetted out to create 10 ppm, 25ppm, 50ppm, 75ppm and 100ppm nicotine concentration solutions. A fixed amount of 0.5ml of Quinoline was added to each of the solutions. Then, 1ml of stock solutions were pipetted into a 1ml vial for GCMS analysis. For the electronic cigarette liquids, 950ul of the liquids were transferred into a 1ml vial. Then, 50 ul of quinoline was added to all the vials. The vials were run through the GCMS overnight. The results from GCMS was entered into the Microsoft Excel and SAS program for inferential statistic results.

Inclusion and exclusion criteria

There was no inclusion or exclusion criteria as different liquids from different manufacturers were tested. Because there was no prior knowledge about the

different manufacturers and liquids, the choosing of the liquid was dependent on cost and the vendor's recommendation.

Ethical consideration

Because there was no exposure of electronic cigarette liquids to humans, ethical consideration was not required.

Proposed statistical analysis:

The type of data was numerical data for this research study. The following statistical analysis was conducted on SAS software. A one- tail one sample t-test was used, which compared the mean of one sample to the mean of the standard. First, an Excel spreadsheet, which contained the actual data, was entered in the SAS program. The Excel spreadsheet data can be found in Appendix Section A. Table 1 presents the descriptive statistics, which included the sample size, mean, standard deviation, standard error and minimum and maximum value. The full SAS statistical printout can be found in Appendix Section B.

Based on the normality test, the data was normally distributed for the actual nicotine concentration because the p-value generated were all less than 0.05. Thus, the parametric results were used to interpret whether the results were statistically significant or not.

After analyzing the parametric results the p values for equal and unequal variance were greater than 0.05. Therefore, the null hypothesis cannot be rejected and the results were not statistically significant (Heacock & Karakilic, 2015). Therefore, the mean concentration of nicotine in nicotine free electronic cigarette liquid was equal to zero ppm.

Table 1: Descriptive statistic sample size, mean, standard deviation, standard error and minimum and maximum value for nicotine concentration in nicotine free electronic cigarette liquid

Labeled	N	Mean	Std Dev	Std Error	Min	Max
Actual	10	32.4	92.2	92.2	0	293.3

Type 2 error:

Although the p-values 0.2811 and 0.2953 were relatively larger than 0.05, there was a high probability for type 2 error because the sample size was only ten samples. To decrease this error, a larger sample size should be taken which includes at least 30 samples (Heacock & Karakilic, 2015).

Discussion

After chemical analysis, it was found that one manufacturer had nicotine mislabeling. On the labels, if the sample is nicotine free, the label should be labeled with 0 mg of nicotine. If the sample contains nicotine, the label will either have two, four or 16 mg of nicotine present. There were two liquids from that same manufacturer that were tested positive for nicotine concentration with 30 ppm and 293 ppm. However, the nicotine found in the liquid may not equal to the nicotine in the vapour due to the heating coil of in the electronic cigarette evaporating the nicotine (Clark, 2014). In this situation, there might not be any nicotine found in the vapour because the heating element can evaporate nicotine in the parts per million range. Therefore, further research should be conducted which focuses on health effects due to long term exposure of nicotine concentration vapour in the ppm range.

WorkSafe BC (2016) created a guideline, which included the nicotine concentration for an 8 hour time weighted average exposure limit (TWA) to be 0.5 mg/m^3, which is 0.08 ppm. A 8 hour TWA is defined as a concentration of a substance in air which can not exceed over a normal 8 hour work period (WorkSafe BC, 2016). Thus, by using the results from this experiment, if the 300 ppm in the liquid did lose some

nicotine concentration while undergoing a thermal reaction, the vapour entering the body should still be significantly higher than 0.08, which is the WorkSafe exposure limit. This guideline is more applicable to the people who work in an electronic cigarette store which allows people to vape inside the store. But, it is inaccurate for the general population as they are not being exposed nicotine vapour for eight hours. Instead, to further augment the WorkSafe BC guideline, short term studies should be conducted to determine short term exposure limit (STEL), which is defined as the time weighted average concentration of a substance which may not exceed over any 15 minute period (WorkSafe BC, 2016).

Although in this experiment, statistical analysis showed that nicotine level was not statistically significant between the label and the actual nicotine content, two out of the ten samples were found to have nicotine in the parts per million range. This has confirmed some of the observations that were found in the literature studies. For example, Hutzler et al. (2014) analyzed 28 different brands, which ten of those brands were declared nicotine-free, from a German retailer. Results from the analysis indicated that seven out of the ten brands were identified to have nicotine content from 0.1 – 15 ug/ml. The other eighteen brands, which did not declare nicotine-free, had nicotine levels between 0.1-324 ug/ml (Hutzler et al. 2014).

Nicotine impurities and other tobacco specific toxicants were found in the electronic cigarette liquid composition. Trehy et al. (2011) also discovered that electronic cigarette liquids produced by manufacturers such as NJOY and CIXI found nicotine content in liquids varied significantly than what the manufactured had labeled. This suggested nicotine impurities were present in the liquids. Perhaps the same machinery may be used for producing nicotine and nicotine free liquids. This may mechanically transfer nicotine in nicotine free liquids while preparing the liquids with specific nicotine concentration. Herrington et al. (2015) found that there were numerous chemical components present in addition to what was labeled on the product. The chemicals were present due to the flavoring of the liquids and some of these chemicals are derivatives of nicotine. For example, pyridines, which

is one of the chemical, is known to be a tobacco-derived nicotine. Farslinos (2013) described the variety of nicotine derivatives were very similar to nicotine. For instance, the difference between a nicotine derivative compound, nomicotine, and nicotine is a hydrogen substituted in place of a methyl group. But, according to Hahn et al. (2014), these nicotine impurities were below the levels which were likely to cause harmful human effects.

Limitations

According to the retailer Eastsidevape (n.d.), there were more than ten e-cigarette's manufacturers selling to this retailer. For each manufacturer brand, there were at least five different electronic cigarette liquids (Eastsidevape, n.d.). Ideally, if there were enough samples obtained from each manufacturer, the sample set would be more representative and have more accountability towards addressing the topic of nicotine mislabelling in nicotine free electronic cigarette liquids. Heacock & Karakilic (2015) required that at least 30 samples were needed to control type 2 error. However, because the total sample size contained only four samples from one manufacturer and only six samples from another manufacturer, it was insufficient to conclude that there was no nicotine in nicotine free electronic cigarette liquids on the basis of this sample size. Focusing strictly on one manufacturer will alleviate type 2 error because there will be prior knowledge about which manufacturer is known to have nicotine in their nicotine free liquids. However, there is major bias as one manufacturer is not representative for all the electronic cigarette liquid manufacturers. Ideally, if this research was to be done on a larger scale with a larger budget, bias and type 2 error would be reduced.

There can be improvements towards developing better lab procedures for this experiment. During the data analysis, it was noted that the calibration curve from the standard nicotine solutions was not linear. Theoretically, as the nicotine concentration in the solution increases, the signal from Gas Chromatography Mass Spectrometry (GCMS) should increase. However, that was not the case for one of the standard nicotine solution, as that point on the graph did not fall linearly. And, due to time constraint,

preparing another set of nicotine standard solutions was not possible.

Recommendations

Although inferential results indicated there were no concerns with improper labelling of nicotine concentration in electronic cigarettes, more studies should be done because the safety of electronic cigarette is still widely questioned. Testing for nicotine in nicotine free electronic cigarette liquids is a good indicator of how manufacturers are producing their liquids. But, it does not explain how humans can be affected because the exposure is the vapor generated, and not the liquid. It would be recommended that long-term study should be conducted on whether vaping nicotine free electronic cigarette liquid can lead to addiction. Other parameters such as volatile organic compounds (VOCS), formaldehyde, glycols, and tobacco specific nitroasmines should be tested because the aforementioned chemical parameters are also known to create adverse human effects (Hynes, 2015) These chemicals are present despite the fact that if the liquids are nicotine free or not. According to the *Food and Drugs Act* (2015), electronic cigarettes are regulated as the sale of nicotine delivery advice is illegal without a prescription. The sale of nicotine delivery devices must get authorization before any retailer can sell them.

The labelling of products is also regulated under the *Food and Drugs Act* (2015). But because the amount of nicotine that was found in nicotine free electronic liquids were in the ppm range, it is very close to zero. Perhaps, there should be a section under the *Food and Drugs Act* which addresses the maximum allowable discrepancy percentage between labeled and actual nicotine concentration in electronic cigarette liquids.

In terms of professional involvement, unless the *Tobacco Control Act* includes electronic cigarettes under the definition section (Tobacco Act, 2015), bylaw officers can only regulate electronic cigarettes under the city's bylaws. But, there are positive movements towards regulating electronic cigarettes. For instance, the electronic cigarette stores have a 18+ sign which prohibits the sale to minor as well as prohibiting advertisement of electronic cigarettes

(Meissner, 2015). It is necessary for these signs to be placed in order to reduce the access of electronic cigarettes to youth because electronic cigarettes is a source of nicotine delivery gadget which can induce addiction for cigarettes. With regards to EHO's involvement, EHOs can support public education by suggesting there are precautions with using electronic cigarette's because there are associations with electronic cigarette's and harmful chemicals such as volatile organic compounds, nicotine and toxic heavy metals.

Future Research Suggestion

- Future studies can study involve testing the exposure of nicotine to humans by doing a vapour analysis. The methodology which Hynes (2014) used for heavy metals can be similarly applicable to the analysis of vapour for nicotine in nicotine free electronic cigarette liquids. In fact, a topic of significant concerns is the inhalation of particulate matter such as PM 10 and PM 2.5. Students can sample for PM while inhaling vapour from electronic cigarette.
- The generation of carbonyl compounds for electronic cigarettes is an interesting topic. Because one of the carrier solvents of electronic cigarette liquid is propylene glycol and glycerin, the thermal reaction of propylene glycol and glycerin can generate carbonyl compounds such as formaldehyde and actealdehyde. Bekki et al. (2014) concluded that propylene glycol as a carrier solvent generates the most carbonyl compounds. Kosmider et al. (2014) also confirmed that as the battery voltage increases, the higher carbonyl compounds are generated. Future students can confirm that this is the case by doing a vapour analysis under several battery voltage settings.
- Participants can conduct a brief survey to the population who vapes nicotine free electronic cigarette liquids and ask about if there are any behavioral changes. For instance, the survey can be designed to study whether there are any associations vaping nicotine free electronic cigarette

liquids with signs of nicotine withdrawal symptoms.

- Participants can also survey how manufacturers prepare their nicotine free liquids. Several questions to consider are their quality control and assurance. In addition, surveys about the general public's knowledge of concerns with electronic cigarettes as well as youth's usage of electronic cigarettes should also be pursued.

Conclusion

By using the software SAS to generate inferential statistical results from the ten samples, the null hypothesis failed to be rejected in this study. Therefore, the mean concentration of nicotine found in nicotine free electronic cigarette juices was not statistically significant and what was sampled was equal to what was label. However, there are many confounding parameters to this study. Mainly, type 2 error is a significant issue in this study as there was not enough samples tested to meet minimum of 30 samples. As well, bias is a huge issue because the samples came from two manufacturers. In order for these results to be representative, at least 30 samples need to be obtained from a vast majority of manufacturers. In this research, only 10 samples were obtained from two manufacturers. Thus, using these results from this research alone to generate conclusions will generate a bias.

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Competing Interest

The author declares that he has no competing interests.

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