University of Nevada, Reno

The Public Health Implications of Nicotine Containing Products

A thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in

Environmental Sciences

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December 2019
THE GRADUATE SCHOOL

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The Public Health Implications of Nicotine Containing Products

be accepted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Abstract

Background: Tobacco has been embedded into the history of the Americas since before European colonization. Throughout American history, the plant has been used for a culmination of reasons, mainly being what is commonly perceived as the pleasurable effects following the use of the product. Following the plant’s massive manufacturing and distribution throughout the world in the 19th century, there have been several issues surrounding the use of tobacco, such as its irrefutable association to several diseases, including cancer. The association between tobacco use and cancer was argued between the tobacco industry, politicians, and scientists between the 1950s to the 1980s. The United States legislation has been stained by tobacco industry tactics aimed to disinform the public and limit the decline of tobacco products, which includes the lack of information provided on product labels, which could otherwise deter public interest in the product itself. We have addressed the lack of information provided on tobacco product labels in a quantitative survey of nicotine within smokeless tobacco products (STPs) while taking literature-based toxicity, metabolism, and the addictive properties of nicotine into account following a thorough literature review of the history behind the use and dispersion of tobacco itself.

Methods: Using a modified Cooperation Centre for Scientific method, we analyzed 15 different smokeless tobacco products using gas chromatography to determine: nicotine concentrations, nicotine concentration variabilities between and within different smokeless tobacco products, and inconsistencies with provided manufacturer labeled nicotine concentrations.

Results: The data from our experiment provided us with several outcomes, including the following (1) Nicotine concentrations within and between different types of smokeless tobacco products typically varied widely. (2) fourteen of fifteen of the total pack nicotine
contents within smokeless tobacco products exceeded the potential dose of nicotine that could be toxic for an average adult male 30.0-60.0 mg. (3) Literature claims that about 20% of nicotine is bioavailable following the use of tobacco products, when taking the literature-claimed percent bioavailability of nicotine into account, the majority of the total pack nicotine contents still exceeded the potential dose of nicotine that could be toxic for an average adult male. (4) Manufacturer labeled nicotine concentrations differed significantly from analyzed nicotine concentrations within multiple products.

Conclusions: The use of nicotine-containing products (NCPs) is known to be a significant public health hazard around the world and has been thoroughly investigated. Health advocates have battled against manufacturer discrepancies within the tobacco industry for decades. Some of these industry tactics include neglecting to inform the public of the chemical contents within their products which may result in adverse health effects throughout the population. Contrary to this, the tobacco industry still practices several of these tactics today. Our data shows that there is a lack of consistency in nicotine concentrations between and within several different types of nicotine-containing products. Consumers not only typically have no idea what quantity of toxic nicotine that they are ingesting, but even if labels are present, these concentrations differ from analyzed concentrations significantly. We hope that this project may contribute to changes in the public policy requiring manufacturers to provide accurate labels on nicotine NCPs not only for nicotine, but for any other chemical contents present within STP to the aid of the public, which this study does not take into account.
Acknowledgments

I am very thankful for all the people that helped me accomplish completing my thesis in the Environmental Science program at the University of Nevada, Reno. Firstly, I would like to thank my family the Killarneys and Quinlans for supporting me throughout College and setting me on a pedestal for success, without you all this would have been entirely impossible. Secondly, I would like to thank my advisor Dr. Jeff Angermann who did everything in his power to make me a better scientist and ensure that I was able to achieve my goal of earning my master’s degree. I would also like to thank Dr. Glenn Miller, who was there at any time I had issues with all my projects; both he and Professor Angermann are an inspiration for what a good Professor should represent. I would also like to thank my program director Dr. Stanley Omaye, for continuously helping me throughout graduate school and always being there for me when I needed help. I would like to thank Dr. Judith Sugar for her interest in the project and her incredible involvement in the final thesis and her service within the defense committee. Lastly, the hours that it took to finish this project would not have been achievable without the aid of each undergraduate working in the laboratory who contributed as Co-Authors to the major thesis project in order of position: Devin Miner, Ryan Hoben, Ryan Becker, and Stephanie Zunini. Without the culmination of all of these people, completing my thesis and potentially earning my master’s degree would not have been possible. I am beyond appreciative of each and every one of you, thank you very much.
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Major Acronym Key

NCP - Nicotine Containing Product

NSDP - Nicotine Salt Derived Product

PTD - Potentially Toxic Dose of Nicotine

SD - Standard Deviation

SEM - Standard Error of Mean

ST - Smokable Tobacco

STP - Smokeless Tobacco Product

%RSD - Percent Relative Standard Deviation

TPNC - Total Pack Nicotine Content

TPW - Total Product Weight

[X](s) - Concentration(s) of X, i.e. Concentration(s) of Nicotine
1.0.0 Introduction

The primary focus of this study was to review and assess the public health issues surrounding nicotine-containing products (NCPs) in general. Since the commercialization of NCPs, there has been a long history of industry tactics geared towards manipulating the public to believe that NCPs are not a hazard but rather a necessary and pleasurable everyday activity. Studies have determined that although the use of NCPs has declined over the turn of the century, more than 66.9 million or 25.5% of Americans over the age of 12 are still reliant on these products currently.\(^1\) 55.5 million of those addicted to tobacco products are cigarette smokers while about 11.4 million are smokeless tobacco users.\(^2\)

Chemicals generated during the use of NCPs, via oral ingestion or inhalation, have been thoroughly investigated and are known to cause several diseases including heart disease, cardiovascular disease, oxidative stress, several types of cancer, DNA-level mutations, teratogenesis, and early-onset of death.\(^3\)–\(^5\) In terms of death and disease, the use of NCPs is the leading cause of preventable death in the United States and several other countries. Smoking NCPs accounts for 20%-1 in 5 deaths-every year.\(^6\) Oral ingestion of NCPs is linked to many deaths resulting from tobacco-related illness such as esophageal and oral cancers.\(^7,8\) Nicotine, is not known to play a major role in tobacco-related illnesses. However, nicotine is the addictive chemical within NCPs which makes it the proximate cause of tobacco-related diseases and illnesses such as cancer, diabetes, and high blood pressure.\(^1\) In the United States, more people are reliant on nicotine than any other drug; the chemical is arguably as addictive as heroin, alcohol, and cocaine.\(^6\) Nearly 14% of adults in America are addicted to NCPs, 4% of whom are addicted to smokeless tobacco product (STP), and 96% of whom are addicted to smoking tobacco.
(ST) with prevalence varying from state to state.\textsuperscript{9} Over 80\% of NCP users attempt to quit use only to return within the month, likely resulting from bodily chemical imbalances following discontinuation of NCP use.\textsuperscript{1,6,10} Many people find the withdrawal symptoms resulting from the discontinuation of nicotine-use unbearable, symptoms which include: headaches, nausea, moodiness, anxiousness, anxiety, depression, and general stress.\textsuperscript{11}

Many factors influence both the absorption and metabolism of nicotine including age, race, sex, kidney-function, diet, weight, pregnancy, and use of medications. Nicotine is primarily absorbed through airways in the alveoli of the lungs, likely resulting from the large surface area of the alveoli itself.\textsuperscript{12} Nicotine levels peak in the brain about 10 to 20 seconds after each puff or through oral ingestion in 15 minutes.\textsuperscript{12}

In STP nicotine absorption is highly dependent upon: the length of use, type of product, and gum-line conditions among many of the other conditions described above.\textsuperscript{12,15,16} Biologically, nicotine absorption across membranes is dependent on pH conditions. Nicotine is a weak base (pKa 8.0) with a low pH, and with a pH lower than 8.0 the chemical is predominantly ionized and crosses biological membranes slowly. For example, when nicotine is found within a more acidic product such as flue-cured tobacco, absorption across biological membranes is low. Conversely, when nicotine is found within a less acidic environment, such as within air-cured tobaccos, the chemical becomes primarily un-ionized and is more readily absorbed through biological membranes.\textsuperscript{17} Manufactured STPs are buffered to alkaline pH which facilitates the passage of nicotine through biological membranes into the oral membranes and mucosa.\textsuperscript{14} This makes manufactured STPs more readily absorbed like many nicotine-replacement therapy products such as nicotine-inhalers, patches, and sprays.\textsuperscript{12,14} Although the use of STPs results in increased rates of nicotine absorption from added chemical properties allowing the chemical to pass biological membranes more easily, brain-nicotine levels
still increase more slowly than they do with the use of smoking tobacco resulting from the differences in the routes of bodily absorption between smoking and mucosa.\textsuperscript{12,15,16,18} Overall the pH adjustment of STPs enables a more rapid input of nicotine into the human envelope. Thus, the addictive chemical properties of the STP, such as nicotine, are enhanced.\textsuperscript{14} If NCP users had a better understanding of the risk of ingesting nicotine in any form, it might aid in the cessation of nicotine addiction.

In terms of toxicity, nicotine’s generally accepted lethal dose at 50\% of a population (LD\textsubscript{50}) is approximately 30-60 mg or 0.8 mg/kg for an average adult male.\textsuperscript{18} In ST 30-60mg of nicotine equates to about 5 cigarettes. In STP this quantity is not commonly stated within peer-reviewed literature. Following the excessive use of nicotine, overdose symptoms may include nausea, vomiting, increased heart rate, and sudden death.\textsuperscript{18} Manufacturers of NCP do not typically label nicotine concentrations on their product nor any serving size. A lack of serving size on product containers leaves consumers oblivious to the amount of nicotine they are ingesting contrary to the relatively low toxicity of the chemical. In this study, we have addressed manufacturer labeling discrepancies within STP while taking literature-based toxicity, metabolism, and the addictive properties of nicotine into account. The overall purpose of this study is to provide a survey of nicotine concentrations and variability between and within several types of STP, which were analyzed by gas chromatography- mass spectrometry (GC-MS).
2.1.0 Literature Review Nicotine: Public Health Implications

2.1.1 The History of Tobacco

The name tobacco is given to plants within the family of Solanaceae (nightshade), genus “nicotiana.” Prior to Native American’s history of using tobacco, there is little information known about the origin of the use of tobacco. Although it has been seen in ancient Mayan drawings, its utility prehistorically is unknown. In its earliest known stages, the tobacco plant originated in the Americas. Tobacco leaves were chewed by Native Americans as a delicacy, and their leaves were smoked. Before European colonization, Native Americans were already using several types of tobacco, such as smokable, chewable, snus, and snuff. Natives used the plant as a medicine or hallucinogen, and within ritualistic practices after discovering what the perceived as a euphoric feeling resulting from nicotine input. As the use of tobacco increased, the plants spread rapidly throughout the Americas via trade routes used by indigenous people.

Tobacco became introduced to the remainder of the world soon after Columbus arrived in the Americas. The plant was first introduced outside of the Americas in England by Sir Walter Raleigh. Raleigh, a close friend of the Queen at the time, had religiously promoted the plant and claimed that it was a necessary recreational activity for men. Soon after Raleigh’s return to England, both chewing and smoking had become a type of trademark for high-class men in both country and hunting clubs. Later, after having easy access to the commodity, Spanish, English, and Portuguese sailors began using tobacco as a form of exchange. Early explorers were enticed as tobacco was used by Natives Americans not only recreationally, but also as a remedy for nearly every disease known to humans at the time, a universal cure. By the 1500s, tobacco became a cash crop, and further spread as these sailors introduced the product at ports and soon brought them back to their own home countries. Although banned for a short period
by kings and queens, tobacco was adopted rather quickly in the home-bound sailor’s countries, the product was well accepted as a drug of choice in Europe. By the 16th century, tobacco was not only used throughout the world but had played a pivotal role within the global economy. Many have even claimed that it was the foundation for America’s economy in and of itself.

Nevertheless, even in early centuries there was pushback against the use of tobacco. Many within royalty had disdain for the use of the plant. Rightly so because, contrary to their lack of knowledge of the actual biological effects resulting from the abuse of tobacco, royals claimed that using it was dangerous for the lungs and that it had a foul smell. Many rulers followed harsh measures to deter the use of tobacco. For example, during the 16th century, the ruler of Turkey had people ripped from their homes and strangled in the streets for smoking. In Russia, those smoking a pipe with tobacco would have the pipe jammed into their noses. Contrary to the pushback from rulers, as went on, tobacco use became more popular, commercialized, manufactured, and more readily available as the plant held its grip over the world, and addiction ran its course on its inhabitants. By the early 20th century, billions of cigarettes were being sold every year, and by 1995, 500 billion cigarettes were sold in the United States alone.

2.1.2 The Chemical Properties of Nicotine

Figure 1) Nicotine Chemical Structure.

Nicotine is naturally found within the tobacco plant, acting as a botanical insecticide in tobacco leaves. Nicotine (C_{10}H_{14}N_{2}) is a plant alkaloid with a molecular
weight of 162.236 g/mol.\textsuperscript{15} The chemical itself is an addictive central nervous system (CNS) stimulant. Due to nicotine’s chemical properties, it has the ability to pass through all membranes of the human envelope through inhalation, ingestion, and skin absorption.\textsuperscript{15} The liquid form of the chemical is clear to light-yellow when exposed to air and has an odor like fish. Nicotine has a boiling point of 246.7°C and a melting point of -78.9°C.\textsuperscript{15}

2.1.3 Nicotine Metabolism

Nicotine is absorbed through airways in the alveoli of the lung, likely resulting from the large surface area of the alveoli.\textsuperscript{12} Nicotine levels peak in the brain about 10-20 seconds after each puff. Nicotine is metabolized primarily by the liver enzymes CYP2A6, UDP-glucuronosyltransferase (UGT), and flavin-containing monooxygenase (FMO).\textsuperscript{14,21} Factors that influence both the absorption and metabolism of nicotine include age, race, sex, kidney function, diet, weight, pregnancy, and use of medications.\textsuperscript{13} In smoking tobacco, nicotine is carried on tar droplets either by routes from the combustion of cigarettes, or in STP by passage through the gums, buccal membrane, and oral mucosa.\textsuperscript{12} The overall absorption of nicotine in a smokable product is dependent on the user-specific smoking topography variables, including puff interval, puff duration, and puff length.\textsuperscript{14} STP nicotine absorption is mainly dependent upon the range of use, type of product, and conditions of the gumline.\textsuperscript{12,16} Biologically, nicotine absorption across membranes is dependent on pH conditions.\textsuperscript{22} The pH of nicotine is a weak base pKa 8.0. When ionized the chemical does not cross biological membranes rapidly.\textsuperscript{12} For example, when nicotine is found within a more acidic product such as in flue-cured tobacco, absorption across biological membranes is low.\textsuperscript{12} Conversely, when nicotine is found within a less acidic environment such as air-cured tobaccos, the majority of the chemical becomes un-ionized and is more readily absorbed through biological membranes.\textsuperscript{12} Manufactured STP are buffered to alkaline pH facilitating the passage of nicotine through biological membranes through the oral membranes, making
them well absorbed, which is identical to nicotine-replacement therapy products such as nicotine: inhalers, patches, tablets, and sprays.\textsuperscript{12} Although the use of STP results in increased rates of nicotine absorption, brain-nicotine levels still increase slower than ST resulting from the differences in the routes of bodily absorption between smoking and mucosa.\textsuperscript{12,14}

Nicotine is metabolized to several metabolites, with 70-80% of the chemical converted within the liver to the lactam derivative, cotinine. The conversion of nicotine to cotinine involves several steps, including mediation by CYP2A6 producing nicotine-delta$^1$ (5)$'$-iminium ion in equilibrium with 5$'$-hydroxynicotine. This step is followed by oxidation by cytoplasmic aldehyde oxidase. Another primary metabolite of nicotine is nicotine 1-N-oxide, which accounts for 4-7% of nicotine absorbed by smokers and 3% absorbed by STP users.\textsuperscript{13} The trans-isomer of nicotine 1-N-oxide nicotine is formed via flavin-containing monooxygenase 3 (FMO3). Nicotine 1-N-oxide is not further metabolized by the biological system except by reduction back to nicotine in the intestines, this has been proven by urinary analysis.\textsuperscript{21} Some nicotine (3-5%) is converted to nicotine glucuronide, with the remainder being converted to many other metabolites including the smoking caused metabolite; 3$'$-hydroxycotinine.\textsuperscript{12} Nicotine is metabolized via two nonoxidative pathways: methylation of pyridine nitrogen and glucuronidation.\textsuperscript{12} Nicotine can be converted throughout the body into about 14 different primary metabolites which may result in several secondary metabolites, using 6-7 different known pathways.\textsuperscript{12} We are not going to focus upon each of these pathways but instead understand that the majority of the chemical (70-80%) is converted to cotinine.\textsuperscript{12}

2.1.4 Nicotine General Toxicity: Overdose

Generally, literature within textbooks, databases, and safety sheets claims that the lethal dose of nicotine for a human is around (30-60 mg). This claim states that around five cigarettes could be potentially fatal for an adult.\textsuperscript{18,23} The lethal dose at 50% (LD$_{50}$) is
0.8 mg per kg, which is an amount smaller than for laboratory animals, which is 3.3 mg per kg in mice and 50 mg per kg in rats. Nicotine at 3.3 mg per kg in mice would make nicotine toxicity similar to that of cyanide at 1-3 mg per kg, and yet overdoses resulting from nicotine are rare, even though many people abuse nicotine with amounts five times higher than those seen at the commonly known lethal doses on a consistent basis. For example, there is evidence of people consuming far more than 60 mg/kg on a daily basis, even some cases where average sized men have ingested 60 milligrams of the chemical in one sitting without dying. One factor that may be the result of the seemingly unrepresentative lethal dose of nicotine is the loss of bioavailability of the chemical itself following the symptoms of an overdose. Those symptoms are vomiting and or sweating. Another note worth mentioning is that acclimation to nicotine is commonly observed, in that smokers are less susceptible to nicotine than non-smokers. Making it possible for habitual NCP users to tolerate more nicotine than non-users.

There are several postmortem reports following suicides or accidental deaths resulting from the overuse of nicotine. These postmortem reports made it possible to determine new lethal doses for nicotine. Scientists concluded that there was a major loss in nicotine following death, which led to an underestimation of the actual lethal dose of the chemical. However, the postmortem quantification of nicotine could still be used to determine the lower bound of the range for the chemical’s toxicity. The final quantification for the lower bound of nicotine toxicity was around two milligrams per liter in blood plasma. Continued studies determined that about 20% of nicotine is bioavailable after ingested in blood plasma. A cigarette contains roughly two milligrams of nicotine, which represents 30 nanograms per milliliter that truly becomes bioavailable. As discussed previously, the lethal dose for nicotine has historically been claimed to be up to 60 milligrams. Assuming that the entirety of nicotine available in a product is not wholly bioavailable once consumed, as proven by continued investigation, this level of
intake (60mg of nicotine) would give rise to about 0.18 milligrams per liter in blood plasma. In the lower limit of reports on the toxicity of nicotine postmortem, the toxic dose is claimed to be two milligrams per liter equating to four milligrams per liter in blood plasma. This amount is about 20-fold higher than bodily concentrations of nicotine caused by ingesting 60 mgs worth of the chemical.\textsuperscript{18} When taking into account the amounts of nicotine bioavailable after ingestion, further estimates using postmortem values as a lower limit have concluded that the true potential LD\textsubscript{50} of nicotine is 0.5-1g of ingested nicotine or about 6.5-13mg/kg for a human, this amount agrees with some animal assays.\textsuperscript{5} Similar to the common literature stated LD\textsubscript{50} for nicotine (0.8 mg/kg) there are several limitations to the post-mortem LD\textsubscript{50} (6.5-13mg/kg), which include variables that do not get taken into account when estimating the LD\textsubscript{50} of nicotine such as tolerance. Overall, there is a discrepancy between the widely accepted toxic dose of nicotine and those documented by daily users and postmortem reports, which is an important variable to mention when discussing the chemicals toxicity.\textsuperscript{18} Later, within our study, we will discuss nicotine concentrations in comparison to the widely accepted lethal doses of the chemical.

2.1.5 The Physical/Biological Effects of Smoking Tobacco

Manufacturers develop smoking tobacco through cured tobacco leaves.\textsuperscript{25} Although, there are many different types of smoking tobacco, all of them are not homogenous regarding: nicotine, tar, additives, tobacco, and even sugar as a flavoring additive. Some notable characteristics of tobacco types that determine the quality for consumers include: how the tobacco is cured (flue, cured), the shade of the leaf (hue), and thickness.\textsuperscript{25} The effects of using tobacco are well documented. In this section, we will segregate ideas and focus on what the main effects biologically and physically are from the use of ST. For the sake of relevance, about 25% of the United States population smokes cigarettes to date, 55.5 million people.\textsuperscript{26}
Smoking Tobacco and Cancer

The leading cause of death from cancer in 1999 among both men and women in the United States was lung cancer; there was an estimated death toll of about 158,000 people per year. Estimates claim around 90% of male deaths and 80% of female deaths from lung cancer directly result from the use of ST. Among many of the cancers that are affected, by ST, lung cancer is one of the most common and well-documented forms known. The burning of tobacco results in both pyrolysis and combustion, creating thousands of different chemicals that end up in the body. It is unclear whether nicotine itself is a carcinogen; however, many of the other chemicals found within tobacco cause lung and many other forms of cancer. Twenty chemicals within tobacco are specifically known to cause lung cancer in both laboratory animals and humans. In tobacco smoke, 300 chemicals have been identified as carcinogens that will dissolve within saliva, some of which are created as a product of combustion. Many of the saliva dissolvable carcinogens within tobacco smoke are also found within STP. These chemicals are similar to aromatic hydrocarbons such as benzopyrene and N-nitrosamines such as (methyl nitrosamino)-1-3-(3-pyridyl)-1-butanon and N-nitrosonornicotine. These chemicals cause damage to several organs, may cause oxidative stress, and potentially disrupt the replication of cells following absorption.

2.1.6 The Physical/Biological Effects of Smokeless Tobacco Products

Smokeless tobacco products may be separated into several different ‘types,’ with each ‘type’ of product being developed uniquely. The types of STP are variable around the world, including betel quid, chimo, creamy snuff, dry snuff, gudhaku, gul, gutka, iq’mik, and many others. Each type of product is manufactured and ingested differently. However, we will focus on the most common of STPs which include loose-leaf chewing tobacco, moist snus, dry snus, and snuff, which are also the STPs that we will focus on later in the major project of this thesis. Loose-leaf tobacco itself, the primary STP used in the United States, is developed in manufacturing plants. The leaves of the tobacco
plant are dried, cured, shredded into small flakes, and then treated with additives for flavor. The product is then primarily used by men during recreational activity via oral ingestion. Manufacturers develop moist snuff with finely cut dark and cured tobacco. Snuff, which is called ‘snus’ in several other countries is used through nasal ingestion and has become the most popular form of tobacco in the United States, with sales increasing 77% in the last 15 years, due to claims of the health benefits and celebrity use of the product. Dry snuff is cured tobacco developed similar to moist stuff other than that being more finely pulverized than for any other STP in the hopes of increasing nicotine absorption; it is also intended for nasal ingestion. In this section, we will focus on each of the discussed types of STP and the risks associated with their use.

Smokeless Tobacco and Cancer

Although smokeless tobacco products (STP) contain many different chemicals, on average, they contain fewer carcinogens than ST because they lack any form of a combustion reaction. Smokeless tobacco causes cancer and diseases mostly within the cardiovascular and respiratory systems. Smokeless tobacco is specifically known to cause both oral and esophageal cancer, as seen within clinical trials resulting from the rapid absorption of chemicals and metals with no metabolic function that are not typically ingested by humans. STP contains nonvolatile nitrosamines, N-nitrosamine acids, and many other constituents. Hundreds of chemicals are found within STP including 19 different carcinogens and at least 30 different metals. These discovered chemicals include lesser-known metals known to cause cancer such as cadmium, arsenic, copper, mercury, selenium, and lead. Accidental exposure to these metals is well-documented and is typically seen in countries with a lack of water filtration systems that would protect the public from exposure. Copper is responsible for fibrosis within mouth cavities. Lead is not only a teratogen but may cause IQ deficiencies in young adults, which are non-reversible. Arsenic exposure has many known effects such as cancer, skin pigmentation, ulcers within the mouth,
leukemia, renal failure, seizures, and damage to nerves.\textsuperscript{33} Lastly, cadmium is known to cause lung and bone damage along with high blood pressure; it may also cause cardiovascular disease.\textsuperscript{34} And these are just the more commonly studied metals found within STP.

2.1.7 Addiction: The Psychological Effects (Mechanism) of Nicotine Containing Products

The use of many different NCPs including cigars, cigarettes, electronic cigarettes, and chewing tobacco are linked to several phenomena such as cancer, oxidative stress, DNA-level mutations, diabetes, high blood pressure, osteoporosis, reproductive disorders, and many others.\textsuperscript{1} A major contributing factor to the widespread use of NCPs is how effectively addicting the nicotine chemical within tobacco products is. More people in the United States rely on nicotine than any other drug; the chemical itself is arguably just as addictive as heroin, cocaine, and alcohol.\textsuperscript{6} One key concept for understanding why nicotine is so addictive is the mechanisms surrounding the nicotinic receptor. Following absorption, nicotine is metabolized through the liver and leaves the body renally.\textsuperscript{30} Before excretion, the chemical makes its way to the nicotinic receptors which are located in the peripheral and central nervous system (CNS) and are activated naturally by acetylcholine (a major NE transmitter in the brain) or its agonist, the active form of nicotine, following the use of a tobacco-containing product.\textsuperscript{35,36} When the nicotinic receptor is bonded to naturally, the ionic channel opens, selecting for Na\textsuperscript{+} (sodium). The channel depolarizes, closes, and then goes into a state of standby where it will regenerate.\textsuperscript{6} When the active form of nicotine substitutes for acetylcholine the chemical overstimulates the nicotinic receptor, and the receptor becomes desensitized (inactivated), nicotine prevents the receptor from being regenerated as a result of being desensitized.\textsuperscript{37} The binding to the nicotinic receptor is known to modulate dopamine release as seen in mice; this mechanism may produce the pleasurable effect of the use of tobacco products.\textsuperscript{38} However, the overstimulation of the nicotinic receptors results in a downregulation of the
receptors themselves as nicotine causes the desensitization of nicotinic receptors. With fewer receptors present for nicotine or acetylcholine to bind to, it takes more and more nicotine to bind to the few receptors left to increase the production of dopamine to produce the desired pleasurable effects. This mechanism may increase dependence on, and tolerance for tobacco.

55.5 million Americans smoke tobacco-containing products today, and, as discussed previously, 80% of users try to quit using NCPs every year and begin using again by the end of a month. When individuals attempt to quit using tobacco products, their nicotinic receptors remain desensitized, which decreases the efficiency of the production of dopamine, which may result in withdrawal effects. These withdrawal effects, which many people find intolerable and which may take weeks to subside, include, headaches, nausea, moodiness, anxiousness, anxiety, depression, and general stress. There are a few gaps in the public literature regarding nicotine absorption as discussed in section (2.1.4) and the durations for nicotinic receptor regeneration following the use of tobacco containing products at different topographies. If tobacco users had clear directions on the length of time it would take for withdrawal symptoms to cease, and information about chemical concentrations within the products that they are using, it could make quitting smoking easier for the 55.5 million Americans who smoke today.

2.1.8 Nicotine Containing Products and the Economy

The United States is the 5th largest importer and 4th largest exporter of tobacco in the world. The US’s most significant exports include Japan, Western Europe, Turkey, and industrialized countries of East Asia. For the sake of blending the United States imports lower grade tobacco from Brazil, Malawi, Zimbabwe, and several countries in Latin America. As a result, NCPs constitute a significant contributor to the U.S
economy; estimates claim that in 1998, 59.3 billion dollars were spent by consumers on NCPs alone.\textsuperscript{25} Tobacco sales support businesses, industry, retail stores, and an estimated 6,234 farms (2017) rely on the commodity contrary to the health implications surrounding it.\textsuperscript{25,40} The taxes surrounding NCPs are also a significant source of revenue for the government.\textsuperscript{25} The tobacco leaf itself and the farming of the product only makes up 4 cents per dollar spent by consumers while the rest is split between the marketing of the product, exporting, domestic manufacture, and imports.\textsuperscript{23} Contrary to adverse public health implications for the United States and the remainder of the world, with the decline of the tobacco industry comes a decline in types of work, farms, communities, income, and businesses.\textsuperscript{25} Many communities within the United States have been reliant on the tobacco industry for hundreds of years, As the demand for the product decreases, steady transitions that many cannot afford must take place.\textsuperscript{25} This subject may be an area for continued investigation; however, I believe it is an important topic to discuss while confronting the issues surrounding the tobacco industry.

2.1.9 Manufacturers Discrepancies. Industry Tactics.

There has been a long history of industry tactics against the public for the sake of monetary reasons while ignoring health issues surrounding NCPs. STPs and ST were first studied in the 1940s for connections to negative public health outcomes, mainly cancer.\textsuperscript{41} When confronted with evidence of the implications of the use of tobacco products, the tobacco industry was faced with two options, either watch the decline of a major product or create doubt within the population.\textsuperscript{42} The tobacco industry believed that if uncertainty could be established within academics and the public, then people would continue NCP use. By creating uncertainty and indirectly disassociating the link between NCPs and cancer, the tobacco industry was able to avoid a decline in the use of their product.\textsuperscript{41} Although the tobacco industry was successful in creating doubt within the populations for some time, by funding scientists and politicians within disinformation campaigns and
creating conflicts of interest throughout society, during the early ‘50s, the statistical link between tobacco products and cancer was definitively made. For the following thirty years, most of the tobacco industry continued not only to deny the possibility that the use of tobacco could cause cancer but advertised their products as a solution to other stress-related problems. The tobacco industry continued to ignore statistical evidence of the high risks behind the use of NCPs. Examples of tobacco industry tactics at the time included attempts to remove scientific links between tobacco and cancer, and attempts to both control and undermine science by directing money towards scientific funding in support of tobacco use in a positive light, thereby harming public confidence for the opposing argument. The tobacco industry also built networks of interests and influence within the scientific and political communities. Eventually the tobacco industry went as far as simply denying emerging scientific facts against their interests entirely. When the link between NCPs and cancer became irrefutable, the tobacco industry asserted individual NCP users’ responsibility for their own industrially produced health risks. The majority of the tobacco industry’s tactics were accomplished by funding disinformation campaigns. These tactics were accomplished while keeping the industry seemingly as far away from campaigns as possible.

In time, with the governmental crackdown on the tobacco industry in the United States, several accomplishments were made such as: making tobacco advertisements illegal, increasing taxes on the product, and producing warning labels on NCPs. Each of these accomplishments have hurt the tobacco industry. Warning labels introduced in 1966 specifically have proven to be effective in reducing smoking throughout populations. Multinational tobacco companies accepted changes in legislation requiring them to provide warning labels on their product because it could reduce their risks of losing lawsuits. After some time, the tobacco industry launched an attack on changes to legislation after more thorough warnings were introduced in 1985, which drastically
reduced tobacco sales. There is an ongoing battle between the government and the tobacco industry concerning these warning labels.

It is our belief that the pushback by the tobacco industry against warning labels has allowed them to avoid a quantitative analysis of their product provided on labels. Similar to the basic labels that became required in the United States in 1966, a quantitative label of the chemical contents within NCPs could aid in the steady decline of NCP use. When considering the literature behind NCPs’ lethal doses, addictive properties, and prevalence of use, the Angermann laboratory addressed this manufacturer labeling discrepancy as described below.

3.0.0 Addressing Manufacturer Discrepancies (Angermann Laboratory)

To address the manufacturer discrepancies described in section (2.2.0), we extracted quantified nicotine concentrations within 15 STP using gas chromatography (GC-MS) in a single-blind experiment. The purpose of this project was to give the public a better sense of awareness of the variance of nicotine found within STPs. All but two STPs did not have any information pertaining to the actual concentration of nicotine within their product. These included: snus, snuff, chewing tobacco, and nicotine-salt derived products. With a better idea of the quantity of nicotine that it takes to overdose as described in (2.1.4), and an idea of how nicotine becomes addictive psychologically as described in (2.1.7), the public may have a better chance at combating a chemical that has been compared to cocaine, heroin, and alcohol in terms of how addictive it is.

3.0.1 Materials and Methods

The CORESTA method no. 87 was chosen as a standard method to extract nicotine from STP. The CORESTA method no. 87 has been proven in collaborative studies as an appropriate tobacco-nicotine extraction method by 18 laboratories throughout the United States and has shown both repeatability and reproducibility within
values. A second CORESTA document, CRM no. 11, was used as a guide for proper handling, storage, and preparation of STPs. A final CORESTA document, CRM no. 71, was used as a guide for proper sampling of STP.

3.0.2 Sample Collection/Handling

The STP samples (N=15) were collected based on the suggestions in CRM No. 71, which involved three different smoking shops across Reno, Nevada. The STP samples were collected and sorted based on type, such as chewing tobacco, snus, snuff, and nicotine-salt derived products (NSDP). The main STP information of interest was type, brand, and total product weight (TPW). Following the purchase, the samples were handled and stored in accordance with CRM No. 11. Modifications of CRM protocol included (1) undergraduate investigators covered, randomized, and relabeled all samples before the experiment in order to reduce the risk of bias from the investigators; and (2) due to nicotine’s tendency to degrade under light sources STPs were individually transferred to amber ultraviolet protection bags and stored based on their STP type before the experiment. After completion of nicotine quantitation and statistical analysis, the labels were removed to identify the sample types and brands.

10 students (age 20-25), among my friends at the University of Nevada, Reno surveyed for STP usage topography. These students were selected based on having at least minimal experience using STPs. Each student was presented with the chewing tobacco Longhorn Longcut Cherry Blend, which was previously quantified for its nicotine concentration. This STP was chosen due as a result of its ready availability, and similarity in nicotine concentrations towards the average nicotine concentration of all STP 7.80 mg/g. Weights of STP administered were taken within test tubes prior to self-administered doses of STP by students. Student STP usage topography measurements included weight of STP self-administered, STP use time period, quantity of uses per day,
and presence of one potential overdose symptom (nausea) experienced during or following (5:00-10:00 minutes) STP usage.

3.0.3 Reagents used

All reagents were the highest available purity and reagent grade. Nicotine (Fisher Scientific, Hampton), quinoline (Sigma-Aldrich, St. Louis), ethyl acetate (Pharmco-AAPER, Brookefield), and sodium hydroxide (Science Company, Lakewood) were our reagents of choice. A 2.0 M sodium hydroxide solution was prepared by adding sodium hydroxide pellets (80.0g, 2.0 mol) to a 1.0 L volumetric flask.

3.0.4 Standard Preparation

Standard preparation followed procedures within CRM no. 87 with minor adaptations. A 50 mg/mL nicotine stock solution was prepared by injecting 500 μL of nicotine into 10 mL of ethyl acetate in a volumetric flask. 1 mL of nicotine stock solution was transferred to a 50 mL volumetric flask and diluted fifty times to make a working nicotine stock solution at a final concentration of 1 mg/mL. A 50 mg/mL quinoline stock solution was prepared similarly, 1 ml of quinoline was transferred to a 50 mL volumetric flask and then diluted 12.5 times to make a quinoline working internal standard solution at a final concentration of 4 mg/mL. Seven standard solutions were prepared with nicotine concentrations of 4, 8, 20, 40, 120, 200, and 400 μg/mL with quinoline at a constant concentration of 40 μg/mL in each. Before injection into the gas chromatograph-mass spectrometer, each standard was sonicated for 30 min.

3.0.5 Nicotine Extraction from STPs

All glassware and equipment were diligently cleaned between the preparation of samples. STP A-O (STP names following re-labeling and randomization) and their respective samples were treated identically regarding sample preparation. STPs were
selected at random, and triplicate samples 250 ± 50 mg of each STP were placed into mortar and pestles. 4.00 mL of the 2.0 M sodium hydroxide solution and 480 μL of the working internal standard solution were both added to the STP. The STP was allowed to sit for 30 min before frozen with liquid nitrogen and ground down to a paste. After thawing, 50 mL ethyl acetate was added, mixed, and the mixture was poured into culture tubes. The culture tubes were then placed into an orbital shaker at 200 RPM for 30 min. A 5 mL portion of the ethyl acetate top layer was pulled through a 0.45 μm nylon filter and placed into an amber autosampler vial. The samples were sonicated for 30 min before GC-MS injection.

3.0.6 Gas Chromatograph

Gas chromatography analysis was performed with a Shimadzu gas chromatography-mass spectrometer (QP2020, Shimadzu, Kyoto) coupled with an autosampler, injector, and multiple ion detection systems. For nicotine analysis, helium was used as a carrier gas with a flow rate of 1.0mL/min. Nicotine separation from extracts was carried out using a 5% methyl silicone capillary column (30 m x 0.25mm x 0.25 μm.) (Shimadzu, Kyoto). Selected ion detection was set at 84.00 nicotine and 129.00 quinoline. Separated nicotine was quantified using a method from CRM No. 87 with temperatures as follows: Inlet 230ºC, transfer line 230ºC, MS Quad 150ºC, MS source 230ºC, Oven initial 110ºC hold for 1 min; ramp 10ºC/min to 235ºC hold for 4.5 min46.
Figure 2. Nicotine Calibration Curve

Standards were injected in triplicate using GC-MS settings as described in section (3.0.6). A multi-ion detector was set for determination of nicotine and quinolone at 84.00 amu and 129.00 amu, respectively. Peak area counts were fit to develop a standard calibration curve by nicotine/quinoline ratio. Peak ratio area counts showed linearity with an $R^2$ of (99.24%). All sample extracts provided nicotine and quinoline peak area ratios and were fit to the calibration curve for initial quantitation before statistical analysis for the variance.

3.0.6 Statistical Analysis

Three samples of each STP were injected in triplicate into the GC-MS system in accordance with CRM No. 87 GC-MS recommended settings as described above. Nicotine/quinoline ratios from sample extracts were fit to a developed calibration curve for quantitation and expressed in parts per thousand (PPT). Statistical analyses used data software in excel and Origin9. The analysis was used to determine: standard error of the mean (SEM), standard deviation (SD), and relative standard deviation (RSD) expressed as $[\text{PPT}] \pm \text{SEM and SD}$. A two-sided t-test was used to determine the lower and upper
bound ranges for nicotine concentrations between each STP. P-values <0.05 were analyzed to determine whether each STP was statistically different from the hypothesized mean (0) as shown in Table 1. The data was then further analyzed to show similarities and dissimilarities between both means and variances within individual STP populations to provide the wide range of nicotine concentrations data variabilities between each STP population using analysis of variance (ANOVA) tests. ANOVA tests are represented in both Table 3 and Table 4. A box and whisker plot of all STP represented in PPT was developed to visually present differences in individual STP variabilities. STP A-O identities are presented in Figure 2. Total pack nicotine content (TPNC) within each STP was quantified (TPW(g) x [Nicotine/g]), [Nicotine]% Content represents total percentage of nicotine within each STP calculated by (((TPNC)(mg)/1000)/(Pack Weight (g)))x100, both are presented in Table 2.
3.0.6 Tables and Figures

<table>
<thead>
<tr>
<th>STP A–O Brand Name and Type</th>
<th>Pack weight G.</th>
<th>PPT (mg/g)</th>
<th>95% (Confidence interval)</th>
<th>Standard Deviation</th>
<th>SEM</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skoal Longcut: Cherry tobacco blend</td>
<td>32.10</td>
<td>10.09</td>
<td>7.35–12.79</td>
<td>±</td>
<td>1.09</td>
<td>0.83</td>
</tr>
<tr>
<td>Copenhagen Longcut: Wintergreen</td>
<td>35.47</td>
<td>6.42</td>
<td>5.36–7.47</td>
<td>±</td>
<td>0.42</td>
<td>0.24</td>
</tr>
<tr>
<td>Zyn: Cool Mint</td>
<td>5.91</td>
<td>12.50</td>
<td>5.70–19.30</td>
<td>±</td>
<td>2.74</td>
<td>1.58</td>
</tr>
<tr>
<td>Grizzly Premium Wintergreen Pouches</td>
<td>24.20</td>
<td>7.16</td>
<td>3.96–13.35</td>
<td>±</td>
<td>2.49</td>
<td>1.44</td>
</tr>
<tr>
<td>Skoal Snus Mint</td>
<td>16.83</td>
<td>10.23</td>
<td>9.13–11.32</td>
<td>±</td>
<td>0.44</td>
<td>0.25</td>
</tr>
<tr>
<td>Kodiak: Moist snuff Premium Wintergreen</td>
<td>33.37</td>
<td>7.46</td>
<td>2.67–12.23</td>
<td>±</td>
<td>1.34</td>
<td>1.12</td>
</tr>
<tr>
<td>Grizzly Longcut: Premium Wintergreen</td>
<td>37.02</td>
<td>7.06</td>
<td>4.90–9.43</td>
<td>±</td>
<td>0.96</td>
<td>0.55</td>
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<tr>
<td>Skoal Longcut: Peach Tobacco Blend</td>
<td>34.51</td>
<td>7.26</td>
<td>4.89–9.84</td>
<td>±</td>
<td>1.04</td>
<td>0.60</td>
</tr>
<tr>
<td>Copenhagen Pouches: Mint</td>
<td>22.07</td>
<td>1.91</td>
<td>0.38–3.43</td>
<td>±</td>
<td>0.61</td>
<td>0.35</td>
</tr>
<tr>
<td>Longhorn longcut: Wintergreen</td>
<td>34.87</td>
<td>3.45</td>
<td>2.11–5.78</td>
<td>±</td>
<td>2.35</td>
<td>1.71</td>
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<tr>
<td>Zyn Pouches: Coffee</td>
<td>5.86</td>
<td>10.87</td>
<td>5.37–15.75</td>
<td>±</td>
<td>1.37</td>
<td>1.14</td>
</tr>
<tr>
<td>Camel Snus: Mint</td>
<td>7.62</td>
<td>3.75</td>
<td>2.55–4.84</td>
<td>±</td>
<td>0.44</td>
<td>0.25</td>
</tr>
<tr>
<td>Grizzly Longcut: Premium dark select</td>
<td>31.09</td>
<td>5.82</td>
<td>4.59–7.05</td>
<td>±</td>
<td>0.50</td>
<td>0.29</td>
</tr>
<tr>
<td>Copenhagen Longcut: Straight</td>
<td>32.47</td>
<td>2.62</td>
<td>2.04–3.20</td>
<td>±</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>Longhorn longcut: Straight</td>
<td>35.54</td>
<td>14.44</td>
<td>4.33–24.55</td>
<td>±</td>
<td>4.07</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Table 1. STP information and Nicotine Concentrations.

STP A–O labels were removed following nicotine extraction and analysis to match and identify each brand and type of smokeless tobacco product. Nicotine/Quinoline ratios were fit to the developed calibration curve and expressed in UG/ML, these units were further converted to parts per thousand (mg/g) using the exact weight used for samples (C/M x V/1000). Statistical analyses were performed on an aggregate of the data collected from three portioned STP samples and included: Two-sided T-test (Confidence interval, P-value), standard deviation, standard error of mean (SEM), and relative standard deviation (RSD%).
Smokeless tobacco products (STP A-O), Table 3 indicates the identities of each STP. Nicotine concentrations (mg/g) along Y-axis.
Table 2. Total Pack Nicotine Content Within STPs Measured.

Total pack nicotine content (TPNC) for each STP were calculated by ([Total Nicotine]x [Total product weight]). [Nicotine]% Content represents total percentage of nicotine within each STP calculated by ((([TPNC](mg)/1000)/(Pack Weight (g)))x100.

<table>
<thead>
<tr>
<th>STP</th>
<th>Pack Weight (g)</th>
<th>TPNC(mg)</th>
<th>[Nicotine] %Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longhorn Longcut: Straight</td>
<td>35.50</td>
<td>513.20</td>
<td>1.45</td>
</tr>
<tr>
<td>Longhorn Longcut: Wintergreen</td>
<td>34.90</td>
<td>329.30</td>
<td>0.94</td>
</tr>
<tr>
<td>Skoal Longcut: Cherry Tobacco Blend</td>
<td>32.10</td>
<td>323.80</td>
<td>1.01</td>
</tr>
<tr>
<td>Grizzly Longcut: Premium Wintergreen</td>
<td>37.00</td>
<td>261.30</td>
<td>0.71</td>
</tr>
<tr>
<td>Skoal Longcut: Peach Tobacco Blend</td>
<td>34.50</td>
<td>250.60</td>
<td>0.73</td>
</tr>
<tr>
<td>Kodiak: Moist Snuff Premium Wintergreen</td>
<td>33.40</td>
<td>249.60</td>
<td>0.75</td>
</tr>
<tr>
<td>Copenhagen Longcut: Wintergreen</td>
<td>35.50</td>
<td>227.90</td>
<td>0.64</td>
</tr>
<tr>
<td>Grizzly Longcut: Premium Dark Select</td>
<td>31.10</td>
<td>181.00</td>
<td>0.58</td>
</tr>
<tr>
<td>Grizzly Premium: Wintergreen Pouches</td>
<td>24.20</td>
<td>173.20</td>
<td>0.72</td>
</tr>
<tr>
<td>Skoal: Snus Mint</td>
<td>16.80</td>
<td>172.10</td>
<td>1.02</td>
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<tr>
<td>Copenhagen Longcut: Straight</td>
<td>32.50</td>
<td>85.10</td>
<td>0.26</td>
</tr>
<tr>
<td>Zyn: Cool Mint</td>
<td>5.90</td>
<td>73.80</td>
<td>1.25</td>
</tr>
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<td>Zyn: Coffee</td>
<td>5.90</td>
<td>63.70</td>
<td>1.08</td>
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<tr>
<td>Copenhagen Pouches: Mint</td>
<td>22.10</td>
<td>42.00</td>
<td>0.19</td>
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<tr>
<td>Camel Snus: Mint</td>
<td>7.60</td>
<td>28.60</td>
<td>0.38</td>
</tr>
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</table>
Table 3. Condensed ANOVA Statistical Comparisons Among Chewing Tobacco, Snus, Snuff, and NDS.

Superscripts in the PPT column represent population independent means that are not statistically different between each STP (P>.05), i.e., STP N 2.62L translates to STP N (Copenhagen Longcut: Straight) population means show no statistical differences to STP I and L (Copenhagen Pouches: Mint and Camel Snus: Mint).

<table>
<thead>
<tr>
<th>STP Names</th>
<th>PPT (mg/g)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Skoal Longcut: Cherry Tobacco Blend</td>
<td>10.09</td>
<td>0.63</td>
</tr>
<tr>
<td>B. Copenhagen Longcut: Wintergreen</td>
<td>6.42A</td>
<td>0.24</td>
</tr>
<tr>
<td>D. Grizzly Premium: Wintergreen Pouches</td>
<td>7.16A,B</td>
<td>1.44</td>
</tr>
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<td>G. Grizzly Longcut: Premium Wintergreen</td>
<td>7.06A,F,E,D,B</td>
<td>0.55</td>
</tr>
<tr>
<td>H. Skoal Longcut: Peach Tobacco Blend</td>
<td>7.26A,G,F,E,D,B</td>
<td>0.60</td>
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<tr>
<td>I. Copenhagen Pouches: Mint</td>
<td>1.91</td>
<td>0.35</td>
</tr>
<tr>
<td>N. Copenhagen Longcut: Straight</td>
<td>2.62L</td>
<td>0.14</td>
</tr>
<tr>
<td>M. Grizzly Longcut: Premium Dark Select</td>
<td>5.82G,F,D,E,H,I,L</td>
<td>0.29</td>
</tr>
<tr>
<td>O. Longhorn Longcut: Straight</td>
<td>14.44CK</td>
<td>2.35</td>
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<tr>
<td>L. Camel Snus: Mint</td>
<td>3.75G,B,I</td>
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<tr>
<td>E. Skoal: Snus Mint</td>
<td>10.23A,D,C</td>
<td>0.25</td>
</tr>
<tr>
<td>F. Kodiak: Moist snuff Premium Wintergreen</td>
<td>10.87A,E,D,B</td>
<td>1.14</td>
</tr>
<tr>
<td>K. Zyn Pouches: Coffee</td>
<td>7.48A,E,C</td>
<td>1.12</td>
</tr>
<tr>
<td>C. Zyn: Cool Mint</td>
<td>12.5A</td>
<td>1.58</td>
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</table>
Table 4. ANOVA of Independent STP Populations

STP populations separated by STP type, including chewing tobacco (set 1, set 2), snus, snuff, and NSDP. (~) Represents no statistical difference in population means between two STP populations (P>.05). i.e., STP B~A: STP B shows no statistical difference in population means with STP A.

<table>
<thead>
<tr>
<th>STP</th>
<th>P-Value</th>
<th>STP</th>
<th>P-Value</th>
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<th>P-Value</th>
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<tbody>
<tr>
<td>A~None</td>
<td>-</td>
<td>J~F</td>
<td>0.99</td>
<td>L~G</td>
<td>0.12</td>
<td>F~A</td>
<td>0.80</td>
</tr>
<tr>
<td>B~A</td>
<td>0.06</td>
<td>J~E</td>
<td>0.99</td>
<td>L~B</td>
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<td>F~E</td>
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</tr>
<tr>
<td>D~A</td>
<td>0.41</td>
<td>J~D</td>
<td>0.86</td>
<td>L~I</td>
<td>0.91</td>
<td>F~D</td>
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</tr>
<tr>
<td>D~B</td>
<td>0.99</td>
<td>J~C</td>
<td>0.09</td>
<td>E~A</td>
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<td>F~B</td>
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<tr>
<td>G~A</td>
<td>0.22</td>
<td>J~B</td>
<td>0.32</td>
<td>E~D</td>
<td>0.32</td>
<td>C~A</td>
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<td>G~F</td>
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<td>N~I</td>
<td>0.99</td>
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<td>N~L</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>D~B</td>
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<td>M~G</td>
<td>1.00</td>
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<tr>
<td>H~B</td>
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<td>M~D</td>
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<td></td>
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<tr>
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<td>O~C</td>
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<tr>
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<td>-</td>
<td>O~K</td>
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<tr>
<td>J~A</td>
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<tr>
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Table 5. Student STP Use Topography.

10 students at the University of Nevada, Reno surveyed for STP usage topography. Quantified overall quantity of STP ingested per use, the average time over which STP is used, # of uses per day, estimated concentration of nicotine ingested per use and per day (mg), and determined whether if nausea (1 nicotine overdose symptom) was expressed by students following use (N=None symptomatic, Y=Symptomatic). Each student ingested skull longcut cherry tobacco blend (10.09 mg/g).

\[
\text{[Nicotine]/use (mg)} = 10.09 \text{ mg/g} \times (\text{g ingested}) \quad \text{[Nicotine]/Day (mg)} = (\text{[Nicotine]/use}) \times (\# \text{ of uses/day})
\]

<table>
<thead>
<tr>
<th>Students (N=10)</th>
<th>Ingested STR(g)</th>
<th>Average ingestion time(min.)</th>
<th>Use/Day</th>
<th>~[Nicotine]/Use (mg)</th>
<th>~[Nicotine]/Day (mg)</th>
<th>Symptomatic</th>
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<td>31.7</td>
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<td>4.00</td>
<td>22.2</td>
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<tr>
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<td>5.0</td>
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<tr>
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<td>15.3</td>
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<td>Standard Dev.</td>
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</table>
4.0.0 Results

The overall data resulting from the nicotine extraction of STPs are provided in Table 1 and includes total product weight (TPW), [nicotine], CI, SD, SEM, %RSD. STP concentrations showed statistical differences in both population-means and variances between and within each of the four types of STPs. Average nicotine concentration and TPW on a data-aggregate of all samples was 7.80 mg/g and 25.9 g. Within samples, the peak nicotine concentration among STPs was the chewing tobacco Longhorn Longcut Straight 14.44 ± 10.1 mg/g while the lowest nicotine concentration was found in Mint Copenhagen pouches 1.91 ± 1.5 mg/g.

Total pack nicotine contents (TPNCs) were quantified based on nicotine concentrations and TPWs of each STP. The average TPNC between an aggregate of all samples was 198.4 mg. After analysis, the highest TPNC was Longhorn Longcut Straight 513 mg, the lowest TPNC was seen in Camel Snus Mint 28.6 mg, the product had a TPNC content showing a eighteen-fold difference in nicotine concentrations between the lowest and highest TPNCs of each STP. Notably, NSDP (Zyn1, Zyn2) showed the highest nicotine concentration in comparison to TPW between all the pouched STPs. NSDP’s average TPW 5.90 g is 75.62% lower in product weight than Grizzly Premium Wintergreen pouches 24.2 g, the highest TPW of pouched product. However, NSDPs, on average, provided 11.40 mg/g while the highest TPW pouch STP provided 7.16 mg/g, a 37.20% difference. NSDPs were the only two products out of the 15 STP that provided a nicotine concentration label, each of which was claimed to be 6.00 mg. The product did not explicitly state whether this concentration was 6.00 mg/package (condition #1) or 6.00 mg/pouch (condition #2), so we compared the STPs concentrations of nicotine under both conditions. Under condition #1: average determined nicotine concentration is 91.27% higher than the manufacturer claimed concentrations. Under condition #2:
average determined nicotine concentration is 23.64% lower than manufacture labeled concentrations.

Levene’s and Tukey’s Analysis of variances (ANOVA) tests were completed to determine differences (P<F, P<.05) and a lack of statistical differences (P>F, P>.05) between both population variance and means within and between individual STP populations as provided in Table 3. No STP other than chewing tobacco (N=10) showed no statistical differences within population means with ≥1 of any products within their product type. Of the 8 STP within chewing tobacco that showed statistical no statistical differences with ≥1 of its type of STP, only 30% showed a lack of statistical differences between average nicotine concentrations with >3 STP within its product type. Snuff data was neglected, resulting from a small sample size for statistical analysis within its group. Similar to the remainder of STPs, snuff was still compared between independent STP populations. NSDPs showed statistical similarities within-population means and variances to chewing tobacco in all of the products, snuff to snus and chewing tobacco, snus to chewing tobacco and NSDP, and chewing tobacco had statistical similarities to at least one of each of the product types. Overall, ANOVA tests determined the population means between all independent STPs are statistically different (α= 0.05, Prob>F=1.45\(^{-28}\), F=24.30). Levene's tests of all STP determined that overall population variances were also all statistically different (α= 0.05, Prob>F=1.54\(^{-10}\), F=7.13).

A survey was conducted to determine STP user topography (n=10) as provided in table 5. 50% of students within the user-topography survey presented with one of many symptoms of nicotine overdose (nausea) within or following 5 minutes of STP usage. The average STP product between all students was 1.52g, the most being 3.52g, and the least being 0.20g in a single self-administered dose. The average dose and duration of usage within students presenting potential symptoms of a nicotine overdose (n= 5) was (1.50g,
8:40min.), while the non-symptomatic students were (1.49g, 12:40min.). The average estimated nicotine concentration ingested by individual users per use was 15.3mg, the most being 39.0mg, and the least being 2.00mg with the average STP uses per day between students being around 2.30 (2-3 times a day).

5.0.0 Discussion

When comparing the potentially toxic dose (PTD)(30-60 mg) of nicotine to some of the data provided from this project, several major public health hazards come to light. (1) All but the two NSDPs within all STPs of this project provided any nicotine concentration or serving size on product labels, with each of the two differing significantly from labeled concentrations. (2) 93%, or 14/15 of STPs TPNCs exceeded PTD of nicotine. (3) The average TPNCs within all STP is 198.4 mg, meaning consumers would only have to ingest 15.1% of the average STP to potentially meet toxicity doses for nicotine (4) ANOVA tests determined that 2/3 types of STPs did not show any statistical similarities between averages or variances with individual STP populations within their product type (Snus and NSDP). Furthermore, chewing tobacco is the only product to show statistical similarities within its product type; however, 7 of the ten products were not statistically similar to >3 other chewing tobacco products. Not only do consumers have no label to determine the nicotine concentration within STPs, but the chances of picking another product in the same type with a similar nicotine concentration would be unlikely. (5) If a nicotine concentration is provided on a label it may still vary significantly within each type of product similar to the only two STP that contained a labeled nicotine concentration (NSDP), which differed from claimed concentrations by (-23.64%, +91.27%) posing yet another potential health hazard. (6) ANOVA statistical tests determined that both the population averages and variances differed significantly between all STP. Possible explanations for nicotine concentration differences within STP
may be a result of differences in types of product overall, manufacturer production methods, originating plant sources, or simply due to exposure to light sources during production leading to degradation of nicotine.

After recognizing the lack of literature in STP use topography, as a personal endeavor recommended to me by Professor Glenn Miller, in order to determine the total amount of tobacco ingested by individual STP users in Reno and individual risks towards nicotine’s PTD, I analyzed the STP use topography between 10 students at the University of Nevada, Reno as presented in Table 5. Each student was presented with the same STP of the chewing tobacco type from the nicotine quantitation project (Skull Longcut: Cherry Tobacco Blend) (nicotine concentration = 10.09 mg/g) to estimate the possible concentration of nicotine ingested per usage and day by individual users. The average dose and duration of usage within students presenting potential symptoms of a nicotine overdose (n= 5) was (1.50g, 8:40min.), while the non-symptomatic students were (1.49g, 12:40min.). The STP topography survey showed little to no apparent correlation between average STP usage and the presence of possible nicotine overdose symptoms.

When comparing the data of the student-survey to multiple literature stated lethal doses of nicotine, some conclusions could be formed. One of the five students (student #5) presenting a potential nicotine overdose symptom ingested an estimated nicotine concentration within the range of the rat assay PTD of nicotine (30-60 mg). However, students with much lower ingested nicotine concentrations (<30.0 mg) equally both did and did not experience a potential overdose symptom at all. When analyzing the estimated daily ingestion of nicotine concentrations, average doses overall were 35.2 mg. Six of the ten students in this survey exceeded the commonly stated nicotine LD\textsubscript{50} daily. However, when accounting for a potential bioavailability reduction, only about 3 of 10 of students within this survey exceeded commonly stated nicotine LD\textsubscript{50}. When comparing
this survey to postmortem reports of nicotine LD\textsubscript{50} 0.5g-1.0g, which disagrees with the commonly stated nicotine LD\textsubscript{50}, none of the ingested daily doses of nicotine between students exceed the PTD of nicotine.

There are several limitations to this study that, if included, would strengthen the case that the nicotine concentrations in STPs is a potential health hazard, including: (1) The data provided does not take bioavailability or tolerance into account. Several factors play a substantial role in nicotine bioavailability such as age, race, sex, kidney-function, diet, weight, pregnancy, gum-line conditions, and medication use.\textsuperscript{12,41} Taking these variables into account would require a clinical trial coupled with an analytical experiment such as this. However, some investigators have claimed only about 20% of nicotine becomes bioavailable after passage into the human envelope following several metabolic variables.\textsuperscript{14} Even when taking this data into account with an 80\% reduction of the TPNCs of each of the STP analyzed in this experiment, 10 of the 15 STP’s TPNCs still exceed the PTD of nicotine so long as most of the product is ingested at once. (2) The data provided does not include the average amount of STP ingested by consumers per use. The student-survey does provide potential topographies, however, the sample size in the survey is not reflective of an actual city or state population. The data does suggest that an overuse of the product is entirely possible due to the wide variability and distribution of nicotine concentration means between and within each STP type and individual STP population. (3) ANOVA tests could not be used within the snuff product type resulting from lacking an appropriate sample size (N=1) which was recognized following the experiment. However, similarities in nicotine concentrations were still able to be made between snuff and every other type of NCP showing statistical similarities to STPs within the chewing tobacco and snus types. (4) Sample sizes may have been small regarding product types: chewing (N=10), snus (N=2), snuff (N=1), NSDP (N=2). However, each sample was portioned three times and further injected into the GC-MS system in triplicate
resulting in an aggregate of (N=9) analysis per STP. As a result of this issue, comparisons of data between STP types could not be made. However, independent populations of STP comparisons of both variabilities and means could still be determined i.e. STPA (N=9) vs STPB (N=9). With independent STP population comparisons, assumptions could still be made of STP type comparisons in regards to nicotine concentrations. (5) The student-survey of NCP users in Reno was a convenience sample, it is not reflective of the population of Reno. However, this side project may provide a potential model for future investigation of the risks the Reno population may subject itself to with regard to possible nicotine overdoses compared to lethal doses of the chemical itself.

6.0.0 Conclusions

The focus of this study was to determine nicotine concentrations, variances, differences in average concentrations, and labeling inconsistencies within STPs for consumers. As discussed, the data provided from our experiment shows that there are statistical variabilities within both the same and different types of STP (chewing, snus, snuff, and NSDP). The experimental design focused on nicotine quantification on multiple STPs. In terms of nicotine concentrations variability and averages, our experiment better represents the population of STP users who purchase multiple types or products of STPs for ingestion. Although STP’s TPNCs mostly exceeded or were nearing nicotine’s PTD (which is a public health concern in itself), nicotine concentrations within the same container of each STP remained similar and consistent between each analysis (N=9). Tukey tests determined that individual runs of each portioned STP analyzed did not differ by more than one variable: population variance, and population means. To manufacturer’s defense, this implies that the nicotine within individual products is likely homologous. However, for the population of habitual STP users that purchase the exact same STP for ingestion, conclusions cannot be made of the nicotine concentration’s
consistencies within a population of the same product without analysis of multiple STPs of the exact same brand and type. This subject may be considered an area for continued investigation.

When recognizing individual nicotine concentrations among STP and taking a bioavailability reduction into account, each STP is still at least nearing the nicotine’s PTD. The data provided from this experiment, coupled with the high prevalence of nicotine addiction throughout the world, sparks the question of how or why there are not more nicotine overdoses throughout society? Although the incidence of unintentional nicotine poisoning within young children is reported, the number of overall accidental nicotine overdoses has not been stated in peer-reviewed literature, The Center of Poison Control, nor the Center for Disease Control, contrary to the high toxicity and prevalence of use of products containing nicotine. Without knowing the average number of tobacco products and average amount of nicotine ingested by the typical user, it is not possible to answer this question.

However, under the assumption that a portion of the population of NCP users ingests>30-60mg of nicotine (~5 cigarettes) (PTD), assumptions may be made resulting from the lack of nicotine-caused mortality. These assumptions include the possible discrepancies in the common nicotine LD50.\textsuperscript{18} The literature-based nicotine LD50 is based on an animal to human comparisons, which can pose issues for several reasons, mainly: (1) Animal experiments including toxicological data do not always translate from animal to human trials. (2) Metabolism is not entirely reflected when comparing animals to humans due to variables that go unaccounted for.\textsuperscript{18} Although the common literature based LD50 may not be entirely precise, it is still a reasonable estimate for the potential toxicity of nicotine in general. Contrary to the possible issues surrounding the nicotine’s LD50, such as contrasting values, and the lacking prevalence of mortality from the overuse of
NCPs, nicotine remains a proximate cause of tobacco-related illness resulting from the addictive properties of the chemical itself. As NCP users continue consumption, addiction increases, the cessation of addiction becomes more difficult, and the likelihood of smoking coupled with the risks of tobacco-related illness persists.\textsuperscript{1,35}

The feasibility of having manufacturers provide labeled nicotine concentrations and serving sizes may be quite tricky. To achieve this task, manufacturers would have to determine an affordable way to massively quantify nicotine concentrations within each STP, including uncertainty values. Manufacturers would also have to discover a way to confirm the literature-based nicotine LD\textsubscript{50} to provide appropriate serving sizes based on several demographical variables related to nicotine metabolism within human beings. The steps to labeling the concentration-based contents of NCPs would require scientific investigation, time, changes in legislation, and the determination of responsibility of NCP use between both the manufacturer and the consumer. We believe that the responsibility of providing the chemical contents of NCPs lies with manufacturers. With the knowledge of NCP’s contents, the responsibility for the use of NCPs should fall on the consumers themselves.

NCPs are projected to kill over 1 billion people over the 21st century; the use of the product is a known significant public health hazard around the world and has been thoroughly investigated.\textsuperscript{17} Although NCPs are a known hazard, scientists have still battled against manufacturer discrepancies within the tobacco industry for decades. And contrary to the ongoing battle, the tobacco industry still practices several of these tactics today, including neglecting to inform the public of the chemical contents within their products resulting in adverse health effects throughout the population.\textsuperscript{41,47} By promoting nicotine concentrations on the labels of STP, along with serving sizes nicotine’s PTD, manufacturers could not only potentially reduce the number of unintentional overdoses
but also give NCP users’ a sense of awareness. Adding clear quantitative warning-based labels to NCPs may aid in the cessation of nicotine addiction under the circumstance that consumers know the exact quantity of the chemicals that they are ingesting. We hope that this project may contribute to changes in the policy requiring manufacturers to provide accurate labels on NCPs not only for nicotine concentrations and allowable variabilities as discussed, but also for any other chemical contents present within STPs to aid the public which this study does not consider.
7.0.0 References


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