AMERICAN UNIVERSITY OF BEIRUT

ASSESSMENT OF THE IMPACT OF CLEANING PROTOCOL AND PUFFING REGIMEN ON IQOS EMISSIONS

by
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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Chemistry
of the Faculty of Arts and Sciences
at the American University of Beirut

Beirut, Lebanon
June 2022

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ACKNOWLEDGEMENTS

I would like to special thank God for giving me good health and the grace to complete this work. “So, do not fear, for I am with you; do not be dismayed, for I am your God; I will uphold you with my righteous right hand” (Isiah 41:10).

I would like to express my heartfelt gratitude to my thesis advisor, Professor Najat Saliba, for accepting me as a graduate student and providing me with the opportunity to advance my research skills under her amiable supervision. Also, I want to appreciate Dr. Ahmad El Hellani, who co-supervised this project. Without any doubt, you have facilitated my growth and development as a graduate student through your mentorship, support, and encouragement.

I also want to thank the members of my committee, Professor Tarek Ghaddar and Dr. Pierre Karam, whose guidance has undoubtedly improved the quality of this thesis and made me a better researcher

My heartfelt gratitude also goes to Mrs. Rachel El Hage Kalakech, the senior research assistant in our laboratory, and all other laboratory members: Fatima El Hajj Moussa, Amira Ajaj Yassine, Abdel El Aziz Kordieh, Mehdi Abo Hamad, Cynthia Antossian, and Mohamed El Hallak for their unwavering support throughout this project.

I would also like to thank the Mastercard Foundation scholarship program for funding my entire education program and providing me with all-around academic support.

Finally, I would like to thank my mother, father, and siblings: Adesola, Adebimpe, and Aderinsola, for their unwavering support, both financially and emotionally. I would also like to thank my amazing Fiancée, Oluwadamilola, for her encouragement and constant push to be the best I can be. These few words could never express how much you all mean to me and how much assistance you have given me on my journey.

ABSTRACT

OF THE THESIS OF

Ayomipo Emmanuel Adeniji for Master of Science
Major: Chemistry

Title: Assessment of The Impact of Cleaning Protocol and Puffing Regimen on IQOS Emissions

A wide variety of tobacco products are currently available in the market. Although they all imbibe the central function of delivering nicotine, they differ in design and mode of operation. These variations could lead to the production of different suites of compounds, and hence different toxicant profiles. To account for any product-specific toxicants, and to comprehensively characterize the emissions of tobacco products, researchers have used non-targeted analysis (NTA) besides targeted analysis methods of predetermined compounds. In chapter two of this thesis, we describe recent NTA studies of tobacco product emissions, highlighting the potential and challenges of NTA in tobacco research. Although the challenges of NTA are multi-layered, cutting across sample generation and collection, to instrumental setup and then data analysis, the chapter emphasizes its potential in the identification of product-specific compounds. In the third chapter we engaged NTA to assess the impact of users' behavior on IQOS emissions using gas chromatography tandem mass spectrometry. The effect of user behavior on toxicant emissions was investigated in two ways: the effect of device cleanliness between consecutive use sessions and the effect of puffing parameters that may alter the heating temperature. The assessment was done under two sampling procedures, one involving NTA analysis of filters derivatized by silylation, and the other using multi-step trapping of IQOS aerosol followed by GC-MS analysis of particle and gas phases of the aerosol. Both analyses led to the detection and semi-quantification of compounds belonging to chemical classes such as alkanes, carboxylic acids, ketones, aromatic acids, esters, and substituted hydrocarbons. Statistical analysis of the effect puffing parameters on IQOS emissions showed that all the three puffing parameters (puff duration, number of puffs, and puffing flow rate) had significant effects on IQOS emissions. However, device cleaning did not have any statistically significant effect on IQOS emissions

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ABBREVIATIONS

2,3,4-trihydroxytetrahydrofuran: THTF
2,4-Dinitrophenylhydrazine: DNPH
2-ethyl-3-hydroxypropionic acid: EHPA
2-hydroxy-2-methylpropanoic acid: HMPA
3-chloro-1.2-propanediol: CPD
4-Hydroxyphenethyl alcohol: HPA
Alternative tobacco products: ATPs
Aromatic amines: AAs
Atmospheric pressure chemical ionization: APCI
Benzoic acid: BA
British American Tobacco: BAT
Carbonyl compounds: CCs
Cleaning protocol 1 and 2: CP-1 and CP-2
Electron impact ionization: EI
Electronic cigarettes: ECIGs
Electrospray ionization: ESI
Flame Ionization detector: FID
Food and drug Administration: FDA
Food and Drug Administration: FDA
Fourier transform ion cyclotron resonance mass spectrometry: FTIR-MS
Harmful and potentially harmful constituents: HPHCs
Head space-solid phase micro-extraction: HS-SPME
Health Canada Intense: HCI
Heated tobacco products: HTPs
Hydrophilic interaction liquid chromatography: HILIC
Hydroquinone: HYD
Hydroxy acetic acid: HAA
Internal standard: ISTD
International Agency for Research on Cancer: IARC
International organization for standardization: ISO
Ion mobility spectrometer: IMS
Lactic acid: LA
Laser/desorption ionization: LDI
Liquid chromatography-high-resolution accurate mass spectrometry: LC/HRAM-MS
Mass spectroscopy: MS
Modified risk tobacco product: MRTP
National Institute of Standards and Technology: NIST
Non-targeted analysis: NTA
One-dimensional gas chromatography: GC-MS/1D-GC
Phenylacetic acid: PAA
Philip Morris International: PMI
Polycyclic aromatic hydrocarbons: PAHs
Premarket obacco Authorization: PMTA
Puffing regimens: PRs
Retention time: RT
Reversed-phase-liquid chromatography-heated electrospray ionization: RP-LC-HESI

Semi-volatile compounds: SVOCs
Solid-phase microextraction: SPME
Thermal desorption: TD
Tobacco-specific nitrosamines: TSNAs
Total particulate matter: TPM
Two-dimensional gas chromatography tandem time-of-flight mass spectrometry
GCxGC-TOFMS / 2D-GC-MS
Unique Compounds and Spectra Database: UCSD
Volatile organic compounds: VOCs

CHAPTER 1

INTRODUCTION

Tobacco consumption has accompanied humans for a very long time. Apart from the early use of tobacco in forms of snuffing, chewing, and drinking (like tea), various tobacco products that rely on burning tobacco and inhaling its smoke such as pipe tobacco, cigars, and cigarettes have been developed for mass consumption. However, newer tobacco products such as e-cigarettes and heat tobacco products, with advanced technology, have recently been introduced into the market, as allegedly safer alternatives to combustible tobacco products that were linked to deleterious health effects after decades of research.

In the first chapter of this thesis, a narrative of the evolution of tobacco products is summarized. The history of various tobacco products that are currently predominant in the population, their product designs, and mode of operations are discussed. The chapter also gives a brief insight into the role researchers and tobacco regulators have played in unmasking the health effects of these products, especially for cigarettes. This chapter also highlights some of the maneuvers that the tobacco industry took to outsmart tobacco regulators and curb down tobacco control efforts.

Due to the recent surge in the number of new tobacco products of different designs and modes of operation, there is a need for a fast and comprehensive method of aerosol assessment, that will identify and quantify known and unknown chemical compounds in tobacco emissions for users' awareness and regulatory purposes. Hence, the second chapter of this thesis introduces non-targeted analysis that has been adopted by both independent and industry-affiliated researchers as a viable method for a rapid and

thorough characterization of tobacco product emissions. Although this method has shown great potential in the identification of not only predetermined analytes of interest, but also product specific compounds, it is currently being challenged with problems that span the range from sample collection and preparation to data analysis, and in between chromatography and mass spectrometry challenges. *Chapter 2 will be submitted as a Perspective to Analytical Chemistry (an ACS journal), due to its timely relevance.*

Chapter 3 highlights the importance of considering user behavior in the assessment of toxicants from tobacco products. In the 1950s and '60s, Light and Ultra-Light cigarettes were introduced as reduced tar and nicotine cigarettes in response to growing public awareness of the health risks of smoking. Later, independent research showed that smoker behavior alteration (i.e., more puffs, larger volume puffs, and cigarette filter vent blocking), also known as compensatory smoking, yielded nicotine, tar, and toxicant levels comparable to Regular cigarettes. A similar influence of user behavior on nicotine delivery and toxicant emissions from an e-cigarette was recently addressed. Our group and others showed that user behavior in response to some product characteristics (e.g., lower nicotine content), in addition to the possibility of customization of some devices, has a substantial effect on the levels of toxicants in ECIG aerosol, and ultimately on user exposure. Similarly, the influence of user behavior, namely the puffing regimen, on toxicant emissions from IQOS was assessed by several groups. Reports showed that using an intense puffing regimen like HCI to quantify toxicant emissions from machine smoked IQOS yielded higher levels of toxicants compared to ISO puffing regime, albeit several folds lower than a combustible cigarette. Other reports also assessed IQOS-specific puffing regimens induced from monitoring actual IQOS user puffing behaviors. In contrast, only one report evaluated the influence

of device cleaning protocols, as part of user behavior, on charring of the tobacco plug and melting of the polymer-film filter. This report showed that leftover residues could build up on the heating blade in consecutive use sessions if the device were not properly cleaned. Heating these residues may increase the formation of toxicants or the emission of unexpected chemical compounds. These observations from IQOS literature highlight the rationale and significance of the work presented in Chapter 3 of this thesis.

We conducted a non-targeted analysis using gas chromatography tandem mass spectrometry on IQOS, a heated tobacco product that is rapidly gaining popularity among smokers. Although IQOS aerosol is reported to be less complex than cigarette mainstream smoke, there is still a need to fully characterize its chemical composition before a concrete comparison can be made. Therefore, we assessed the influence of use patterns on IQOS aerosol composition to ensure that users are always exposed to lower toxicants compared to smokers. The user behavior effect on toxicant emissions was studied under two main categories: the effect of device cleanness between consecutive use sessions and the effect of puffing regimen that may alter the heating temperature.

CHAPTER 2

EVOLUTION OF TOBACCO PRODUCTS

2.1. A Historical Glimpse of Tobacco

Nicotiana tabacum is a native plant of the Americas and its cultivation dates to 5000-3000 BC. By the time the European explorer, Christopher Columbus, and his crew arrived on the shores of the "new world" in 1492, tobacco use had already gained prominence on the American continent.[1] In 1560, Jean Nicot, a French diplomat, introduced tobacco he acquired from the Portuguese colony of Brazil to the French court and then to Northern Europe, to be known as "herba regina" or the queen's herb, and used it as a remedy to several ailments including cough, headache, and asthma.[2] The early use of tobacco included snuffing, chewing, drinking (like tea), and smoking.[1, 2] Over the years, various tobacco products have been developed for mass consumption. Some of the earliest of these products are pipe tobacco, cigars, cigarette, plug chewing tobacco, and snuff. Recently, new products with advanced technology such as E-cigarette and Heated tobacco products have been marketed.

2.2. Combustible Tobacco products: Cigarette and Cigar

In its earliest version, cigarettes involved wrapping raw tobacco leaves in papers,[3] like in Spain and Portugal before the 18th century, where Virginia tobacco cigarettes called "Cigaritos" were very popular.[3] However, the smoking style changed with the introduction of curing (tobacco leaves drying) methods in the middle of the 19th century.[4] Various curing methods including freeze-drying, air-curing, sun-curing, and flue-curing exist and the choice of curing method influences the tobacco quality (aroma,

texture and color).[5] For instance, flue-curing of Virginia tobacco involves hanging tobacco leaves in barns, where heated air is circulated to dry the leaves, while air-curing is used for Burley tobacco and the tobacco leaves are left to dry over time. Curing also affects tobacco constituents, as studies have shown that flue-cured Virginia tobacco has a high content of sugars, that break down to organic acids during smoking leading to protonation of tobacco alkaloids, especially nicotine.[6, 7] Protonated nicotine is less harsh to inhale, making cigarette smoking more appealing compared to tobacco products filled with air-cured tobacco.[8, 9] Unlike cigarettes that are wrapped with paper, cigars are a roll of tobacco that is wrapped with a tobacco leaf. As of the 18th century, the cigar has gained prominence as the prevalent form of processed tobacco in Europe,[10] however, dropped from the top of tobacco products list because of its acrid and strong taste resulting from the predominance of freebase nicotine (unprotonated) in cigar smoke usually made from air-cured tobacco.[10]

After the first and second world wars, there was a surge in tobacco cigarette consumption. The increase was so drastic that 80% of men in Britain were regular smokers and doctors in hospitals often offered cigarettes to patients.[3] Prior to the world war era, cigarettes were majorly considered luxury products, however, the introduction of cigarette rolling machines resulted in an increased production of cigarettes, and reduced cost of purchase.[11] The robust and skillful marketing of cigarette smoking as healthy and tasteful, further played a significant role in the surge of tobacco use in America and other nations of the world.[3]

Public concern about the health implications of smoking began to gain ground due to noticeable increase in the number of lung tumor related death in the early 20th century.[12] In the first monograph on lung cancer published in 1912 by Isaac Adler, the

abuse of tobacco and alcohol was linked to increased occurrence of malignant neoplasms in the lungs.[12] However, causality of Lung cancer by cigarette smoking remained quite alien in the early 20th century. At that time, surgeons were postulating various reasons to be the possible cause of the increased cases of lung cancer. Aside from smoking, other possible causes that were linked to lung cancer included dust from asphalts used in road construction and industrial air pollution.[3] Solid evidence attributing cigarette smoking to be the predominant cause of lung cancer began to emerge a few decades into the 20th century. In 1950 alone, retrospective studies of cancer patient smoking habits were assessed by four different groups.[13-16] The results of these studies established that people who smoked cigarettes are highly prone to develop lung cancer. Another report published by Doll and Hill in 1954 concluded that smoking 35 cigarettes or more per day can increase the chances of developing long cancer by a factor of 40.[17] Apart from population studies that gave evidence of cigarette smoking leading to lung cancer, *in vitro* and *in vivo* studies led to similar conclusions. The combination of experiments conducted by Angel (1931) [18] and Ernst et. al (1953)[19] showed the ability of tobacco cigarette smoke condensate and tar to generate tumors in rabbits and mice respectively. Furthermore, the findings of Anderson Hilding in 1956 established the ability of cigarette smoke to cause pulmonary ciliastasis–the loss of movement of cilia cells, a tiny hair-like structure that is found in the airway of our lungs that are responsible for the screening of particulate contaminants from the lung.[20]

Starting from 1960s, researchers began to identify carcinogens such as polycyclic aromatic hydrocarbons and nitrosamines in tobacco tar emissions.[21] These discoveries further raised questions about the actual composition and safety of cigarette smoking. Tobacco manufacturers, at that time, disagreed with the validity of scientific findings that

linked cigarette smoking to various diseases, however, despite their ardent refusal to admit the facts, more research evidence from various tobacco smoke analyses was pointing in the same direction.[13, 22] To verify the accumulating reports that linked smoking to various diseases, the US Surgeon General opened an investigation in 1962. The report of this investigation substantiates the fact that cigarette smoking is hazardous to health and can lead to illness and death from lung cancer, chronic broncho-pulmonary diseases, and cardiovascular diseases, among other diseases.[23] The 1964 US Surgeon General report was a turning point in tobacco history as it definitively linked tobacco smoking to lung cancer and other ailments.[21]

In response to the enormous evidence that reveals the health impact of cigarette smoking, tobacco manufacturers partnered with health agencies, like the National Cancer Institute, to work on low-emissions cigarettes.[24, 25] The first tobacco harm reduction approach was the introduction of filtered cigarettes that were advertised to emit lower tar and nicotine yields.[26, 27] During the 1950s, many filter modifications were tested and marketed by the manufacturers. For example, they designed and marketed charcoal filter tips to “reduce” volatile compounds in smoke.[28, 29] However, the charcoal filter tip was ineffective and a cellulose acetate filter was introduced as allegedly a better alternative.[30] In addition, filter perforation through ventilating holes was designed to dilute the smoke during puffing. This turned out to be one of a series of industry maneuvers to outsmart tobacco control scientists and regulators.[31] Although smoking machine measurements in the lab showed a significant reduction in tar and nicotine content, the actual user behavior, in terms of compensatory smoking, which involves an increase in puff duration, puff volume, and blocking of ventilating holes, has made this approach yield little to no effect.[32-35]

Furthermore, tobacco cigarette manufacturers began advertising cigars (little, cigarillos, and large) as a less harmful alternative.[36]. However, various studies have refuted the less risky notion, while establishing the association of cigar smoking to health diseases like aerodigestive tract cancer,[37, 38] lung cancer,[39] and other diseases.[40, 41] Cigars can be characterized based on type or size (large cigars, little cigars, or cigarillos) and filter (filtered and unfiltered).[42] [43] The indistinguishable design of little cigars to that of filtered cigarettes coupled with enormous publicity and flavor addition made this product widely accepted among smokers.[43] In the United State, the sale of cigar products is rapidly increasing.[44] For example, between 1993 and 1998, cigar sales in the United States increased by almost 50% to reach 4.5 billion cigars sold,[45] while from 1995 to 2008, sales increased by 316%.[46] Notably, about 14.2% of young adults between the age of 18 to 24 use cigars, therefore making it the second most popular tobacco product after cigarettes in this population.[47]

2.3. Alternative Tobacco Product (ATPs)

Recently, tobacco manufacturers have been making relentless efforts to introduce products that appeal to their increasingly health-conscious consumers who like to switch to less harmful alternative products that will deliver nicotine but reduce toxicant exposure when compared to combustible cigarettes.[48-50] These products are called Alternative Tobacco Products (ATPs) including heated tobacco products (HTPs) and electronic cigarettes (ECIG) among others. Although ATPs are categorized as less harmful, available data have shown that they do not necessarily reduce users' exposure to harmful or potentially harmful constituents (HPHCs).[51, 52]

2.3.1. Electronic Cigarette (ECIG)

Electronic cigarette (ECIG) is a device that was patented in 2003,[53] and operates by aerosolizing a nicotine-containing liquid solution to generate inhalable aerosol.[54] ECIGs are usually made up of a battery (power source), a heating element (coil or atomizer), a cartridge or liquid reservoir for holding ECIG liquid, and a mouthpiece.[55] The liquid solution is a mixture of nicotine (a dependence-producing drug), propylene glycol, vegetable glycerin, flavorings (menthol, coffee, fruit, candy, etc.) and other additives.[53, 56]

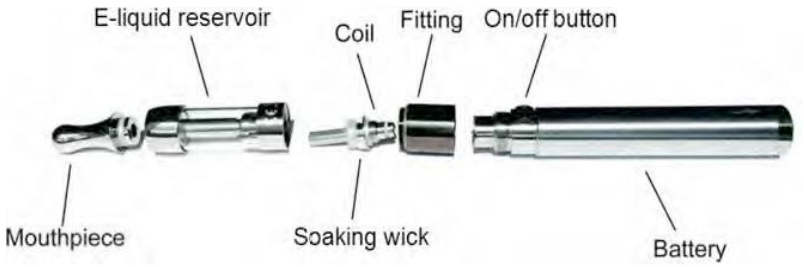


Figure 1: Parts of an electronic cigarette.[55]

The principle by which ECIG operates is still the same despite the wide variation in designs and appearance that is present in the market today. During puffing, the heater coil is activated and the liquid that surrounds the coil is heated up to produce a hot vapor which is directed by the air drawn through the device from coil surrounding to the mouthpiece.[57] A process of recondensation then takes place as when hot vapor hits the cooler air and inner surface of the mouthpiece to produce an aerosol mist that visually mimics tobacco smoke as illustrated in Figure 2. [57]

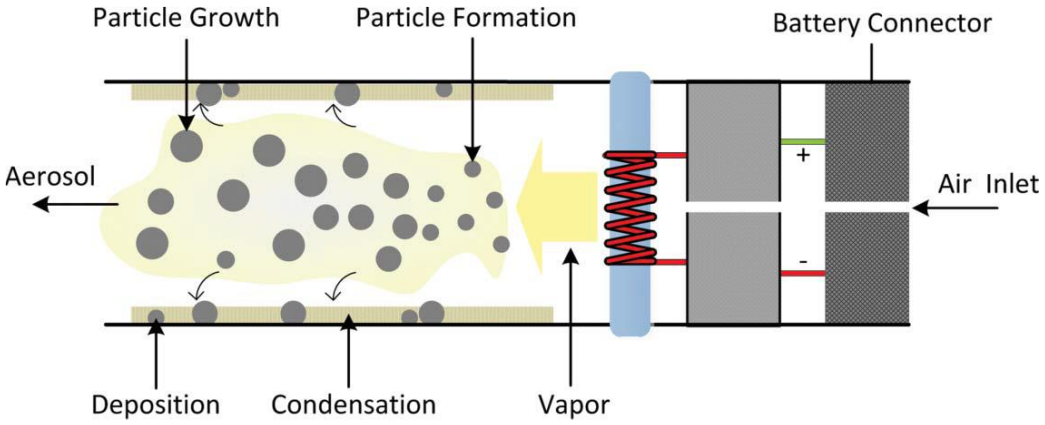


Figure 2: A schematic showing ECIG aerosol generation. [57]

The ECIG market is a moving landscape and various generations of ECIGs have emerged. The first generation of ECIG was cig-a-like in design having a close resemblance to tobacco cigarettes. Most of the first-generation ECIGs were closed systems, i.e., they are not made to be refilled with e-liquid, neither can the battery or the atomizer be replaced, and they were disposable after use.[55, 58] The second generation of ECIG was a pen-like device or "personal vaporizer", and when compared to the first generation, these devices were larger in size and have a larger battery capacity, which allows longer vaping periods of 1-2 days. In addition, some of the product designs allowed the user to adjust the voltage and change liquid constituents.[58, 59] The third-generation ECIG was called a "mod" and could reach very high power because of using very low resistance coils, so they were often referred to as sub-ohm devices. Mod's high power allowed them to produce more aerosol by increasing the heating efficiency of the atomizer coil and delivering more flavor to the user.[58] The fourth-generation came with added features to the mod of the third generation, and they were fitted with automatic temperature control through a temperature sensor.[58, 60] A newer version of ECIGs known as pod-mods has been recently introduced, with one example, *JUUL,* leading the ECIG market for the last few years.[61]

Figure 3: Example of ECIG device and their parts.[55]

Within the last decade, ECIGs have grown in popularity worldwide among smokers who are willing to quit smoke and those who wanted to explore new nicotine delivery methods. However, it has largely become an access point to nicotine-naïve users that never considered being smokers.[62] Between 2011 and 2018, market research group Euromonitor estimates the number of ECIGs adult vapers increased from 7 million to 41 million.[63] Likewise, the global market capitalization of ECIGs has exponentially increased from $6.9 billion in 2013 to about $19.3 billion as of 2018.[63]

The prevalence of ECIGs among youth is alarming. A 2015 report showed that 13% of Hungary's children between 13 to 15 use ECIGS on a regular basis. Also, 8.2% of Poland's high school students and 4.5% of children in South Korea between 13–18 of age are current ECIG users. [64] Similarly, a study among Middle and High School Students in the United States between 2011-2018 showed that 10% of middle and 27% of high school students are current ECIG users.[65, 66]

Although ECIGs are believed to produce fewer and lower toxicants when compared to combustible cigarettes, yet extensive research has questioned ECIG risk potential, especially to non-smokers. ECIGs have shown harmful effects on human health

especially on cardiovascular,[67] pulmonary,[68] nervous,[69] and reproductive systems.[69, 70] Therefore, to make ECIGs less attractive, Food and Drug Administration (FDA) in the United states has recently banned some ECIG flavors and also mandated tobacco industry to write a warning statement on the packaging.[71] In addition, FDA also mandated Premarket Tobacco Authorization (PMTA) for any new tobacco product, forcing manufacturers to provide data showing that their new product protects public health (i.e., users and non-users). It also obliged the manufacturer to scientifically prove that the new product will not encourage non-smokers to begin smoking while reducing the numbers of current smokers.[72]

2.3.2. Heated Tobacco Products (HTPs)

Heated Tobacco Product (HTP) is another alternative tobacco product that big tobacco manufacturers are vehemently promoting among smokers as less harmful than combustible cigarettes.[73-75] As their name implies, HTPs generate aerosols by heating tobacco to a relatively low temperature compared to combustible cigarettes. In 1988, R.J. Reynolds introduced the first HTP called “premier”, however, due to the lack of acceptance of this product among smokers, the production was stopped.[76] In 1998, Philip Morris International (PMI) introduced an HTP named “Accord”, which was made up of a tobacco stick (low tar) and a battery-powered heater. When the user puffs, the tobacco filler is heated, producing an inhalable tobacco-flavored aerosol.[77, 78] However, Accord was not a successful product, and a successor product that relies on the same mode of operation was reintroduced into the market in 2014 after serious design modifications to meet users demand. Also, in 2016, British American Tobacco (BAT)

presented an HTP called “glo” to the market,[79] while Japan Tobacco developed a hybrid of an HTP and an ECIG, called “Ploom TECH”.[79]

The most popular HTP, IQOS, is a heat-not-burn tobacco product that is made up of an electronically controlled heating element, called the holder, and a charger for recharging the holder (Figure 4).[80] The holder is pen-shaped and has a metal blade that can heat up tobacco sticks (called “HEETS”) to a maximum temperature of 350°C.[80] When the maximum temperature of 350°C is reached, the energy supply is cut off.[81] In addition, the device is designed to turn off automatically after 6 min or 14 puffs, whichever comes first.[81] The IQOS stick, called “HEET”, is different in design from normal cigarettes, and it is made up from processed tobacco that has been reconstituted into sheets through the addition of glycerin, water, and polysaccharides.[81] IQOS tobacco stick has two filters, in which the first is a polymer-film filter while the second is a cellulose acetate mouthpiece filter. The tobacco plugs and the polymer-film filter are separated by a hollow acetate tube.[81] Because the temperature of the IQOS is lower than the temperature range of a combustible cigarette (650-950 ºC), reported data showed that toxicant emission is lower in IQOS emissions.[82, 83]

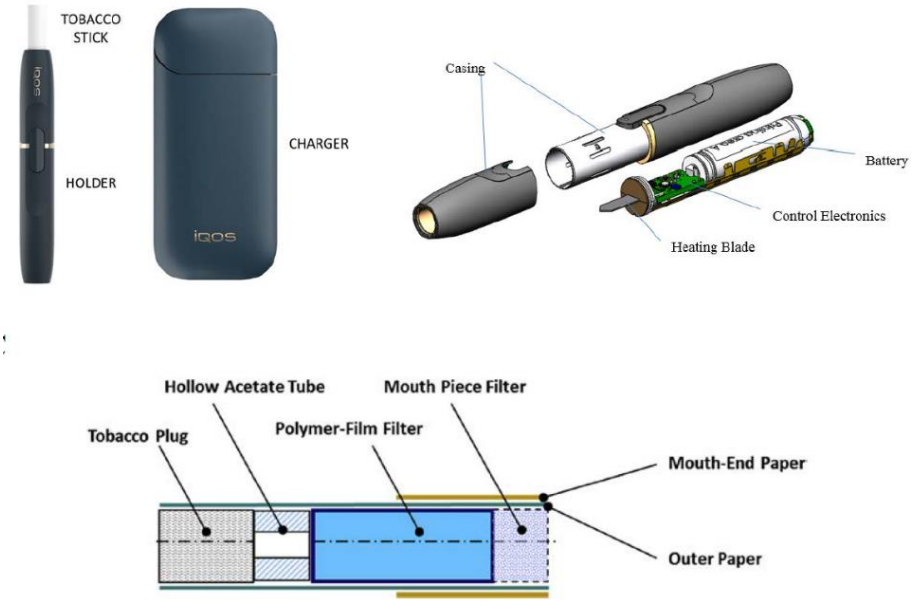


Figure 4: IQOS components (A), a schematic of the Holder (B), and a schematic cross-sectional view of the Tobacco Stick (C).[84]

In 2014, PMI introduced IQOS into the world market, starting with Japan and Italy. Currently, IQOS is leading other HTPs in global market, having about 17 million users across over 60 countries.[85] In 2019, IQOS was introduced to the US market,[86] and a report that same year shows that over 1.6% of students were current IQOS users.[87] In Japan, the percentage of people who utilized IQOS rose from 0.3% in 2015 to 0.6% in 2016 and 3.6% in 2017.[79] Within a year of introduction into the Korean market, PMI sold over 2 million IQOS devices.[88]

The US Food and Drug Administration (FDA) recently announced that tobacco products with the potential to lessen risk when compared to cigarettes could be classified as modified risk tobacco products (MRTP).[89] In response, Philip Morris International (PMI) filed an application in December 2016 to have IQOS classified as a Modified Risk Tobacco Product (MRTP) in the United States.[78] Although PMI was authorized to market IQOS with “reduced exposure” but not “reduced risk” claims, various researchers have contested by re-examining PMI’s data to show that IQOS reduced exposure to carefully selected toxicants by PMI researchers, but increased exposure to other toxicants including carcinogens. Also, reduced exposure to some toxicants does not readily translate into reduced health risk.[90] Furthermore, contrary to PMI’s reduced potential harm biomarker study,[91, 92] when comparing IQOS emissions to cigarette smoke,[93, 94] a statistical assessment of this data showed no statistically significant reduction in most of the biomarkers of potential harm.[95, 96]

2.4. Tobacco Research Agenda

The newly introduced ATPs hold promise to public health if used by smokers as a less harmful alternative and complete substitute to smokers' deadly habit of cigarette smoking. On the other hand, ATPs can cause an irreversible harm to public health if they hook a new generation of nicotine addicts, setting a long journey of suffering premature morbidity and mortality to youth that started using these products yet could have never considered using any traditional tobacco product. More epidemiological research is needed to highlight trends and features of uptake and continued use of these products among vulnerable population like youth to better tailor regulatory interventions. Also, longitudinal data on long-term health effects of these products are direly needed to better understand their impact on public health.

In addition, preclinical data and basic science research are needed to better understand the toxicant profile of these products. For example, a recent report showed that although IQOS operates at lower temperature compared to a combustible cigarette, there is a possibility to have product-specific toxicants.[80] The study reported the detection of a toxic chemical (formaldehyde cyanohydrin) resulting from heating the polymer-film filter of the tobacco stick, and showed evidence of charring on the tobacco plug, leading the authors to claim that IQOS is not precisely a "heat-not-burn" product.[80] This report highlighted that chemical analysis of tobacco product emissions that rely on a known set of targeted analysis, usually inherited from decades of research on combustible cigarettes, may underestimate the toxicity risk of ATPs. Therefore, complimentary to targeted chemical analysis of tobacco product emissions, non-targeted analysis (NTA) is critical to highlight the unique profile of toxicant emissions for any new tobacco product. The next chapter of this thesis addresses the importance of NTA in

tobacco research, and the last chapter presents empirical data of NTA applied to IQOS emissions under different use conditions, encompassing puffing behavior and device cleaning.

CHAPTER 3

NON-TARGETED ANALYSIS IN TOBACCO RESEARCH

3.1. Introduction

Decades of research on combustible cigarettes have yielded extensive characterization of the chemical profile of cigarette smoke, with about six thousand (6000) toxicants including carcinogenic, mutagenetic, and respiratory toxicants detected and quantified.[97, 98] Several methods have been developed specifically to detect tobacco emissions. For example, the detection of nicotine forms in cigarette smoke was widely studied by industry affiliated and independent researchers.[99-103] Both parties developed different methods that varied in techniques, scope, and repeatability to quantify nicotine forms in smoke. On the other hand, for the detection and quantification of other smoke emissions, methods were adopted from other fields and were continuously revisited and optimized.[32, 104-107] However, the number of detected chemicals in cigarette smoke does not readily translate into the number of used analytical methods, as some of these emissions could be classified into families and subcategories that could be detected simultaneously using a single analytical method. Also, several targeted screenings were historically used to compare cigarette brands and design features to reflect variability in cigarette smoke constituents that could impact abuse liability and toxicity of these products. However, for the generic profiling of cigarette smoke, non-targeted analysis (NTA) was only recently employed.

To have a comprehensive chemical characterization of cigarette smoke, researchers developed and applied NTA methods that rely on the detection and sometimes semi-quantification of chemically unknown compounds identified or postulated without the need for analytical standards.[108, 109] NTA relies on molecular features that are

indicative of unknown individual compounds like accurate mass, retention time [RT], and molecular fragmentation induced from mass spectrum to be compared to databases of chemical suspects for the identification of a plausible match.[108, 110] NTA application in tobacco research caught interest with the advent of alternative tobacco products (ATPs) and the continuous development in the technology of high-end chromatography instrumentation.

In comparison to the extensive work done over the years on tobacco cigarettes, more work needs to be done to determine the toxicant profile of the new and emerging tobacco products. The bulk of the research that has been done on ATPs is targeted toward specific classes of compounds; analytes of interest are predetermined, and methods of detection are known beforehand or optimized accordingly. Although targeted analysis has the advantage of accurate compound identification and quantitative data precision, they are unable to detect unknown compounds. Since most of the targeted analyses of these new products are focused on toxicants identified in tobacco cigarette smoke, it may be limited in providing a robust toxicity profile of new tobacco products as this approach does not take into consideration the peculiarity of each product and the ability to give product-specific toxicants. Cigarette, for instance, generates smoke through combustion, reaching a temperature of 900 °C. However, HTPs function by heating a tobacco filler (usually made up of reconstituted tobacco, cellulose, and polymer filters) to a lower temperature compared to combustible tobacco cigarettes. In addition, ECIGs generate aerosols by vaporizing a nicotine-containing liquid on a battery-powered heated coil. This gradient of operating temperature between combustible cigarettes and other emerging tobacco products is suggestive of a product-specific toxicant profile.

Although there is an increasing interest in the use of NTA for the detection of new toxicants in tobacco emissions, there have been lots of challenges that are associated with different methods used for the characterization of a large matrix of unknown chemicals.[108] The Various combinations of chromatographic methods (*i.e.,* liquid chromatography and gas chromatography), ionization methods (electro-spray, electron, chemical), add mass analyzers (time-of-flight, quadrupole, ion trap) adds to the complexity of the qualitative and quantitative characterization of suites of chemicals from tobacco products. To have an inclusive screening, it is expected that the analytical method should have a good resolution and the sample preparation should be broad enough to cover chemicals with different polarity and volatility in the sample matrix. These challenges will be discussed in this chapter highlighting the potential of NTA in tobacco research. *Based on a recent communication with the editorial office of Analytical Chemistry, and viewing its timely relevance, this chapter will be submitted as a Perspective to this ACS journal (impact factor = 6.986; The Most Cited Journal in Analytical Chemistry).[111]*

3.2. Screening of Cigarette Smoke

Cigarette smoke is a complex mixture of gases and particles that contains more than 30,000 compounds, most of which have been confirmed as detrimental to human health.[112] The complexity of this matrix is far higher than ATP emissions. However, despite this complexity, various research groups have made efforts to comprehensively analyze the smoke composition. Some reports focused on the particulate phase,[32, 104, 105] while others focused on the volatile component of the smoke [107, 112-114]. Furthermore, to reduce the complexity of the sample matrix, sample pretreatment that

separates the tobacco smoke complexes into acid and base fractions has also been used.[32, 104, 105]

In a series of three reports, Lu et al. characterized the chemical profile of the particle phase of cigarette smoke trapped on filter pads using two-dimensional gas chromatography tandem time-of-flight mass spectrometry (GCxGC-TOFMS).[32, 104, 105] These reports were classified according to the sample preparation technique, splitting the smoke condensate into an acidic fraction [104], a basic fraction[32] and a neutral fraction.[105] Out of the analytical methods tested for the three fractions, GCXGC/TOFMS gave a better result compared to one-dimensional gas chromatography (GC-MS). However, when GCxGC-TOFMS was used, a total of 4000 compounds were detected of which 1800 were tentatively identified.[105] Furthermore, in the acidic fraction, more than 1000 compounds were detected including 139 organic acids and 150 phenols which were tentatively identified,[104] and in the basic fraction, 377 nitrogen-containing compounds (including 155 pyridine derivatives, 104 quinoline/isoquinone derivatives, and 56 pyrazines) were tentatively identified.[32] To be noted is that Lu et al. are affiliated with the tobacco industry.

Another group affiliated with the tobacco industry, this time to PMI (the manufacturer of Marlboro cigarettes and IQOS), reported an NTA approach that integrates multiple analytical methods and compound identification strategies to characterize the chemical profile in the particulate phase of cigarette smoke.[115] They used liquid chromatography coupled to high-resolution accurate mass spectrometry (LC/HRAM-MS) with a combination of several chromatographic and ionization techniques including a reversed-phase LC with heated electrospray ionization (RP-LC-

HESI), RP-LC with atmospheric pressure chemical ionization (APCI), and hydrophilic interaction liquid chromatography (HILIC) with HESI. They used their in-house developed Unique Compounds & Spectra Database (UCSD) and the National Institute of Standards and Technology (NIST) MS libraries for compound matching. An *in-silico* fragmentation method was used when a compound cannot be found in the two databases. The semi-quantified concentration for each compound was estimated by comparison with an internal standard (ISTD) of known concentration allowing the detection and semi-quantification of 331 compounds of which fifty (50) are novel compounds.

An industry-independent group reported the use of a Fourier transform ion cyclotron resonance mass spectrometry (FTIR-MS) coupled to laser/desorption ionization (LDI) for the analysis of the particle phase of cigarette smoke trapped on filter pads. However, since polar compounds are poorly detected by LDI, electrospray ionization (ESI) was used as a complementary method. This approach was very effective in the detection of heteroaromatic and highly oxygenated compounds in cigarette mainstream smoke.[116]

The NTA of volatile organic compounds (VOCs) and semi-volatile compounds (SVOCs) in mainstream cigarette smoke has been done using various methods. A study reported using a solid-phase microextraction (SPME) coupled to GC–MS for VOC sampling, leading to the identification of 70 VOCs.[112] However, SPME coupled with GCxGC-TOFMS led to the identification of double the number of VOCs.[106] Other VOC and SVOC trapping techniques that were employed in NTA included thermal desorption (TD) coupled to GC–QTOF allowing the detection of 133 compounds related to characterizing flavors in cigarettes.[114] Automatic peak deconvolution and detection

were done by comparing acquired mass spectra with reference spectra in the NIST library and the comparison of linear retention indexes with those reported in the literature.[114] Also, when TD was coupled with GCxGC-TOFMS, a better resolution was obtained and 130 compounds were reliably identified.[107] A recent study reported the accurate identification of 11 VOCs in mouthpiece cigarette adhesives using headspace sampling with GC–MS.[113] It is noteworthy that the majority of this work is reported by tobacco industry researchers.

3.3. NTA for Screening of HTP Emissions

The use of NTA for the assessment of the toxicant profile of HTPs, as new tobacco products, is critical and was recently addressed by several industry-affiliated research groups. The affiliates of PMI, the manufacturer of IQOS, reported using NTA to answer the question of a likely formation of compounds with toxicity concerns in IQOS aerosol.[117] To comprehensively characterize the IQOS aerosol, the particle phase was trapped on filter pads and the gas phase in cryogenic impingers, and the samples were analyzed on GC×GC-TOFMS and LC-HRAM-MS. For the LC-HRAM-MS method, the authors adapted four methods: HILIC-HESI with positive mode ionization, RP-LC-HESI using both positive and negative mode of ionization, and RP-LC-APCI with positive mode, to cover a wider suite of compounds. In order to analyze the different classes of compounds found in IQOS aerosol, Bentley, et. al incorporated three GC×GC-TOFMS analytical methods for capturing non-polar, polar, and volatile compounds. Each of these three methods makes use of different GC-columns and separation parameters that will improve the quality of the chromatographic spectrum. Also, different sample preparation steps were used to fit each of the analytical methods.[117] For the polar compound

method, the team did not use a derivatizing agent as it is common in targeted analysis, rather, they used a GC column that is suitable for polar compounds. This comprehensive approach allowed the detection and semi-quantification of 529 chemical constituents in IQOS aerosol (Figure 5).[117]

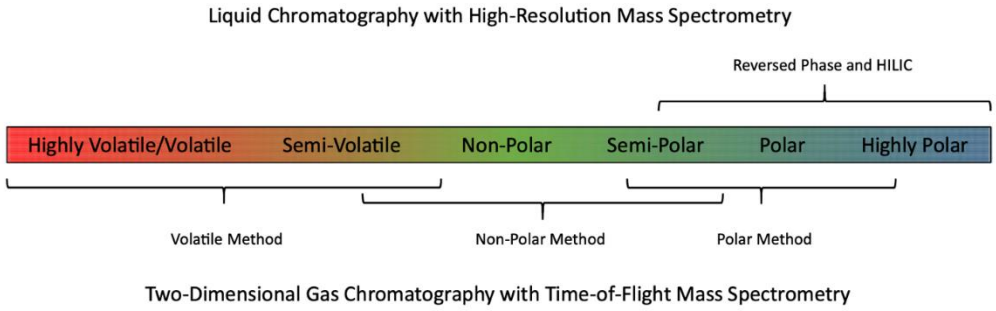


Figure 5: Illustration of the NTA approach used for the comprehensive chemical characterization of IQOS aerosol.[117].

Similarly, a group of researchers affiliated with British American Tobacco (BAT), another manufacturer of HTP, reported three NTA analyses of HTP aerosol.[118-120] Two of these papers investigated the presence of VOCs in the vapor phase and particulate phase of Glo (BAT HTP) aerosol respectively,[119, 120] while the third assessed both the volatile and semi-volatile organic composition (VOC/SVOC) of the particulate extract of IQOS.[118] Complimentary methods of thermal desorption (TD) and GC×GC-TOFMS/FID and TD-GC×GC-HRTOFMS were used in the assessment of VOCs present in the vapor phase fraction of Glo aerosol.[119] TD was used as a non-solvent approach for sample collection and preparation.[119, 120] In comparison to solvent-based sampling methods, the solventless approach, prevents the problem of low sensitivity due to multiple extractions and dilution steps.[119] Head space-solid phase micro-extraction (HS-SPME) is another solvent-free method that has been used for trapping particulate-

phase faction of HTP aerosol.[118] Individual methods that include GC×GC-TOFMS and GC×GC-TOFMS/FID were reported in the other two reports.[118, 120]

Since data analysis is a critical step in NTA that can affect the study outcome, different peak deconvolution software has been integrated into the analytical workflow for effective and efficient compound identification. This type of software performs the collision of retention times and peak areas into a related peak table,[120] aiding library search and spectral matching.[117-119] Unlike targeted analysis where direct quantification is done using a specific standard for each compound present in the sample matrix, NTA often uses a semi-quantification approach to determine the quantities of the identified compounds. Semi-quantification methods are usually done by constructing an external calibration curve using compounds that represent different chemical classes,[119] and employing the closest response factors to the internal standard.[118]

3.4. Chemical Profiling of Electronic Cigarette Aerosol

The role of NTA in the comprehensive chemical profiling of electronic cigarette aerosol for the identification of previously identified tobacco-related compounds and/or product-specific compounds is vital in informing users about the actual composition of ECIG aerosol and liquid.[121] Both industry-affiliated and independent researchers have reported NTA of electronic cigarette aerosol and liquid composition.[109, 121-124] To assess the presence of flavor compounds in e-liquid, Augustini et. al used GC coupled to ion mobility spectrometer (IMS) and mass spectroscopy (MS). Since the flavors are essentially volatile, the investigators used a headspace sampling method to reduce the effect of the predominant less volatile e-liquid composition (i.e. propylene glycol (PG),

glycerin (VG), and nicotine) which might mask the desired analytes.[124] In a similar report, a group of researchers used a unit-mass resolution GC-MS in the electron impact (EI) ionization mode to assess the volatile and semi-volatile compounds present in various ECIG liquids and aerosols.[109] They mixed the particulate phase that was collected on a filter pad, and the gas phase that was cryogenically trapped in a solvent together before an aliquot was injected in GC for analysis. [109]

Furthermore, a group affiliated with BAT, reported the characterization of the volatile and semi-volatile organic constituents of e-cigarette aerosol using a GC connected to two detectors (TOFMS and FID). A dean switch was used so that the more abundant components (PG, VG, and nicotine) that were isolated by a cutting sequence using their specific retention time could be sent to the FID for analysis rather than the TOFMS. This process prevented the contamination of the GC-column and oversaturation of the MS by these predominant compounds.[123]

Moreover, to assess the semi-volatile and non-volatile compounds of tobacco-flavored e-liquids and aerosols using Liquid chromatography (LC) coupled with high-resolution mass spectrometry (LC-HRMS), Tehrani et al used an aerosol condensation method that allowed the trapping of ENDs aerosol as liquid condensate, thereby preventing intermediate extraction and sample preparation for LC- HRMS analysis.[122, 125] A combination of analytes unique information such as retention time (RT), accurate mass, and MS/MS fragments were used for the identification of unknown compounds while quantification of identified compounds in e-liquids and aerosol fractions was computed by standard addition method using a confirmation mix stock solution of analytical standards.[122] Their report showed the presence of potentially hazardous

compounds such as tributyl phosphine oxide and the stimulant caffeine which would not have been detected using targeted analysis.[122]

Based on the various analytical methods used, different methods of compound identification were reported. Augustini et. al, identified an unknown analyte using retention index (RI) and reduced mobility that was computed from the IMS drift time and GC-retention time.[124] Furthermore, in combination with the NIST database that has been used by most NTA researchers, [121, 123] some researchers incorporated the use of external standards [121] and in-house developed libraries [109] into peak identification processes to improve compound identification. Besides, where peaks were unidentifiable using libraries, a group of researchers reported the use of a GC-Orbitrap MS system that gives a tentative chemical structure that was then confirmed using reference standards or custom synthesis.[109]

3.5. Challenges of NTA in tobacco Research

3.5.1. Sample generation and collection

NTA has overlapping challenges that begin with the sample generation and collection, although different research groups have considered various approaches to generate, capture and analyze desired analytes. For instance, NTA using SPME fiber is often challenged by the low sample concentration.[107] This is because only a fraction of the target analyte is extracted from the sample matrix. Also, the extraction capacity of the fiber is quite limited, therefore, extensive work is usually required to optimize collection factors like fiber type, temperature, and time required for extraction, incubation, and fiber desorption.[106, 112, 118] Furthermore, since SPME fibers are

usually designed to extract a specific class of compounds, their ability to trap a wider range of compounds, as required by NTA, is limited.[123] Trapping on sorption tubes is another sample collection method that has a limited trapping ability. However, to increase the number of compounds that can be trapped, some groups used a combination of sorbents.[121, 123] For example, Herrington and Myers et. al. were able to trap analytes within the range of C2-C32 when they used three sorbent combinations (Tenax TA, Carbograph 1-TD, and Carboxen 1003), [121] however, a similar analysis conducted by Rawlinson et. al using Tenax TA/Sulficarb sorbent was unable to trap highly volatile compounds (e.g. formaldehyde).[123] Also, tedlar bags and impingers are other sampling methods that are often been used for aerosol collection. The tedlar bag method is usually affected by contamination from background levels of compounds in the sampling bags, [107] while the impinger method suffers from loss of highly volatile compounds during storage and transportation.[126]

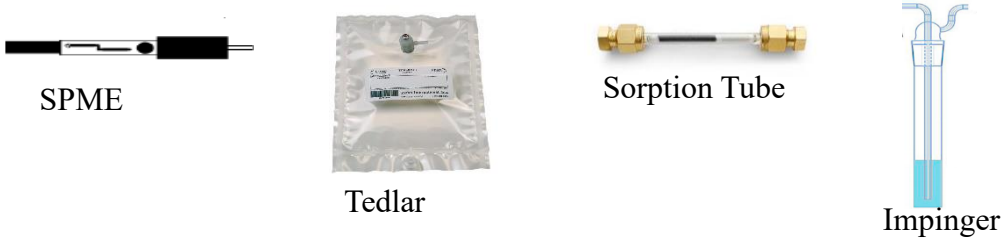


Figure 6: Examples of sampling devices.

Furthermore, because of the differences in tobacco products design and operation, the mode of sample generation for each new tobacco product defers from the commercially available smoking machines and topography recording devices that were designed for cigarette research. In addition, the puffing regimes that can accurately mimic the topography of the actual users of most of the newly emerging tobacco products are still very much lacking. Hence, the current standardized machine smoking regimes such

as the ISO, Massachusetts, or Health Canada Intense are not representative of the topography of users of these new tobacco products.[127] Also, since recent reports have shown that puffing parameters can affect aerosol composition, generating and comparing the toxicity profiles of tobacco under these standard puffing regimes might be misleading.[128]

3.5.2. Chromatography

Despite the huge advancement that has been made on chromatography instruments, the complexity of NTA matrices often results in instrumental damage of some critical parts such as the column and the MS filament among others, hence, reducing the lifespan of the equipment.[117, 121] Furthermore, when a sorption tube is used for sampling, the thermal desorption system prone to contamination from complex matrix, resulting in carryovers between samples when proper cleaning is not performed.[121] To have a less busy chromatogram, and to prevent overloading of the GC-column and contamination of the TD-unit, Savareear, et. al. splitted the vapor phase fraction that was collected in a TD tube into three degenerate TD tubes prior to TD-GC×GC-TOFMS analysis.[119] Similarly, the particulate fraction of HTP aerosol collected on thermal desorption tubes was split across ten second level TD tubes.[118] Because most of the analytical instruments are quite expensive to purchase and maintain, there is a limit to the number of replicate analyses that can be performed on a sample extract, lowering the analytical data robustness.[117]

Depending on the resolution capability of the gas chromatography that is being used, NTA often suffers from coelution, which is due to the bulkiness of the sample matrix.[32, 104, 119, 123] The coelution of chromatographic peaks limits the number of

compounds that can be detected. A group of researchers showed the ability of a 2D-GC-MS to effectively separate a complex sample when compared to 1D-GC-MS. Their results showed a 350% increase in the number of compounds detected when GCxGC was used as opposed to 1D-GC.[32] While 2D-GC-MS has significant advantages over 1D-GC-MS, a large number of peaks remain unresolved.[32] /

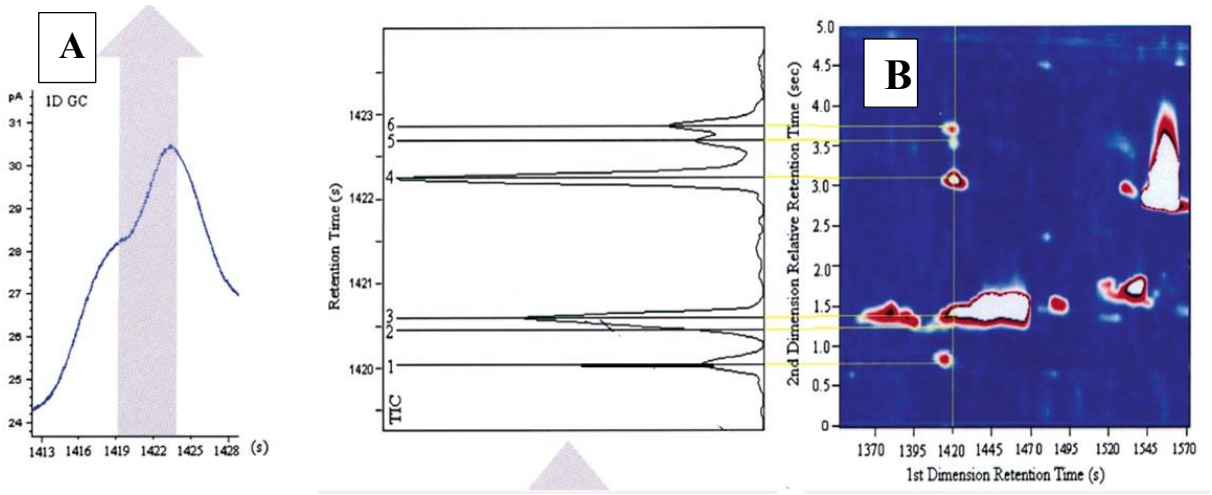


Figure 7: (A) 1D-GC-MS chromatogram with peak coelution. (B) The coeluted peak was separated using 2D-GC-MS into identifiable component.[104]

Moreover, the presence of artifacts in the chromatogram which are derivatives of thermal degradation during thermal desorption or analytical system bleeding can lead to false identification.[113, 123] Rawlinson et. al reported a sharp increase in artefact intensity, and corresponding decrease in analyte abundance when multiple injection of same sample that was recollection in a tube was analyzed.[123]

3.5.3. Mass spectrometry

The mostly used detector in NTA analysis is the mass spectrometer. This is because of its importance in the identification of unknown compounds owing to the uniqueness of each compound's mass fragmentation. However, a major limitation of this detector is its inability to detect electrically uncharged molecules, inorganic acids, and

elemental state metals.[117] Also, because the sensitivity and resolution capability of the MS varies, it can affect the process of compound detection. Therefore, for better compound detection during NTA analysis, there is need for high-resolution mass spectroscopy instruments.

Another limitation associated with MS in NTA is oversaturation of the detector by highly concentrated species such as PG, VG, and nicotine. The ion intensities of these compounds exceed the detection limit of the system thereby preventing accurate calculation of mass-per-charge ratio and intensity values. To overcome this challenge, Shan et. al introduced a heart-cutting process with a deans-switch, that isolates these compounds from entering the MS and sends them to FID for analysis.[121] However, this process might prevent the identification of compounds that are co-eluting with these abundant compounds.[109] Another method that has been used to avoid oversaturation of the MS detector is the reduction of ionization energy at the retention time of the abundant compound. [114]

3.5.4. Data analysis

Data analysis which includes chromatographic peak identification and quantification is usually stressful and time-consuming when done manually.[104, 117, 123]. Hence, deconvolution software has been integrated into their workflow to ease the process.[104, 117, 122, 123] Compared to manual deconvolution, workflow automation fosters reproducible and high data output. For instance, a report showed twice as many chromatographic features when data was processed automatedly as compared to manual processing under the same library match threshold.[123] Despite the benefits of automation in data processing, they can be prone to false-positive signals.[123]

Furthermore, this softwares could frequently be unable to distinguish between true peaks and any other peaks caused by column bleeding and artifacts.[119]

Moreover, the size of a mass spectral library can either increase or decrease the likelihood of accurate compound detection.[123] Therefore, a combination of different library sources are usually used to improve the chances of compound detection.[118-120]. Since most commercially available libraries are made-up of chemical compound spectral data from GC-MS analysis, other analytical techniques, such as LC-HRAM-MS and GC-IMS, do not have such a comprehensive spectral database for compound matching.[117, 122, 124] Therefore, the unavailability of spectral library for analytical instruments other than GC-MS reduces the possibility of identifying unknown compounds.[117] To overcome this challenge, some researchers used a combination of accurate mass and molecular formula information for compound identification,[122] while others used retention index.[124] In addition, the difference in ionization methods used during library build-up and experimental spectral generation often results in low matching factors during compound identification.[109, 117] Also, issues with distinguishing between isomers during peak identification have been reported.[116]

Because there is no standard threshold value for the match factor used for tentative compound identification, there have been minor variations in use among different researchers. This variation may limit the ability to compare results. Some of the values that have been reported include a forward and reverse match factor of > 700 [119, 120] while others used >800.[118]

3.5.5. Semi-quantification

The use of specific standards for the quantification of individual compounds or families of compounds during NTA becomes challenging and cost-ineffective because of the large suite of compounds that is often detected. Besides, suitable standards for some of the detected compounds may not be readily available for purchase. Therefore, semi-quantification is the most widely used alternative to determine compound concentration.[109, 117, 123] However, the closeness of semi-quantified concentration to those of a targeted analysis remains a concern.[117] Bentley, et. al., reported that the semi-quantification analysis of some known HPHCs using GC x GC-TOFMS gave up to ±4-folds deviation from targeted analysis concentrations.[117]

3.6. Conclusion

NTA is a great tool for the comprehensive chemical profiling of tobacco product emissions. It holds potential as a good method for the identification of product-specific toxicants. There is a need for the development of more experimental methods and/or optimization of the existing ones to increase the ability of NTA to detect and quantify more classes of compounds. Researchers must collaborate to find long-term solutions to some of the issues that are currently limiting NTA's potential. As more new tobacco products are expected to emerge in the future, NTA remains the most viable method for a quick determination of their chemical composition in order to inform users of the actual aerosol composition, as well as tobacco regulators for policy making and regulation.

CHAPTER 4

ASSESSMENT OF THE IMPACT OF USER BEHAVIOR ON IQOS EMISSIONS

4.1. Introduction

Decades of research have linked cigarette smoking to deleterious health effects such as lung and liver cancer, cardiovascular, and chronic pulmonary diseases.[129-131] Cigarette smokers are usually aware of the health risks of their deadly habit, yet they fail to quit, due to their addiction to nicotine, the cigarette's main ingredient, which is a strong psychoactive drug.[132] Recently, smokers have been offered "safer" alternatives that are promoted as efficient nicotine delivering products and less harmful compared to combustible cigarettes.[41] The main examples of alternative tobacco products that are now available in the market include HTPs and ECIGs among others. [133, 134]

One of the newly introduced HTPs is IQOS, manufactured by Philip Morris International, Inc. (PMI). IQOS is a "heat-not-burn" device that releases nicotine-containing aerosol by heating a tobacco stick to a temperature (350 °C) much lower than the cigarette combustion temperature (950 °C). IQOS is leading other HTPs in the global market, having about 17 million users across over 60 countries.[85] In July 2020, the US Food and Drug Administration (FDA) authorized IQOS to be marketed with claims of "reduced exposure" but not "reduced risk" in comparison to combustible cigarettes as part of the FDA modified risk tobacco product (MRTP) regulatory mechanism.[135] Thereafter, IQOS popularity and prevalence in the US population is anticipated to grow fast and the FDA is required to closely monitor IQOS market expansion and marketing activities directed towards subpopulations, especially youth.[136] Although IQOS sales in the United States were halted on November 29, 2021, due to infringement on patent

rights of a rival tobacco company, IQOS return to the US market is expected in 2023.[136, 137]

The MRTP application relied on data presented by PMI including chemical analysis of IQOS emissions, toxicity assessment, and assessment of the reduction in biomarkers of potential harm in smokers switching to IQOS for a short period. Although the FDA deemed the evidence presented by PMI sufficient for a "reduced exposure" claim authorization, independent researchers examining PMI data or presenting their own data criticized the FDA decision.[77, 138, 139] Moreover, the MRTP application requires the assessment of the influence of use patterns on aerosol composition to ensure that the candidate MRTP consistently lowers user exposure to toxicants.[140, 141] User behavior is important in two aspects: the device cleaning between use sessions and the puffing regimen that may alter the heating temperature. While most studies looked at the effect of the puffing regimen on toxic emissions from IQOS, [95, 139, 142-145] only one study looked at the effect of device cleaning.[146] According to this report, if the IQOS device is not cleaned properly, leftover residues can accumulate in the heating chamber.[146] We hypothesized that heating these residues may increase the emissions of toxicants or the formation of unexpected chemical compounds. In this chapter, we will discuss our experimental work to test these two hypotheses.

Most studies on IQOS are largely focused on specific compounds of toxicological interest, such as the FDA list of HPHCs,[142, 143, 147, 148] only a few non-targeted analysis have been reported by the manufacturer.[117, 118] The chemical assessment of toxicants in IQOS aerosol was widely guided by that of combustible cigarette smoke.[149-151] Hence, several reports analyzed a comprehensive list of HPHCs to assess their reduction in IQOS aerosol in comparison to the mainstream smoke of a

combustible cigarette.[152, 153] This list includes nicotine, humectants (PG and VG), tobacco-specific nitrosamines(TSNAs), small gases (carbon monoxide, ammonia, and hydrogen cyanide), radicals, carbonyl compounds (CCs), volatile organic compounds (VOCs), phenols, aromatic amines(AAs), and polycyclic aromatic hydrocarbons (PAHs).[128] These reports showed equivalent delivery of nicotine from IQOS compared to cigarettes with a significant decrease in the levels of toxicants like VOCs, AAs, small gases, radicals, phenols, and PAHs, and partial reduction in CCs and TSNAs.[147, 153-155] This significant reduction in the levels of the majority of the analyzed HPHCs is attributed to the low temperature reached in IQOS preventing high-temperature pyrolysis and pyrosynthesis usually encountered in cigarette combustion.[155] Indeed, the more moderate reduction in TSNAs is due to their original presence as contaminants in the tobacco filler and thus not related to the heating process during IQOS activation.[156, 157] Also, the lesser decrease of CCs is attributed to the presence of high amounts of humectants in IQOS that pyrolyze to give carbonyls in the generated aerosol.[145, 158] Another approach of the analytical assessment of toxicants in IQOS aerosol is the non-targeted analysis (Figure 8). This includes the generic toxicity assessment of reactive oxygen species (ROS) and chemical screening or NTA of the trapped IQOS aerosol using GC-MS.[149, 159] An industry-funded report of chemical screening inferred that the chemical profile of IQOS aerosol is a subset of that of cigarette smoke with no new toxicologically relevant compounds deducing that IQOS has lower risk to users.[149] However, such conclusions from targeted and non-targeted analysis are contested by independent researchers saying that the HPHC list assessed by PMI is not complete as per the FDA HPHC list of tobacco products, and also lower exposure does not necessarily mean lower risk.[139, 160]

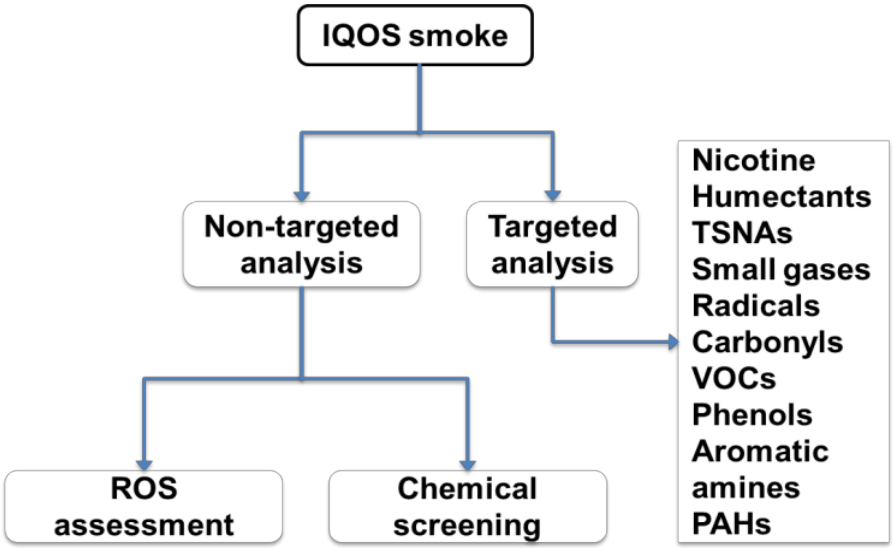


Figure 8: A general scheme of chemical assessment of IQOS emissions.

The above-mentioned studies overlook the impact of user behavior in terms of puffing and device cleaning on the toxicant profile of IQOS emissions. The current study was designed to evaluate the effect of user behavior, including crossover conditions of puffing regimen and device cleaning, on toxicant emission from IQOS. Specifically, we assessed the effect of residue build-up and intense puffing on the emission of selected toxicants (phenols and CCs) as markers of pyrolysis of IQOS Heat stick constituents (i.e., glycerol and cellulose), and we assessed the possibility of formation of IQOS-specific toxicants using non-targeted analysis.

4.2. Materials and Methods

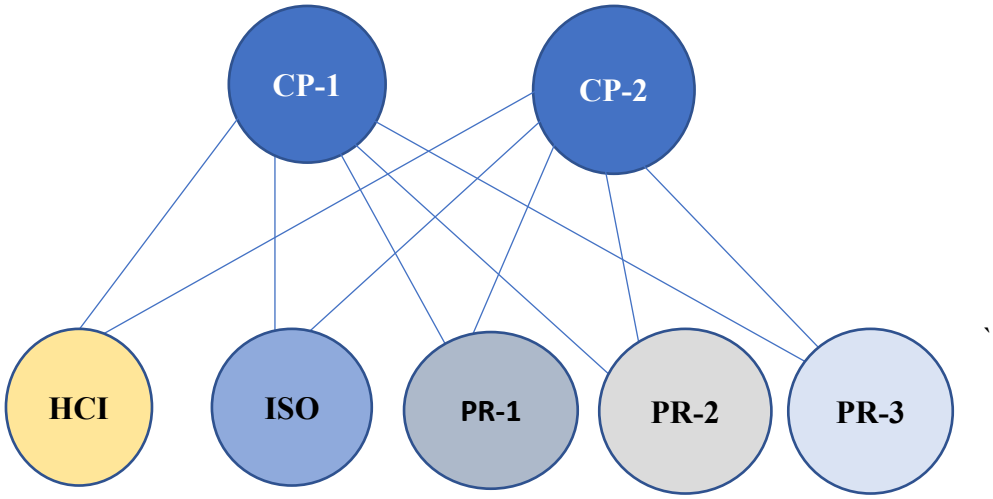
4.2.1. Materials

HPLC-grade Ethyl acetate, and Methanol were procured from Sigma-Aldrich. Quartz Fiber Filter pads (QR-100, 47 mm) were obtained from ADVENTEC. Deuterated standards of *p*-cresol-d8 and benzene-d6 were bought from Absolute Standards and used

for semi-quantification in the non-targeted analysis. Hydrochloric acid (HCl; 37%; CAS 7647-01-0), ascorbic acid (CAS 50-81-7), and sodium bicarbonate (CAS 144-55-8) were procured from Sigma-Aldrich and Fluka. IQOS devices, including Marlboro HEETs (aka HeatSticks), were bought from IQOS official website for the US market.

4.2.2. Study design

To assess the impact of puffing parameters and device cleaning, the study was designed to include two cleaning protocols (CP-1 and CP-2) and five puffing regimens (PRs) (i.e., Health Canada Intense (HCI), PR-1, PR-2, PR-3, and International Standardization Organization (ISO)). The details of the design are shown in Figure 9. The recommended cleaning protocol by the manufacturer (CP-2) will be tested with all the puffing regimens, unlike CP-1 that was tested with HCI and ISO only.[80] This is done to comprehensively understand the combinatorial effect of device cleanness and puffing parameters on the generated toxicants, mainly phenols and CCs. The cleaning protocol CP-1 represents the behavior of an extremely cautious user that cleans her device after each use. Puffing regimens were selected for direct comparison with a combustible cigarette (HCI & ISO), and as induced from our assessment of the limited published literature on IQOS users' puffing behavior (PR-1 to PR-3).[161, 162] These regimens cover the variations that may be experienced by users in puffing rate (puffing flow rate/puff duration) and frequency (number of puffs and inter-puff intervals).



Cleaning protocol

CP-1	After each stick
CP-2	After 20 sticks

Puffing regime

	VOL (ML)	PD (SEC)	IPI (SEC)	PUFF NB	FR (LPM)
HCI	55	2	30	10	1.65
ISO	35	2	60	6	1.05
PR-1	110	4	30	10	1.65
PR-2	55	4	30	10	0.825
PR-3	35	2	30	10	1.05

Figure 9: Study design with details of cleaning protocols and puffing regimens.

Sampling will take place before and after cleaning the device for CP-1. However, for CP-2, sampling will be conducted after the first, fifth, tenth, and twentieth sticks before cleaning and one stick after cleaning the device. Every sample will be tested in triplicates for quality assurance.

4.2.3. Experimental setup

Two sets of sampling (S1 and S2) were carried out depending on the aim of the experiment (Figure 10). For targeted analysis, phenols were quantified in the particle phase collected on filter pads, and gas phase CCs were trapped using DNPH impregnated

silica cartridges placed downstream the filters. For NTA, an accumulation of smoke from five consecutive sticks was used for each sampling session.

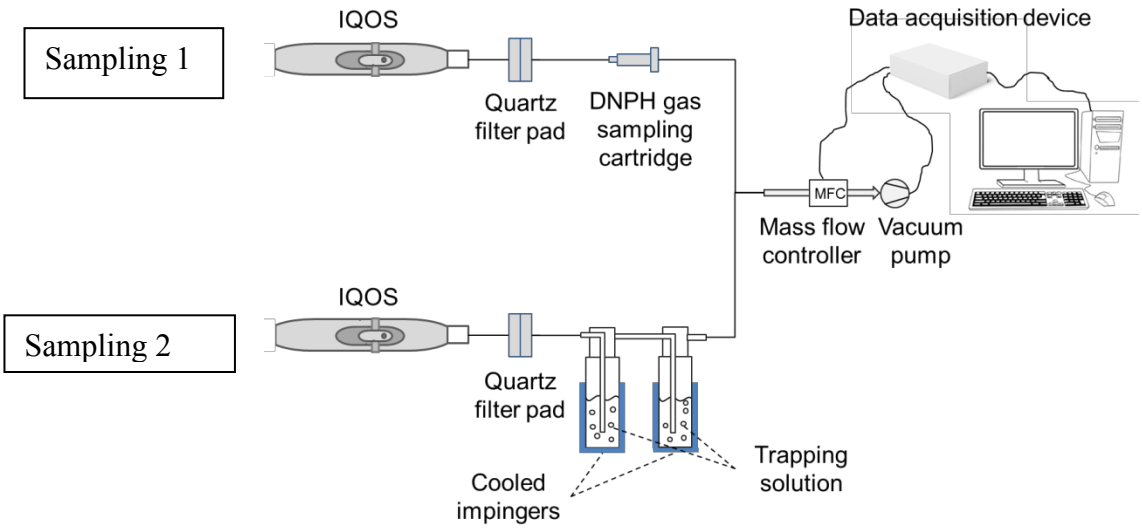


Figure 10: Schematic of the experimental setup for targeted and NTA sampling.

The IQOS cleaning tools including disposable ethanol-soaked cleaning sticks, a plastic cleaning tool, and a brush were used to clean IQOS devices. The cleaning was done according to the manufacturer's specifications mentioned in the IQOS user guide.

4.2.4. Aerosol generation and sampling

Our in-house developed smoking machine, designed by the Aerosol Research Lab at the American University of Beirut (AUB), was used to generate IQOS aerosols.[163] The generated aerosols were analyzed by targeted and NTA methods, under different smoking parameters, for comprehensive analysis of IQOS emissions. The particulate phase of IQOS aerosol was collected on filter pads for both sampling sets and used for the quantification of phenols and posthoc NTA analysis for S1, and real-time NTA analysis for S2. However, the gaseous phase for S1-sample set was trapped using a DNPH cartridge for targeted analysis of CCs (*note: not covered in this Thesis*) while the

gaseous phase for S2 was cryogenically trapped at -50^{o}C using two methanol-containing impingers connected in series. *Only NTA analysis will be discussed in this Chapter.*

4.2.5. Non-Targeted Analysis

For S1 particle phase, phenols were quantified using a silylation derivatization method developed in our group.[164] In brief, this method includes immersing the filter pad in an acidic solution to remove nicotine, then, an organic solvent, ethyl acetate, was added to extract the phenolic compounds to the organic phase from which an aliquot was injected on GC-MS for analysis. The total particulate matter (TPM) collected on the filter pad was calculated gravimetrically. The collected GC-MS spectra were used for post-hoc NTA in which derivatized compounds were screened against NIST library.

For S2, an aliquot of the cooled solvent used to trap the gaseous phase was injected into a GC-MS without any sample preparation to detect VOCs. Benzene-d6 was used as ISTD for semi-quantification. A two-step extraction was done for the particle phase that was captured on quartz filters to remove PG and VG and reduce interference. For the first step, the filter was extracted with ethyl acetate and an aliquot of the extract was injected into GC-MS for NTA analysis. This step was supposed to remove PG/VG interference. The remaining organic phase was further extracted with acidified water to remove nicotine and then an aliquot of the organic phase was injected into GC-MS. Both extracts were spiked with benzene-d6. For all NTA experiments, peak deconvolution was done manually using the NIST mass spectral library. A peak that has a match and a reverse match factor of >750 was considered acceptable. Semi-quantification was done using the ISTD peak areas and concentration

4.2.6. GC-MS method

The GC-MS analysis was performed on a Thermo Trace GC ITQ-900 instrument equipped with a thermostatically controlled AI 3000 autosampler and a TG-5MS Thermo Scientific fused silica capillary GC column (30 m × 0.25 mm × 0.25 μm). The mass spectrometer ionization mode was electron ionization (EI) at 70 eV. The carrier gas was helium delivered at a flow rate of 1 mL/min. The injector temperature was 250 °C, with a split-less injection of 1 μL using a single tapper gooseneck, deactivated, glass wool free liner. The oven temperature program was as follows: hold at 70 °C for 1 min, ramp up 10 °C/min to 200 °C, ramp up 40 °C/min to 250 °C, and hold for 1 min. The total run time was 45min, and the solvent delay time was 3 min and 4.5min for methanol and ethyl acetate sample, respectively.

4.3. Result and Discussion

4.3.1. NTA on phenol filters from S1

Across the different sampling regimes that was used, a total number of fifty-six compounds were identified, as shown in Table 1. Compounds were deemed "identified" when the match factor and the reverse match factor is >750 according to the NIST 2011 library database. The chemical classes included alkanes, carboxylic acids, ketones, aromatic acids, esters, and substituted hydrocarbons.

Although the toxicity data of most of the compounds are largely unknown, some of the identified compounds have been reported to have detrimental effects on human health. For instance, the International Agency for Research on Cancer (IARC) has classified both catechol and 3-chloro-1.2-propanediol as Group 2B, possible human carcinogens.[165, 166]

Table 1: List of unique compounds found in NTA of phenol filters.

Compounds	CAS number
1.3-diethyl-Benzene	141-93-5
2-hydroxy propionic acid (Lactic acid)	50-21-5
hydroxy acetic acid	79-14-1
2,2-Dihydroxyacetic Acid	563-96-2
2.4.5-trimethyl-Benzenaldehyde	4460-86-0.
n-undecane	1120-21-4
1-ethyl-2.3-dimethyl-Benzene	933-98-2
4-ethyl-1.2-dimethyl-Benzene	934-80-5
1.2.3.4-tetramethyl-Benzene	488-23-3
2-hydroxy-2-methylpropanoic acid	594-61-6
2-Furancarboxylic acid	88-14-2
3-hydroxy-propanoic acid	503-66-2
3-chloro-1.2-propanediol	96-24-2
2-methoxyphenol	90-05-1
2-ethyl-3-hydroxypropionic acid	4374-62-3
2-hdroxyl-4-methylpentanoic acid	20312-37
2-hydroxy-hexanoic acid	6064-63-7
benzoic acid	65-85-0
Octanoic acid	124-07-2
phenylacetic acid	103-82-2
2,3,4-trihydroxytetrahydrofuran	
3-Methyl-2-furoic acid	4412-96-8
Catechol	120-80-9
Glycerol	56-81-5
Triacetein	102-76-1
1,2,3-butanetriol	131388 - 1
2-Furanone, 3,4-dihydroxytetrahydro	21730-93-8
3-Methoxy-4-hydroxylbenzaldehyde	121-33-5
lactyl lactate	617-57-2
Hydroquinone	106-42-3
Pentanedioic acid	110-94-1
Acetylpyruvic acid	5699-58-1
6-hydroxyl hexanoic acid	1191-25-9
2-Furanone, 3,4-dihydroxytetrahydro	
(E)-5-isopropyl-8-hydroxy-8-methyl-non-6-en-2-one	60828-13-9
2-Acetyl-3-methylbutanoic acid	59144-21-7
4-hydroxyphenylglycol	67423-45-4
2-hydroxyl- 2-Pentenoic acid,	3639-22-3
Medazepam	2898-12-6

5-Hydroxy-2-methyl-4H-pyran-4-one	501-30-4
Heptanoic acid	111-14-8
Malic acid	6915-15-7
5-methoxy-2-furoic acid	94084-62-5
4-hydroxylbenzoic acid	99-96-7
4-Hydroxyphenethyl alcohol	501-94-0
2,3,4-trihydroxybutanoic acid	10191-35-2
3-ethoxy-4-hydroxylbenzenaldehyde	121-32-4
4,6-Dioxoheptanoic acid	51568-18-4
1,2-benzenedicarboxylic acid mono(2-ethylhexyl) ester	4376-20-9
Dodecanoic acid	143-07-7
4-hydroxyl-3-methoxylbenzoic acid	499-76-3
5-octadecene	112-88-9
1-nanodecene	18435-45-5
Azelaic acid	123-99-9
L-ascorbic acid	50-81-7
Hexadecanoic acid	57-10-3

To assess the impact of user behavior on the toxicity of IQOS aerosol, a list of ten compounds of toxicological concern, out of the identified fifty-six compounds that are common across conditions was compared. This list includes Lactic acid (LA), hydroxy acetic acid (HAA), 2-hydroxy-2-methylpropanoic acid (HMPA), 3-chloro-1.2-propanediol (CPD), 2-ethyl-3-hydroxypropionic acid (EHPA), benzoic acid (BA), phenylacetic acid (PAA), 2,3,4-trihydroxytetrahydrofuran (THTF), Hydroquinone (HYD), and 4-Hydroxyphenethyl alcohol (HPA).

4.3.1.1.Puff duration:

To assess the effect of puff duration, HCl with a puff duration of 2s was compared with PR-1 with puff duration of 4s. For both samples collected after the 1st and the 20th sticks, puff duration had a significant impact on the concentration of most of the compounds tested. There was up to 3-fold increase in the concentration of compound

tested as puff duration increases (Table 2). This is consistent with previous reports showing that puff duration has a substantial impact on the levels of toxicants under various conditions.[167, 168]

Table 2: Effect of puff duration on IQOS emissions

	HCI 1st stick		PR-1 1st stick		HCI 20th stick		PR-PD 20th stick		p-value	
Compounds	**Avg ± STD (ug/stick)**	**%RSD**	**Avg ± STD (ug/stick)**	**%RSD**	**Avg ± STD (ug/stick)**	**%RSD**	**Avg ± STD (ug/stick)**	**%RSD**	**1st Sticks**	**20th Sticks**
LA	19.22±5.7	29.43	40.90±7.3	17.93	31.20±4.1	13.08	43.29±1.7	**3.86**	**	**
HAA	5.06±3.3.7	72.73	14.62±3.6	24.64	13.07±0.8	5.88	15.26±1.1	**6.92**	*	*
HMPA	2.11±0.6	26.33	3.00±0.4	12.57	2.69±0.4	12.92	3.84±0.3	**7.03**		**
CPD	17.54±2.0	11.62	22.66±4.4	19.52	18.18±1.4	7.95	29.92±2.8	**9.47**		**
EHPA	2.33±0.1	4.88	2.85±0.5	16.23	2.55±0.2	8.40	3.75±0.41	**11.16**		**
BA	4.68±0.4	8.39	4.74±0.5	11.04	4.03±0.3	8.24	5.82±0.3	**5.21**		***
PAA	10.84±1.1	10.00	12.21±1.6	13.44	10.88±0.6	5.42	16.08±0.6	**3.98**		***
THTF	7.64±1.0	12.44	8.56±1.1	13.31	7.52±0.4	5.56	12.79±1.9	**14.52**		*
HYD	4.47±3.3	74.67	6.49±2.9	45.29	6.8±2.9	42.13	8.05±1.28	**15.98**		
HPA	7.14±0.34	4.75	7.49±0.8	10.02	7.23±0.6	8.18	10.30±0.8	**7.42**		**

Average *: p < 0.1, **: p < 0.05, ***: p < 0.01

The statistical evaluation between the 1st sticks showed no significant statistical differences for most of these compounds except for two compounds (LA and HAA). However, the comparison between the 20th sticks showed significant statistical differences for all the compounds assessed except for HYD.

4.3.1.2.Numbers of Puff

Comparison was drawn between the 1st and the 20th sticks of ISO with 6-puffs and the 1st and the 20th sticks of PR-3 that have 10-puffs with other parameters hold constant (Table 3). Only three compounds showed near significant statistical difference when the 1st sticks of the two-puffing regime were analyzed. Similarly, comparison between 20th sticks results did not show any significant impact on the IQOS emission (Table 3).

Table 3: Effect of numbers of puffs on IQOS emissions

	ISO 1st stick		PR-NP 1st stick		ISO 20th stick		PR-NP 20th stick		p-value	
Compounds	AVG ± STD (ug/stick)	%RSD	AVG ± STD (ug/stick)	%RSD	AVG ± STD (ug/stick)	%RSD	AVG ± STD (ug/stick)	%RSD	1st sticks	2nd sticks
LA	16.63±11.9	71.4	54.76±16.0	29.2	17.66±5.71	32.34	24.33±11.00	45.22	*	
HAA	15.14±11.7	77.6	12.79±5.4	42.2	5.04±2.94	58.35	5.39±2.10	38.99		
HMPA	1.58±0.9	49.8	4.96±2.0	39.3	1.11±0.80	72.21	1.87±0.89	47.40		
CPD	6.33±2.8	43.5	22.18±6.1	27.6	5.26±3.98	75.61	10.93±5.25	48.00	**	
EHPA	0.78±0.6	71.6	4.28±1.6	38.2	1.48±0.21	13.86	2.16±0.81	37.63	*	
BA	2.32±0.43	18.9	7.27±3.1	42.3	1.77±0.81	45.69	3.42±1.23	35.93		
PAA	5.11±1.78	35.1	22.69±10.5	46.3	3.53±2.64	74.68	11.37±3.83	33.64		*
THTF	4.48±1.9	41.5	15.06±5.8	38.4	3.30±2.43	73.63	7.04±2.60	36.87		
HYD	1.03±0.7	69.8	19.50±9.3	47.7	1.09±1.02	92.89	7.34±3.16	43.00		*
HPA	3.36±1.6	48.6	15.58±6.7	42.9	2.57±2.09	81.09	6.50±2.69	41.34		

Average *: p < 0.1, **: p < 0.05, ***: p < 0.01

4.3.1.3.Flow rate

There was no significant impact of flow rate on aerosol emissions for all the compounds tested (Table 4). This was tested at both 1st and 20th sticks when the flow rate was doubled from 0.82 in PR-2 to 1.65 under PR-1. (Table 4)

Table 4: Effect of flow rate on IQOS emissions.

	PR-PD 1st stick		**PD-FR 1st stick**		**PR-PD 20th stick**		**PD-FR 20th stick**	
Compounds	**Avg ± STD (ug/stick)**	**%RSD**	**Avg ± STD (ug/stick)**	**%RSD**	**Avg ± STD (ug/stick)**	**%RSD**	**Avg ± STD (ug/stick)**	**%RSD**
LA	40.90±7.3	17.93	39.73±7.6	19.22	43.29±1.7	3.86	30.90±2.8	9.18
HAA	14.61±3.6	24.64	13.20±2.7	20.74	15.26±1.1	6.92	11.84±1.8	15.62
HMPA	3.00±0.4	12.57	3.18±0.1	4.43	3.84±0.3	7.03	2.75±0.3	9.43
CPD	22.65±4.4	19.52	20.61±1.0	4.62	29.92±2.8	9.47	20.01±0.7	3.43
EHPA	2.84±0.5	16.23	2.69±0.0	0.05	3.75±0.4	11.16	2.21±0.1	2.67
BA	4.74±0.5	11.04	4.37±0.3	6.60	5.82±0.3	5.21	3.96±0.3	6.94
PAA	12.21±1.6	13.44	12.10±0.6	4.92	16.08±0.6	3.98	11.34±0.6	5.10
THTF	8.56±1.1	13.31	9.90±0.5	5.51	12.79±1.9	14.52	8.73±0.2	2.17
HYD	6.49±2.9	45.29	5.13±1.8	35.63	8.05±1.3	15.98	6.02±0.6	9.96
HPA	7.48±0.7	10.02	7.90±0.7	9.14	10.30±0.8	7.42	6.78±0.7	9.90

4.3.1.4.Effect of cleaning

Statistical analysis to test for the effect of cleaning between the 1st and the 21st stick shows no significant difference in the IQOS emissions. These means that less cleaning, or aging of residue build-up, does not lead to more toxicants in IQOS emissions.

4.3.2. NTA on Gas and particulate phase from sample set 2 (S2)

Ninety-eight compounds were found across the different puffing regimes. Table 5 shows the compounds found for each puffing regime and their concentration. Using ISO Puffing regime, forty-three (43) compounds were identified. Eight (8) of these compounds belong to the gas phase while thirty-five compounds were found in the particulate phase. However, under HCI, fifty-six compounds were found in total; nine compounds were identified in the gas phase while the particulate phase has 47 compounds. When PR-1, PR-2 and PR-3 were used, a total number of 52, 36 and 42 compounds were detected, respectively. For most of the puffing regime test, majority of the compounds were found in the particulate phase as compared to the gas phase. This observation is in alignment with a recent report published by PMI-affiliated researchers.[117]

The concentration of compounds which was computed semi-quantitatively using benzene-d6 as internal standard is reported as ug/stick. When comparing the concentration of common compounds between the two standard puffing regimes, HCI and ISO, HCI shows up to 10-fold increase in concentration when compared to ISO. For example, toluene increases from 3.1 ug/stick under ISO puffing regime to 7.02 ug/stick

using HCI. However, compounds like 2,3-dipyridyl showed a much higher increase in concentration, moving from 0.87 ug/stick under ISO to 4.53ug/stick under HCI.

Table 5: List of compounds detected and semi-quantified from S2 sampling.

***** Compounds in yellow are found in the gas phase while green colored compounds are common to both the gas phase and the particulate phase**

		ISO	HCI	PR-1	PR-2	PR-3	Bentley et. al (2020)[117]
COMPOUND	**CAS NUMBER**	**AVG ± STD (ug/stick)**	**AVG ± STD (ug/stick)**	**AVG ± STD (ug/stick)**	**AVG ± STD (ug/stick)**	**AVG ± STD (ug/stick)**	
Furaneol	3658-77-3	0.46±0.1	1.27±0.1				1.92
phenol, 2-methoxy	90-05-1	0.65±0.1	1.97±0.2	0.60±0.1	0.17±0.0	0.06±0.0	2.18
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	28564-83-2	13.96±2.2	27.25 ±2.5	28.20 ±1.3	10.92±0.1	9.64±0.3	51.4
Catechol	452-86-8	5.82±1.2	9.21±4.3	0.93±0.1	0.88±0.1	0.56±0.3	
4-tetradecyne	629-59-4	73.53±9.5	147.57±54.6	4.27±0.5			ND
Furfural	98-01-1	21.63 ±2.4	66.85±0.9	38.25± 2.9	17.12±0.80	13.58 ± 0.49	47.4
Propylene Glycol	57-55-6	31.11±1.0	176.5 ±1.4	60.20±0.2	26.59±0.6	10.86±0.8	643
3-hydroxylbutanal	107-89-1	0.98±0.1	3.78±0.1	0.10±0.0	0.03±0.0	0.09±0.0	ND
2-furanmethanol	98-00-0	19.03±0.4	32.60 ±2.2	22.55±0.1	15.17±0.0	9.04±0.0	37.5
Acetic anhydride	108-24-7	1.23±0.5	2.85±0.4	0.41±0.0	0.24±0.0	ND	ND
Methyl glyoxal	78-98-8	1.33±0.8	2.26±0.3	0.16±.0	ND	ND	
Butanoic acid, 3-hydroxy-3-methyl	18267-36-2	0.45±0.1	2.00±0.2	ND	ND	ND	ND
Thioacetic	507-09-5	13.01±8.5	203.62±6.5	1.07±0.3	ND	1.33±0.5	ND

5-Hydroxymethylfurfural	67-47-0	3.52±0.8	6.44±1.6	ND	ND	ND	23.0
1,2,3-propanetriol, 1-acetate	106-61-6	33.24±4.6	305.76±11.9	11.04±0.5	9.60±0.9	10.10±0.4	ND
Triacetin	102-76-1	959.62±36.9	1033.81±26.9	106.70±4.9	65.59±6.1	47.43±17.9	112
Phenol, 4-ethyl-2-methoxy	2785-89-9	0.60±0.1	1.06±0.6	0.35±0.1	0.10±0.0	0.06±0.0	0.137
Hydroquinone	123-31-9	2.20±0.5	3.00±1.3	0.35±0.0	0.29±0.0	0.14±0.1	5.71
Nicotine	54-11-5	427.08±41.9	540.22±77.5	283.83±46.5	57.68±6.9	38.09±10.9	ND
6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)	54868-48-3	1.54±0.4	2.05±1.7	ND	ND	ND	7.75
2,3-dipyridyl	581-50-0	0.87±0.8	4.53±2.0		0.28±0.0	0.19±0.1	1.23
isopropyl Alcohol	67-63-0	1.17±0.1	1.50±0.2	ND	ND	ND	ND
Guanidine, (4-aminobutyl)	138-37-4	30.34±7.3	277.32±18.9	ND	ND	ND	ND
Ethanol, 2,2'-oxybis	34604-52-9	160.10±48.2	81.91±4.2	1.32±0.1	1.63±0.2	1.10±0.8	ND
1,2,3-cycloheptatriene	544-25-2	2.12±0.8	2.42±0.2	ND	ND	ND	ND
Toluene	108-88-3	3.11±1.6	7.07±0.5			0.03±0.0	
O-Xylene	95-47-6	1.18±0.4	1.68±0.4	ND	ND	ND	ND
Oxalic acid, allyl nonyl ester		0.85±0.1	0.56±0.2	ND	ND	ND	ND
Hydroperoxide, heptyl	764-81-8	0.14±0.1	ND	ND	ND	ND	ND
2-Pentene, 3,4,4-trimethyl	598-96-9	0.72±0.1	ND	ND	ND	ND	ND
Allyl methallyl ether	14289-96-4	0.71±0.1	ND	ND	ND	ND	ND
2-furancarboxylic acid	88-14-2	4.75±0.1	ND	ND	ND	ND	ND
3-pentanol, 2,3-dimethyl	595-41-5	0.51±0.1	ND	ND	ND	ND	ND

2-Furancarboxyaldehyde	98-01-1	0.24±0.0	ND	ND	ND	ND	ND
2H-Pyran-2,6(3H)-dione	5926-95-4	3.84±0.1	ND	ND	ND	ND	ND
3-methyl-2-butanol	598-75-4	16.67±0.3	ND	ND	ND	ND	ND
3-Dodecyne	6790-27-8	48.30±14.1	ND	ND	ND	ND	ND
4-pentenal, 2,2-dimethyl	5497-67-6	0.73±0.1	ND	ND	ND	ND	ND
3-Hepten-2-one (Z)	1119-44-4	1.11±0.1	ND	ND	ND	ND	ND
Hept-3-yne-2-one	26059-43-8	0.57±0.1	ND	ND	ND	ND	ND
Ethylbenzene	100-41-4	0.79±0.2	ND	0.35±0.3	0.01±0.4	0.02 ± 0.2	0.354
Hydroperoxide, pentyl	74-80-6	1.09±0.3	ND	ND	ND	ND	ND
1,2-Benzenedicarboxylic acid, bis(1-methylethyl)ester	605-45-8	0.21±0.0	ND	ND	ND	ND	ND
1-Propanol, 2-methyl-	78-83-1	ND	1.91±0.1	0.05±0.0	0.05±0.0	0.05±0.0	ND
Propanal, 2-methyl	78-84-2	ND	1.14±0.1	ND	ND	ND	116
2-methoxy-4-vinylphenol	7786-61-0	ND	0.01±0.0	0.06±0.0	ND	ND	0.177
4-ethylcatechol	124-39-6	ND	0.67±0.1	ND	ND	ND	0.124
Naphthalene, 1,2-dihydro-1,5,8-trimethyl	30364-38-6	ND	0.01±0.0	ND	ND	ND	ND
Benzaldehde, 3-hydroxyl-4-methoxy	621-59-0	ND	0.02±0.0	ND	ND	ND	ND
Triethylcitrate	77-93-0	ND	0.28±0.1	ND	ND	0.16±0.1	ND
Oxime, methoxy-phenyl		ND	0.54±0.2	ND	ND	ND	ND
2-Propanol-1-methoxy	107-98-2	ND	1.29±0.1	ND	ND	ND	ND
2-furancarboxaldehyde, 5-methyl	620-02-0	ND	0.79±0.1	ND	ND	ND	14.2
phenol	108-95-2	ND	1.01±0.1	0.29±0.0	0.03±0.0		3.74
3-pentanol, 2-methyl	565-67-3	ND	0.21±0.0	ND	ND	ND	ND

3-methylcyclopentane-1,2-dione	765-70-8	ND	0.02±0.0	ND	ND	ND	ND
Maltol	118-71-8	ND	0.68±0.1	ND	ND	ND	0.175
benezene ethanol	60-12-8	ND	0.01±0.0	ND	ND	ND	0.133
Benzenecarboxylic acid	65-85-0	ND	0.60±0.1	ND	ND	ND	ND
Propane, 1-Nitro	108-03-2	ND	1.45±0.1	0.06±0.0	0.05±0.0	0.05±0.0	ND
Ally acetate	591-87-7	ND	281.07±14.8	8.95±0.6	8.48±0.1	9.07±0.5	ND
propanoic acid, 2-methyl	54340-93-1	ND	16.83±13.7	0.06±0.0	0.22±0.0	0.28±0.0	ND
Oxirane, 2,3-dimethyl-	3266-23-7	ND	2.32±1.0	ND	ND	ND	ND
pyrrolidin-1-acetic acid	6628-74-6	ND	4.94±0.5	0.10±0.0	ND	0.05±0.0	
1-chloro-2-methylpropane	513-36-0	ND	1.77±0.1	0.04±0.0	0.04±0.0	0.04±0.0	ND
2-cyclohexen-1-one, 4-(3-hydroxyl-1-butenyl)-3,5,5-trimethyl	7070-24-8	ND	3.75±1.5	ND	0.21±0.0	0.15±0.1	ND
Tetradecane, 2,6,10-trimethyl-	14905-56-7	ND	1.63±0.8	ND	ND	ND	ND
4-Penten-2-one	13891-87-7	ND	5.13±3.2	0.53±0.7	ND	ND	ND
p-xylene	106-42-3	ND	0.43±0.1	0.35±0.4	0.01±0.0	ND	2.39
styrene	100-42-5	ND	0.22±0.2	ND	ND	ND	1.15
Methanethiol	74-93-1	ND	1.36±1.2	ND	ND	ND	11.7
2-furancarboxyaldehyde. 5-methyl	620-02-0	ND	ND	0.21±0.0	0.14±0.0	ND	14.2
Hydroperoxide, 1-methylethyl	3031-75-2	ND	ND	0.01±0.0	0.12±0.0	0.13±0.0	
Butanoic acid, 3-methyl	32118-53-9	ND	ND	0.48±0.1	ND	ND	ND
Benzene acetic acid, hexyl ester	5421-17-0	ND	ND	0.04±0.0	0.14±0.2	0.04±0.0	ND
Glycerol 1,2-diacetate	25395-31-7	ND	ND	0.75±0.1	0.77±0.0	0.53±0.2	2.47
1,2-Benzenediol, 3-methyl	488-17-5	ND	ND	0.04±0.0	0.16±0.2	ND	ND

1-Propene, 3-(ethenyloxy)	3917-15-5	ND	ND	0.91±0.0	0.79±0.1	0.93±0.1	ND
Ethane, 1,1-diethoxy	2678-54-8	ND	ND	1.61±0.1	1.38±0.1	1.55±0.0	ND
Propanoic acid, 2-hydroxy-2-methyl	594-61-6	ND	ND	0.06±0.0	ND	ND	ND
3,4-Dihydro-6-methyl-2H-Pyran-2-one	3740-59-8	ND	ND	0.05±0.0	ND	ND	ND
Pentanedioic acid, dimethyl ester	1119-40-0	ND	ND	0.08±0.0	0.03±0.0	ND	ND
2-Hexene	592-43-8	ND	ND	0.15±0.0	0.03±0.0	ND	ND
Butanal, 3-hydroxyl	107-89-1	ND	ND	0.03±0.0	0.06±0.0	0.03±0.0	ND
3,5-Hexadien-2-ol	3280-51-1	ND	ND	0.08±0.0	ND	ND	ND
2-pentanone, 3-methyl	565-61-7	ND	ND	0.04±0.0	0.03±0.0	0.04±0.0	ND
5-Hydroxymethylfurfural	67-47-0	ND	ND	0.38±0.0	0.41±0.0	0.10±0.1	23.0
2-isopropoxyethylamine	81731-43-3	ND	ND	0.06±0.0	ND	0.01±0.0	ND
Acetic acid, buytl ester	123-86-4	ND	ND	7.57±1.0	ND	7.90±0.6	ND
1-methylbutyl-Hydroperoxide	14018-58-7	ND	ND	0.17±0.0	ND	ND	ND
3-Hexene-2,5-diol	4436-75-3	ND	ND	0.04±0.0	ND	ND	ND
2-Furanmethanol, 5-methyl	3857-25-8	ND	ND	0.01±0.0	ND	ND	0.164
Sec-Butylnitrite	544-16-1	ND	ND	0.17±0.0	ND	0.03±0.0	
cyclopent-ene-1,3-dione	930-60-9	ND	ND	0.22±0.2	ND		8.40
1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5	ND	ND	0.20±0.3	ND	ND	ND
Formic acid, 1-methylethyl ester	625-55-8	ND	ND	ND	0.03±0.0	0.03±0.0	ND
cyclobutane, 1,2-bis(1-methylethenyl)-, trans	19465-02-2	ND	ND	ND	0.03±0.0	ND	ND

1-propane, 2-methyl	513-44-0	ND	ND	ND	ND	0.82±0.1	ND

When our data was compared with the data reported by PMI-affiliate, Bentley et. al,[117] the only available comprehensive paper on the NTA analysis of IQOS, 28% of out compounds had earlier been identified and reported in IQOS emission. However, a larger percentage of our compounds were not reported in the PMI's data. While the confidence level of the compound identified is above 75%, there is still need for the confirmation of these new compounds using a reference standard.

4.3.2.1 Impact of User Behavior on Toxicants

To assess the impact of user behavior on toxicants, statistical analysis was done on common toxicants as was mentioned for NTA analysis of phenol filter (S1). As shown in the table below, all the puffing parameter tested, i.e puff duration, flow rate, and number of puffs showed statistical significance for most of the compounds tested. This result indicates that there is a significant impact of puffing parameters on IQOS emissions. However, no significant impact of device cleaning on IQOS emissions.

Table 6: Statistical analysis to assess the effect of user behavior on emission from S2.

Compounds	Puff duration	Flow rate	Numbers of puff	Cleaning
phenol, 2-methoxy	***	***	***	
Catechol	*		**	
Furfural		*	***	
Propylene Glycol	***	*		
Butanal, 3-hydroxy	***	***	***	
2-furanmethanol	*	***	*	
1,2,3-propanetriol, 1-acetate	***	*	**	**
Triacetin	***	***	***	
Phenol, 4-ethyl-2-methoxy		***	***	
Hydroquinone	*		**	
Nicotine	**	**	**	**

Average *: p < 0.1, **: p < 0.05, ***: p < 0.01

Our statistical data agrees with a recent report from British American Tobacco affiliates, that highlighted the impact of puff volume and number of puffs on IQOS emissions (puff volume = flow rate * duration).[169] They reported that the choice of puffing parameters has a significant impact on the magnitude of aerosol and smoke emissions. Recently, Robinson et al.[170] showed that standard smoking regimes do not reflect the mean, or the range of parameters recorded from smokers in a natural environment.

Based out findings, there is need for specific puffing regimens obtained from human puffing recording studies for the accurate assessment of IQOS emissions. Otherwise, studies that compare IQOS to cigarettes using "standard" regimes could be misleading.

4.4. Conclusion

NTA analysis is a great tool for the identification of unknown compounds. Here, we have described its use for the identification of compounds present in the particulate and gaseous phases of IQOS aerosol. The effect of user behavior on the emission of toxicant was assessed statistically. We showed that puffing parameters such as the puff duration, flow rate, and the number of puffs can affect the composition of IQOS aerosol. However, as described in the chapter two, this research suffers from some limitations that is peculiar to NTA analysis. Because of the low sensitivity of the one-dimensional GC that was used, and due to the complexity of the analyte matrix leading to co-elution of peaks, the number of compounds that can be identified was lowered. Also, due to the lack

of workflow software that uses algorithm for peak deconvolution and library searching, this work was done manually, hence lowering the accuracy of peak identification.

CHAPTER 5

CONCLUSION

The tobacco market is saturated with a wide variety of nicotine delivery products. While some of these products have been around for over a century, others were introduced within the last decade. As described in chapter one of this thesis, these products differ in terms of product design, aerosolization mechanism, nicotine delivery, and toxicant emissions. Cigarettes and cigars rely on self-sustained combustion to produce aerosol while HTPs and ECIGs, on the other hand, require an external power source, such as a battery, to generate heat for aerosolization. The differences might lead to the production of different compositions of the aerosol, which could be revealed by non-targeted analysis (NTA).

NTA is an analytical technique that is currently being used by independent and industry-affiliated researchers for the identification of chemical suspects or compounds that have not been previously identified in tobacco emissions. The increased number of new tobacco products with varying aerosol generation mechanisms has necessitated the need for a rapid and efficient method for aerosol characterization. Despite its enormous potential for identifying product-specific compounds, NTA is fraught with difficulties. In the second chapter of this thesis, we discussed these challenges and opportunities.

In the third chapter we used NTA to study the impact of users' behavior on IQOS emissions using gas chromatography tandem mass spectrometry. Users' behavior in terms of device cleanliness between consecutive use sessions and puffing parameters was hypothesized to affect emissions in IQOS aerosol. Two sampling procedures were used, one involves NTA analysis of filters derivatized by silylation, while the other is a multi-step trapping of IQOS aerosol followed by GC-MS analysis of particle and gas phases of

the aerosol. Compound chemical classes such as alkanes, acids, ketones, aromatic acids, esters, and substituted hydrocarbon were identified and quantified in the two samplings. Statistical analysis showed that all puffing parameters including Puffing duration, number of puffs, and puffing flow rate have significant effect on the composition of the aerosol. However, device cleaning did not have any statistically significant effect on IQOS emissions.

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