Regulatory Toxicology and Pharmacology 95 (2018) 153–160

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

An evaluation of electronic cigarette formulations and aerosols for harmful and potentially harmful constituents (HPHCs) typically derived from combustion

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ARTICLE INFO

Keywords:
E-cigarette
E-liquid
ENDS
Combustion
Harmful and potentially harmful constituents
HPHC

ABSTRACT

U.S. FDA draft guidance recommends reporting quantities of designated harmful and potentially harmful constituents (HPHCs) in e-cigarette e-liquids and aerosols. The HPHC list comprises potential matrix-related compounds, flavors, nicotine, tobacco-related impurities, leachables, thermal degradation products, and combustion-related compounds. E-cigarettes contain trace levels of many of these constituents due to tobacco-derived nicotine and thermal degradation. However, combustion-related HPHCs are not likely to be found due to the relatively low operating temperatures of most e-cigarettes. The purpose of this work was to use highly sensitive, selective, and validated analytical methods to determine if these combustion-related HPHCs (three aromatic amines, five volatile organic compounds, and the polycyclic aromatic hydrocarbon benzo[a]pyrene) are detectable in commercial refill e-liquids, reference e-cigarette e-liquids, and aerosols generated from rechargeable e-cigarettes with disposable cartridges (often referred to as "cig-a-likes"). In addition, the transfer efficiency of these constituents from e-liquid to aerosol was evaluated when these HPHCs were added to the e-liquids prior to aerosol formation. This work demonstrates that combustion-related HPHCs are not present at measurable levels in the commercial and reference e-liquids or e-cigarette aerosols tested. Additionally, when combustion-related HPHCs are added to the e-liquids, they transfer to the aerosol with transfer efficiencies ranging from 49% to 99%.

1. Introduction

In 2012, the U.S. Food and Drug Administration (FDA) established a list of "harmful and potentially harmful constituents" (HPHCs) in cigarette smoke, cigarette filler, and smokeless tobacco products that included over 90 constituents (FDA, 2012a, 2011; FSPTCA, 2009). Tobacco product manufacturers are required to report the levels of HPHCs found in cigarettes and smokeless tobacco products (FDA, 2012b). However, due to the potential for large and unmanageable testing volumes in contract laboratories and the lack of validated analytical test methods for all HPHCs listed on the established list, FDA published an abbreviated list for initial HPHC testing and reporting requirements (FDA, 2012b). The abbreviated list comprises constituents for which validated analytical methods were thought to be established and represents several different chemical classes.

E-cigarettes, also known as e-vapor products and electronic nicotine delivery systems (ENDS), are an emerging product category in the global market. On May 10, 2016, FDA published the final rule to deem e-cigarettes (referred to as ENDS) to be subject to the Federal FD&C Act, as amended by the FSPTC Act. This provides FDA authority to regulate e-cigarettes and e-liquids (FDA, 2016a). Most, if not all e-cigarettes, ultimately require a market authorization from FDA through the premarket tobacco application (PMTA) pathway to either remain on the market or to enter interstate commerce (FDA, 2016a). FDA recently

Abbreviations: 1-NA, 1-aminonaphthalene; 2-NA, 2-aminonaphthalene; 4-ABP, 4-aminobiphenyl; B[a]P, benzo[a]pyrene; CFP, Cambridge Filter Pad; CORESTA, Cooperation Centre for Scientific Research Relative to Tobacco; CRM, CORESTA Recommended Method; d, days; DG, diethylene glycol; EG, ethylene glycol; EI, electron ionization; ENDS, electronic nicotine delivery system; FD&C Act, Federal Food, Drug, and Cosmetic Act; FDA, U.S. Food and Drug Administration; FSPTC Act, Family Smoking Prevention and Tobacco Control Act; GC-MS, gas chromatography with mass spectrometry; h, hours; HCI, Health Canada Intense; HPHC, harmful and potentially harmful constituent; ICH, International Conference on Harmonisation; ISO, International Organization for Standardization; LOD, limit of detection; LOQ, limit of quantification; NBW, nicotine by weight; NCI, negative chemical ionization mode; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; P&T, purge and trap; PAH, polyaromatic hydrocarbon; PG, propylene glycol; PMTA, premarket tobacco application; s, seconds; SPE, solid phase extraction; TSNAs, tobacco specific nitrosamines; VG, vegetable glycerol; VOC, volatile organic compound

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https://doi.org/10.1016/j.yrtph.2018.03.012
Received 1 December 2017; Received in revised form 15 March 2018; Accepted 18 March 2018
Available online 20 March 2018

Table 1
The HPHCs provided in the ENDS PMTA draft guidance (FDA, 2016b) and their probable origin.

Origin	HPHCs
Base formulation (e-liquid)	nicotine, glycerol, propylene glycol
Formulation impurities	diethylene glycol, ethylene glycol
Flavors	menthol, diacetyl, acetyl propionyl, ammonia
Nicotine and tobacco-related impurities	anabasine, NNK, NNN
Leachables/metals	cadmium, chromium, lead, nickel
Thermal degradation products	formaldehyde, acetaldehyde, acrolein, crotonaldehyde
Combustion-related compounds	benzo[a]pyrene, 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl, acrylonitrile, benzene, 1,3-butadiene, isoprene, toluene

HPHCs, harmful and potentially harmful constituents; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine.

published draft guidance on PMTAs for ENDS (FDA, 2016b). Within this ENDS PMTA draft guidance, FDA specifies the studies that should be conducted in order to demonstrate that the product is "appropriate for the protection of public health" including the constituents that should be considered for analysis in e-cigarette e-liquids and aerosols (FDA, 2016b). The HPHCs provided in the original ENDS PMTA draft guidance (FDA, 2016b) can be categorized based upon the constituent's potential origin (Table 1). With the exception of carbon monoxide, all of the constituents listed in the abbreviated HPHC list for conventional tobacco cigarette smoke (FDA, 2012b) are included in the ENDS PMTA draft guidance (FDA, 2016b).

ENDS products are available in a variety of configurations including small devices resembling cigarettes that are disposable or have rechargeable batteries with disposable cartridges or larger formats with rechargeable batteries and disposable prefilled or refillable tanks. When a user puffs on an e-cigarette, the e-liquid is heated, aerosolized, and inhaled. E-liquids typically contains propylene glycol (PG) and/or glycerin (often referred to as vegetable glycerol or VG), water, nicotine, and flavors.

Several researchers have studied and elucidated the chemical compositions of many commercial and prototype e-liquids and aerosols (Bates and Farsalinos, 2015a, 2015b; Etter et al., 2013; Farsalinos et al., 2015a, 2015b; 2015c; Flora et al., 2016b; Geiss et al., 2016; Goniewicz et al., 2014; Gupta et al., 2015; Guthery, 2016; Herrington and Hays, 2012; Hutzler et al., 2014; Jensen et al., 2015; Kim and Shin, 2013; Kosmider et al., 2014; Laugesen, 2009; Lauterbach and Laugesen, 2012; Lauterbach et al., 2012; Lerner et al., 2015; Lisko et al., 2015; Margham et al., 2016; Ohta et al., 2011; Pellegrino et al., 2012; Schripp et al., 2013; Talih et al., 2016; Tayyarah and Long, 2014; Theophilus et al., 2014; Uchiyama et al., 2013; Westenberger, 2009; Williams et al., 2013). In 2014, Dr. Cheng from FDA Office of Science, Center for Tobacco Products conducted a comprehensive and systematic review of the available scientific evidence evaluating chemicals in e-cigarette e-liquids and aerosols (Cheng, 2014). The author noted, "Wide ranges in the levels of chemical substances such as tobacco-specific nitrosamines, aldehydes, metals, volatile organic compounds, phenolic compounds, polycyclic aromatic hydrocarbons, flavours, solvent carriers, tobacco alkaloids and drugs have been reported in e-cigarette refill solutions, cartridges, aerosols and environmental emissions." It is also stated that many of the methods used in the review were not validated. Dr. Cheng (2014) also noted, "… additional studies based on scientifically validated aerosol generation methods, aerosol physical property measurement methods and chemical analysis methods would be helpful in generating reliable estimates of chemical quantities and, thus, the toxic potential of e-cigarettes."

The scientific evidence demonstrating the presence of the constituents listed in the ENDS PMTA draft guidance (FDA, 2016b) (Table 1) in e-cigarette e-liquids and aerosols continues to increase using a variety of analytical techniques. It has been well demonstrated in the scientific literature that trace levels of potential impurities (HPHCs) from tobacco-derived nicotine such as tobacco specific nitrosamines (TSNAs) and nicotine-related impurities such as anabasine can be present in e-cigarette e-liquids and aerosols (Flora et al., 2016a, 2017; Goniewicz et al., 2014; Kim and Shin, 2013; Margham et al., 2016; Sleiman et al., 2016; Westenberger, 2009). While the potential formulation impurities ethylene glycol (EG) and diethylene glycol (DG) were not observed in the 20 commercial products tested by Etter et al. (2013), Westenberger (2009) observed DG in one product tested as reported in a 2009 Department of Health and Human Services memorandum. Additionally, trace levels of EG and DG were detected in select commercial e-liquid refill solutions as reported in a poster presentation by Shah et al. (2015). It has also been demonstrated by Farsalinos et al. (2015b) and Sleiman et al. (2016) that flavor compounds listed in the ENDS PMTA draft guidance (FDA, 2016b) (Table 1), such as diacetyl and acetyl propionyl, have been identified in select commercial e-vapor products. Williams et al. (2017, 2013) and Goniewicz et al. (2014) have demonstrated that some metal compounds (leachables) have also been observed in select commercial e-cigarettes depending upon the components used in the container closure system or e-cigarette cartridge (often referred to as a cartomizer). Several researchers have also established that thermal degradation products such as carbonyl compounds (e.g., formaldehyde, acetaldehyde, and acrolein) can be detected in e-cigarette aerosols at various concentrations under realistic and unrealistic use conditions, and their formation is clearly related to the temperature of the e-cigarette device during aerosol formation (Farsalinos et al., 2015c; Flora et al., 2016b, 2017; Geiss et al., 2016; Goniewicz et al., 2014; Gupta et al., 2015; Guthery, 2016; Jensen et al., 2015; Kosmider et al., 2014; Margham et al., 2016; Sleiman et al., 2016; Talih et al., 2017; Talih et al., 2016; Uchiyama et al., 2013). Talih et al. (2017) recently demonstrated that some refillable high power sub-Ohm devices (coil resistance well below 1 Ohm) can produce carbonyl levels far greater than conventional cigarettes and explored the interaction between power and device geometry on carbonyl formation. However, it is well recognized that for commercial rechargeable e-cigarettes with disposable pre-filled cartridges (often referred to as cig-a-likes) the levels of these HPHCs found in e-cigarette aerosols are typically far below those found in conventional tobacco cigarettes (Farsalinos et al., 2015b; Flora et al., 2017, 2016b; Goniewicz et al., 2014; Laugesen, 2015; Margham et al., 2016; Tayyarah and Long, 2014).

While the body of evidence regarding some potential HPHCs in e-cigarette e-liquids and aerosols continues to grow, as discussed above, the presence of combustion-related HPHCs in e-cigarette e-liquids and aerosols remains relatively unclear. The combustion-related HPHCs listed in the ENDS PMTA draft guidance (FDA, 2016b) include three aromatic amines (1-aminonaphthalene (1-NA), 2-aminonaphthalene (2-NA), and 4-aminobiphenyl (4-ABP)), five volatile organic compounds (VOCs) (1,3 butadiene, isoprene, acrylonitrile, benzene, and toluene), and the polyaromatic hydrocarbon (PAH) benzo[a]pyrene (B[a]P) (Baker and Bishop, 2004; FDA, 2016b; Fowles and Dybing, 2003; McGrath et al., 2007; Piadé et al., 2013). The mechanism of aerosol formation by e-cigarettes does not involve temperatures at or near those of combustion. Typical rechargeable e-cigarettes with disposable pre-filled cartridges (e.g., cig-a-likes) have low operating temperatures relative to conventional tobacco cigarettes at less than 350 °C (coil temperature) compared to 900 °C, respectively (Baker and Bishop, 2004; Flora et al., 2017; Geiss et al., 2016; Zhao et al., 2016). For this reason, it is highly unlikely that these types of e-cigarette would reach the temperatures found in conventional tobacco cigarettes where pyrolysis and combustion occur (> 400 °C) (Baker, 1975).

Select VOCs in e-cigarettes have been reported in a few publications with mixed results (Goniewicz et al., 2014; Han et al., 2016; Herrington and Hays, 2012; ISO, 1999; Kim and Kim, 2015; Lim and Shin, 2017).