Regulatory Toxicology and Pharmacology 74 (2016) 1–11

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

# Characterization of potential impurities and degradation products in electronic cigarette formulations and aerosols

Jason W. Flora*, Naren Meruva, Chorng B. Huang, Celeste T. Wilkinson, Regina Ballentine, Donna C. Smith, Michael S. Werley, Willie J. McKinney

*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, United States*

ARTICLE INFO

*Article history:*
Received 17 July 2015
Received in revised form
18 November 2015
Accepted 19 November 2015
Available online 30 November 2015

*Keywords:*
E-cigarettes
E-vapor
Harmful and potentially harmful constituents (HPHC)
Formulation
Aerosol

ABSTRACT

E-cigarettes are gaining popularity in the U.S. as well as in other global markets. Currently, limited published analytical data characterizing e-cigarette formulations (e-liquids) and aerosols exist. While FDA has not published a harmful and potentially harmful constituent (HPHC) list for e-cigarettes, the HPHC list for currently regulated tobacco products may be useful to analytically characterize e-cigarette aerosols. For example, most e-cigarette formulations contain propylene glycol and glycerin, which may produce aldehydes when heated. In addition, nicotine-related chemicals have been previously reported as potential e-cigarette formulation impurities. This study determined e-liquid formulation impurities and potentially harmful chemicals in aerosols of select commercial MarkTen® e-cigarettes manufactured by NuMark LLC. The potential hazard of the identified formulation impurities and aerosol chemicals was also estimated. E-cigarettes were machine puffed (4-s duration, 55-mL volume, 30-s intervals) to battery exhaustion to maximize aerosol collection. Aerosols analyzed for carbonyls were collected in 20-puff increments to account for analyte instability. Tobacco specific nitrosamines were measured at levels observed in pharmaceutical grade nicotine. Nicotine-related impurities in the e-cigarette formulations were below the identification and qualification thresholds proposed in ICH Guideline Q3B(R2). Levels of potentially harmful chemicals detected in the aerosols were determined to be below published occupational exposure limits.



## 1. Introduction

The U.S. Food and Drug Administration (FDA) recently established a list of harmful and potentially harmful constituents (HPHCs) for currently regulated tobacco products (e.g., cigarettes) (FDA, 2012a). FDA guidance defined harmful and potentially harmful constituents as "any chemical or chemical compound in a tobacco product or in tobacco smoke: a) that is or potentially is inhaled, ingested, or absorbed into the body; and b) that causes or has the potential to cause direct or indirect harm to users or non-users of tobacco products" (FDA, 2011). Subsequently, a list of more than 90 HPHCs was communicated (FDA, 2012a).

However, there is a lack of in-house laboratory capabilities, a potential for large and unmanageable testing volumes in contract laboratories, and a lack of validated analytical test methods for all of the HPHCs listed on the established list (FDA, 2012a). Therefore, FDA published an abbreviated HPHC list for initial regulatory reporting requirements (FDA, 2012b). The abbreviated list comprises constituents for which analytical methods were thought to be established and which represent several different chemical classes. Table 1 shows the abbreviated list of HPHCs that are initially required by FDA to be reported for cigarette smoke and cigarette filler (i.e., tobacco that is a component of a cigarette).

FDA requires reporting the quantities of all HPHCs on the abbreviated list for a regulated tobacco product. Currently, the regulated tobacco products that require HPHC reporting are cigarettes (smoke and tobacco filler), smokeless tobacco, and roll-your-own tobacco.

E-cigarettes, also referred to as electronic nicotine delivery systems or e-vapor products, are gaining popularity in the U.S and other global markets. FDA (2014) published a proposed deeming rule that would extend the agency's regulatory authority to include e-cigarettes; at this time in manuscript preparation (June 2015), the

* Corresponding author. Altria Client Services LLC., 615 Maury Street, Richmond, VA 23224, United States.
*E-mail addresses:* Jason.W.Flora@altria.com, SciencePublications@altria.com (J.W. Flora).

http://dx.doi.org/10.1016/j.yrtph.2015.11.009

**Abbreviations**

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| B[a]P | benzo[a]pyrene |
| BLOQ | below the limit of quantitation |
| CFP | Cambridge filter pads |
| CO | carbon monoxide |
| CORESTA | Cooperation Centre for Scientific Research Relative to Tobacco |
| CRM | CORESTA-recommended method |
| DFG | Deutsche Forschungsgemeinschaft |
| DNPH | 2,4-dinitrophenylhydrazine |
| EPA | U.S. Environmental Protection Agency |
| FDA | U.S. Food and Drug Administration |
| GC | gas chromatography |
| GC-MS | gas chromatography-mass spectrometry |
| HCI | Health Canada Intense |
| HPHC | harmful and potentially harmful constituent |
| IC-CD | ion chromatography with a conductivity detector |
| ICP-MS | inductively coupled plasma mass spectrometry |
| ICH | International Conference on Harmonisation |
| ISO | International Organization for Standardization |
| LC-MS/MS | liquid chromatography-tandem mass spectrometry |
| LOD | limit of detection |
| LOQ | limit of quantitation |
| MDPH | Massachusetts Department of Public Health |
| MS/MS | tandem mass spectroscopy |
| NIOSH | National Institute of Occupational Safety and Health |
| NNK | 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone |
| NNN | N-nitrosonornicotine |
| NSRL | No Significant Risk Level |
| OEHHA | Office of Environmental Health Hazard Assessment |
| OSHA | Occupational Health and Safety Administration |
| PEL | permissible exposure limit |
| RfC | reference concentration |
| SIM | selected ion monitoring |
| SPE | solid-phase extraction |
| TLV | threshold limit value |
| TSNA | tobacco specific nitrosamine |
| TWA | time-weighted average |
| UPLC-MS/MS | ultra performance liquid chromatography-tandem mass spectrometry |
| UV | ultraviolet |
| VOC | volatile organic compound |
| WHO | World Health Organization |

rule is under review and has not yet been finalized. E-cigarettes are available in both disposable and rechargeable formats. The rechargeable devices have either a refillable or disposable cartridge. When a user puffs on an e-cigarette, a liquid is heated, aerosolized, and inhaled. E-cigarette formulations (often called e-liquids) typically contain propylene glycol and/or glycerin, water, nicotine, and flavors.

The number of publications focusing on the chemical characterization of e-cigarette formulations and aerosols is increasing. Most studies seek to identify impurities in the formulation such as tobacco specific nitrosamines (TSNAs) and nicotine-related impurities (Cobb et al., 2010; Trehy et al., 2011; Westenberger, 2009), while other studies focus on identifying potentially harmful chemicals in the aerosol such as carbonyl compounds, volatile organic compounds (VOCs), TSNAs, metals, and silicates (Goniewicz et al., 2014; Tayyarah and Long, 2014; Williams et al., 2013).

**Table 1**
Abbreviated list of HPHCs in cigarette smoke and cigarette filler (FDA, 2012b).

| HPHCs in cigarette smoke | HPHCs in cigarette filler |
|---|---|
| Acetaldehyde | Ammonia |
| Acrolein | Arsenic |
| Acrylonitrile | Cadmium |
| 4-Aminobiphenyl | Nicotine (total) |
| 1-Aminonaphthalene | NNK |
| 2-Aminonaphthalene | NNN |
| Ammonia | |
| Benzene | |
| Benzo[a]pyrene | |
| 1,3-Butadiene | |
| Carbon monoxide | |
| Crotonaldehyde | |
| Formaldehyde | |
| Isoprene | |
| Nicotine (total) | |
| NNK | |
| NNN | |
| Toluene | |

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

Additionally, a recent study investigated the impact of design parameters such as battery output voltage on the generation of potentially harmful chemicals such as carbonyl compounds in the e-cigarette aerosol (Kosmider et al., 2014).

One of the objectives of this research was to determine and quantify potential impurities and degradation products in the formulations and aerosols of four e-cigarettes (rechargeable with disposable cartridges) manufactured by Nu Mark LLC, an Altria company, and sold as MarkTen®. The other objective was to conduct a risk assessment analysis of these e-liquid formulation impurities and aerosol chemicals. Because the regulation of e-cigarettes has not yet been promulgated and no specific HPHC list exists for e-cigarette formulations and aerosols, this study focused on measuring chemicals that are listed for cigarette tobacco and smoke on the abbreviated HPHC list published by FDA (2012b) (Table 1) as well as chemicals identified in publications focused on analytical characterization of e-cigarette liquid formulations and aerosols (Cheng, 2014; Cobb et al., 2010; Etter et al., 2013; FDA, 2012b; Goniewicz et al., 2014; Kosmider et al., 2014; Tayyarah and Long, 2014; Trehy et al., 2011; Uchiyama et al., 2013; Westenberger, 2009; Williams et al., 2013).

## 2. Methods

### 2.1. E-cigarette test products

Four commercial e-cigarette products available in the U.S. marketplace (at the time of manuscript preparation) produced by Nu Mark LLC (an Altria company) under the MarkTen® brand name were included in all analytical evaluations. The commercial products were the MarkTen® Menthol and Classic sold in Indiana and Arizona during the first quarter of 2014, and all contained 1.5% nicotine by weight. The average nicotine delivery for these devices was 29 μg/puff under the machine smoking conditions discussed below and shown in Table 5 (4 s puffs, 55 mL puff volume, puffed in 20 puff increments to battery exhaustion).

### 2.2. Formulation analysis

All e-cigarette testing methodologies for formulation (e-liquids) characterization were validated based upon the 2005 International Conference on Harmonisation (ICH) guideline "Validation of Analytical Procedures: Text and Methodology Q2(R1)" (ICH, 2005). For chemicals listed on the abbreviated HPHC list (FDA, 2012b) (Table 1), the validations were conducted for cigarette tobacco and adapted for e-cigarette formulations following details provided herein. In the interest of reproducibility, CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) recommended methods (CRMs) were applied (Table 2) when available. For specified nicotine impurities, methods were developed and validated specifically for e-cigarette formulations. Unless specified otherwise, the limits of quantitation (LOQ) are based on the lowest calibration standards, and the limits of detection (LOD) are estimated based upon a signal-to-noise ratio of 3. LOQs and LODs are described throughout on a per gram basis of the e-cigarette formulations. Nicotine was not included in the analysis as it is added to e-cigarette formulations in known quantities and is, therefore, neither an impurity nor a degradation product. Chemical class, analyte, instrumentation, and method summaries are listed in Table 2; analytical details are provided in Sections 2.2.1 through 2.2.4.

#### 2.2.1. Arsenic and cadmium in e-cigarette formulations

This method was developed to quantitatively determine the amounts of arsenic and cadmium in tobacco cigarette filler using inductively coupled plasma mass spectrometry (ICP-MS). The LOQs for arsenic and cadmium were 430 and 220 ng/g of formulation, respectively. Analysis was conducted by Arista Laboratories (Richmond, VA), and LODs were not available.

#### 2.2.2. TSNAs in e-cigarette formulations

This method was developed and validated to quantitatively determine the concentration of TSNAs in tobacco and tobacco products using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The TSNAs analyzed with this method were N-nitrosonornicotine (NNN; CAS #16543-55-8) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; #CAS 64091-91-4). TSNA standards were purchased from Spex Certiprep (Metuchen, NJ), and isotopically labeled internal standards were purchased from Toronto Research Chemicals (Ontario, Canada). All other high-purity chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA). The method of analysis followed CRM N$^{o}$ 72 (CORESTA, 2013a). The method was adapted for e-cigarette liquids by adding 0.45 g formulation to 10 mL 100 mM ammonium acetate extraction solution prior to analysis. The LOQ for both NNN and NNK was 90.0 ng/g, and the estimated LODs were 0.71 and 0.92 ng/g, respectively.

#### 2.2.3. Ammonia in e-cigarette formulations

This method was developed and validated to quantitatively determine the content of ammonia in tobacco products (cigarette filler, pouched snus smokeless tobacco, moist smokeless tobacco, loose dry snuff smokeless tobacco, and loose leaf chewing tobacco) by ion chromatography with a conductivity detector (IC-CD). The method was adapted for e-cigarette liquids from CRM N$^{o}$ 73 (CORESTA, 2011) by adding 0.25 g formulation to 100 mL extraction solution (0.025 N sulfuric acid) prior to analysis. Chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA). The LOQ was 40 μg/g, and the estimated LOD was 2.0 μg/g.

#### 2.2.4. Nicotine-related impurities in e-cigarette formulations

This method was developed and validated to quantitatively determine the amounts of nicotine-N-oxides (*cis* and *trans*; CAS #491-26-9), cotinine (CAS #486-56-6), nornicotine (CAS #5746-86-1), anatabine (CAS #2743-90-0), myosmine (CAS #532-12-7), anabasine (CAS #13078-04-1), and β-nicotyrine (CAS #487-19-4) in e-cigarette formulations using LC-MS/MS. As these analytes are not included in the abbreviated HPHC list and are not routinely measured in tobacco products, additional details are provided for this method. All nicotine-related impurities and internal standards were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). It should be noted that some nicotine impurities such as nicotine-N-oxides are known to be thermally unstable which prevented the use of gas chromatography (GC) for this analysis. Calibration standards ranged from 0.10 μg/mL to 5.0 μg/mL with a coefficient of determination greater than 0.9989. Prior to analysis, e-cigarette cartridges were disassembled and all components, including gauze, heater coil assembly, and metal shell, were placed in 20-mL glass vials. An extraction solution (10 mL of 70:30, methanol:water) was added containing the deuterated internal standards (2.0 μg/mL) listed in Table 3. Vials were then capped and vortexed for 30 min using a Glas-Col (Terre Haute, IN) vortexer at 70% motor speed. Aliquots of the extraction solution were then transferred to auto sampler vials, and 1 μL was injected on the ultra-performance liquid chromatograph coupled to tandem mass spectrometry (UPLC-MS/MS) instrumentation.

Liquid chromatography was conducted on a Waters ACQUITY® UPLC instrument (Milford, MA) with a Waters ACQUITY® X-Bridge

**Table 2**
Summary of analytical methodologies used for e-cigarette formulation analysis.

| Chemical class | Analyte | Instrumentation | Method summary |
|---|---|---|---|
| Metals | Arsenic<br>Cadmium | ICP-MS | Formulation analyzed by Arista[a] |
| Tobacco specific nitrosamines | NNK<br>NNN | LC-MS/MS | CRM N$^{o}$ 72[b] (0.45 g formulation in 10 mL) |
| Ammonia | Ammonia | IC-CD | CRM N$^{o}$ 73[c] (0.25 g of formulation in 100 mL) |
| Nicotine-related impurities | Nicotine-N-oxides<br>Cotinine<br>Nornicotine<br>Anatabine<br>Myosmine<br>Anabasine<br>β-Nicotyrine | LC-MS/MS | Internal method; whole cartridge extraction (10 mL) |

CRM, CORESTA-recommended method; IC-CD, ion chromatography with conductivity detector; ICP-MS, inductively coupled plasma mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine.

[a] Arista Laboratories (Richmond, VA).

[b] CRM N$^{o}$ 72 (see CORESTA, 2013a).

[c] CRM N$^{o}$ 73 (see CORESTA, 2011).

**Table 3**
Specified nicotine impurities and labeled internal standards used for analyte quantitation (Council of Europe, 2012).

| Analyte | Internal standard |
|---|---|
| Myosmine | Myosmine-$d_4$ |
| Nornicotine | Nornicotine-$d_4$ |
| β-Nicotyrine | Myosmine-$d_4$ |
| Anatabine | Myosmine-$d_4$ |
| Anabasine | Anabasine-$d_4$ |
| Cotinine | Cotinine-$d_3$ |
| Nicotine-N-oxide | Cotinine-$d_3$ |

C18 (2.5 μm) 2.1 × 50 mm column. Mobile phase A and the weak wash solvent were 10 mM ammonium acetate (pH 10), and mobile phase B and the strong wash solvent were Optima™ grade methanol (Fisher Scientific, Pittsburgh, PA). Sample temperature was held at 15 °C, and column temperature was at ambient conditions. UPLC mobile phase flow rate was set to 0.3 mL/min. The nicotine impurities were detected by tandem mass spectroscopy (MS/MS) in the UPLC eluent using multiple reaction monitoring. The LOQ for all specified impurities was 4.8 μg/g, and the estimated LODs ranged from 0.025 to 0.39 μg/g.

### 2.3. Aerosol analysis

All testing methodologies for aerosol characterization were validated based upon the 2005 ICH guideline "Validation of Analytical Procedures: Text and Methodology Q2(R1)" (ICH, 2005). For potential impurities listed on the abbreviated HPHC list (FDA, 2012b) (Table 1) (excluding aerosol nicotine), the validations were conducted for cigarette smoke and adapted for e-cigarette aerosols following details provided herein. In the interest of reproducibility, CRMs and/or International Organization for Standardization (ISO) standards were applied (Table 4) when available.

**Table 5**
Smoking machine puff profile settings.

| Parameter | Setting |
|---|---|
| Square wave | Uniform air transfer throughout puff duration |
| Volumetric air flow | 825 mL/min |
| Puff volume | 55.0 ± 0.3 mL |
| Puff duration | 4.00 ± 0.02 s |
| Puff interval | 30.0 ± 0.5 s |

Unless specified otherwise, the LOQs are based on the lowest calibration standards, and the LODs are estimated based upon a signal-to-noise ratio of 3. Chemical class, analyte, instrumentation, and method summaries are listed in Table 4; analytical details are provided in Sections 2.3.2 through 2.3.9.

#### 2.3.1. Aerosol collection

All aerosol collections were conducted under ISO smoking environmental conditions (ISO, 2012) with temperature at 22.0 °C ± 2.0 °C and relative humidity at 60% ± 5%. The protocols for aerosol collection were based upon the Health Canada Intense (HCI) smoking regime (Health Canada, 1999) puff volume and puff interval. However, a square wave puff profile was needed to activate the puff sensors, and ventilation blocking was not applicable to the e-cigarette designs. The regime selected (Table 5) was slightly greater than the average puffing topography of experienced e-cigarette users to the best of our knowledge at this time (Vansickel et al., 2014). At the time of this research, the testing regime was consistent with one of the regimes being evaluated by the CORESTA E-Cigarette Task Force. A technical report from the CORESTA E-Cigarette Task Force (2015) concluded, among other things, that for the puffing parameters evaluated, yields were similar, precision was acceptable, and there was no technical advantage among the puffing parameters tested. All device batteries were fully charged prior to aerosol collection.

**Table 4**
Summary of analytical methodologies used for e-cigarette aerosol analysis.

| Chemical class | Analyte | Smoking machine; aerosol collection details | Instrumentation; CRM/ISO standard |
|---|---|---|---|
| Carbonyls | Acetaldehyde<br>Acrolein<br>Crotonaldehyde<br>Formaldehyde | KC Automation 5-port linear; 5 sets of 20 puffs | UPLC-UV; CRM N° 74[a] |
| Aromatic amines | 4-Aminobiphenyl<br>1-Aminonaphthalene<br>2-Aminonaphthalene | Cerulean 20-port linear; 100 puffs or battery exhaustion | GC–MS; (Cambridge filter pad collection) |
| Volatile organic compounds | Acrylonitrile<br>Benzene<br>1,3-Butadiene<br>Isoprene<br>Toluene | KC Automation 5-port linear; 100 puffs or battery exhaustion | GC–MS; CRM N° 70[b] |
| Tobacco specific nitrosamines | NNK<br>NNN | KC Automation 5-port linear; 100 puffs or battery exhaustion | LC-MS/MS; CRM N° 75[c] |
| Ammonia | Ammonia | Cerulean 20 port linear; 100 puffs or battery exhaustion | IC-CD; (2 impingers with acidic aqueous solution) |
| Polyaromatic hydrocarbons | Benzo[a]pyrene | Cerulean 20 port linear; 100 puffs or battery exhaustion | GC–MS; (Cambridge filter pad collection) CRM N° 58[d] /ISO 22634[e] |
| Carbon monoxide | Carbon monoxide | Cerulean 20 port linear; 2 sets of 50 puffs | CO analyzer (IR) with SM450; CRM N° 5[f]/ISO 8454[g] |

CRM, CORESTA-recommended method; GC–MS, gas chromatography-mass spectrometry; IC-CD; ion chromatography with a conductivity detector; IR, infra-red; ISO, International Organization for Standardization; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine; UPLC-UV, ultra-performance liquid chromatography with ultraviolet absorbance detection.
[a]CRM N° 74 (see CORESTA, 2014a).
[b]CRM N° 70 (see CORESTA, 2013b).
[c]CRM N° 75 (see CORESTA, 2012).
[d]CRM N° 58 (see CORESTA, 2014b).
[e]ISO 22634 (see ISO, 2008).
[f]CRM N° 5 (see CORESTA, 1993).
[g]ISO 8454 (see ISO, 2007).

Aerosol collection methods varied for chemical classes (Table 4). In all cases where Cambridge filter pads (CFP) were used, the CFP was 44 mm with one exception: benzo[a]pyrene (B[a]P) collection used a 92-mm CFP. In most cases, in the interest of increasing method sensitivity, e-cigarette devices were puffed to battery exhaustion (where the device no longer activated), and the total collected aerosol was analyzed. With the 4-s puff duration, this was approximately 80–100 puffs. However, for aerosol collection for the determination of carbonyls, the devices were puffed in 20-puff increments because the analytes in this chemical class are known to be unstable. If carbonyls were collected with more puffs (e.g., approximately 100 puffs), it is highly likely that the values would be underestimated on an estimated per puff basis due to analyte loss. Carbon monoxide (CO) in e-cigarette aerosol was measured using a gas-phase spectrographic technique (discussed in Section 2.3.8) for 50-puff collections.

#### 2.3.2. Carbonyls in e-cigarette aerosol

This method was developed and validated to quantitatively determine the concentration of formaldehyde (CAS #50-00-0), acetaldehyde (CAS #75-07-0), acrolein (CAS #107-02-8), and crotonaldehyde (CAS #4170-30-3) (Supelco, Bellefonte, PA) in mainstream cigarette smoke. The method of analysis was adapted for e-cigarette aerosol from CRM N$^{o}$ 74 (CORESTA, 2014a) for cigarette smoke analysis by implementation of the aerosol collection discussed in Section 2.3.1 and the following procedure. E-cigarette aerosol was collected on a 5-port linear Borgwaldt, KC Automation (Richmond, VA) smoking machine using two impingers containing 30 mL of 2,4-dinitrophenylhydrazine (DNPH) (Aldrich Chemical Company, Milwaukee, WI) and perchloric acid in acetonitrile (Fisher Scientific, Atlanta, GA). The aerosol extracts were analyzed for the respective hydrazones using UPLC (Waters ACQUITY®) with ultraviolet/visible (UPLC-UV) absorbance detection. For carbonyl analysis, samples were collected for 20-puff intervals until the battery was exhausted (approximately five collections). The LOQs and LODs were, respectively, 0.71 and 0.079 μg/puff for acetaldehyde, 0.36 and 0.09 μg/puff for acrolein, 0.19 and 0.080 μg/puff for crotonaldehyde, and 0.036 and 0.013 μg/puff for formaldehyde. As formaldehyde has been detected in background samples due to contamination from the environment and DNPH, all formaldehyde levels reported here have been corrected by subtracting the average concentration of formaldehyde in the blank samples that were collected and analyzed during the same time as the e-cigarette samples (0.18 μg/puff collection).

#### 2.3.3. Aromatic amines in e-cigarette aerosol

A GC–MS method was developed and validated to quantitatively determine the concentration of 1-aminonaphthalene (CAS #134-32-7), 2-aminonaphthalene (CAS #91-59-8), and 4-aminobiphenyl (CAS #92-67-1) in mainstream cigarette smoke. Standards were purchased from Sigma–Aldrich (Milwaukee, WI) and internal standards from CDN Isotopes (Quebec, Canada). The method was adapted for e-cigarette aerosol by implementation of the aerosol collection discussed in Section 2.3.1 and the following procedure. E-cigarette aerosol was collected on a Cerulean (Milton Keynes, UK) 20-port linear smoking machine onto a CFP. The CFP was extracted with 5 mL hexanes. The extract was derivatized with trimethylamine and pentafluoropropionic anhydride (Fisher Scientific, Pittsburgh, PA) and concentrated to 1 mL prior to GC–MS analysis. The LOQs for 1-aminonaphthalene, 2- aminonaphthalene, and 4-aminobiphenyl were 2.0, 1.0, and 0.50 ng/device, respectively (when puffed to battery exhaustion), and the estimated LODs were 0.17, 0.099, 0.068 ng/device, respectively.

#### 2.3.4. Volatile organic compounds in e-cigarette aerosol

This method was developed and validated to quantitatively determine the concentration of five VOCs including 1,3-butadiene (CAS #106-99-0), isoprene (CAS #78-79-5), acrylonitrile (CAS #107-13-1), benzene (CAS #71-43-2), and toluene (CAS #108-88-3) in mainstream cigarette smoke. Custom standard and internal standard stock solutions were commercially prepared by Restek Corporation (Bellefonte, PA). The method was adapted for e-cigarette aerosol from CRM N$^{o}$ 70 (CORESTA, 2013b) for cigarette smoke analysis by implementation of the aerosol collection discussed in Section 2.3.1 and the following procedure. E-cigarette aerosol was collected on a 5-port KC Automation smoking machine after passing through a CFP followed by two chilled impinger traps (chilled with 2-propanol and dry ice) containing methanol. The CFP was extracted with the contents of the impingers. The resulting aerosol extract was analyzed by GC–MS. The LOQs for 1,3-butadiene, isoprene, acrylonitrile, benzene, and toluene were 10, 50, 5.0, 10, and 20 μg/device, respectively (when puffed to battery exhaustion). The estimated LODs were 3.4, 3.3, 3.3, 1.2, and 1.7 μg/device, respectively.

#### 2.3.5. Tobacco specific nitrosamines in e-cigarette aerosol

This method was developed and validated to quantitatively determine the concentration of TSNAs in mainstream cigarette smoke. The TSNAs determined with this method were NNN and NNK. The method was adapted for e-cigarette aerosol from CRM N$^{o}$ 75 (CORESTA, 2012) for cigarette smoke analysis by implementation of the aerosol collection discussed in Section 2.3.1. The TSNAs were collected as described for VOCs in Section 2.3.4 on a CFP, which was extracted with methanol and analyzed by LC-MS/MS. The LOQ for both TSNAs was 40 ng/device (when puffed to battery exhaustion), and the estimated LODs for NNK and NNN were 0.41 and 0.31 ng/device, respectively.

#### 2.3.6. Ammonia in e-cigarette aerosol

This method was developed and validated to quantitatively determine the concentration of total ammonia in mainstream cigarette smoke. Total ammonia was defined as ammonia present in the particulate phase deposited on the CFP and ammonia in the gas phase that is trapped by the impinger solution. The method was adapted for e-cigarette aerosol by implementation of the aerosol collection discussed in Section 2.3.1. Using a 20-port Cerulean smoking machine, e-cigarette aerosol was collected on a CFP in series with two impinger traps containing dilute acidic solution (0.025 N sulfuric acid). After puffing, the CFP was extracted with the impinger solution, converting ammonia to ammonium. The e-cigarette aerosol extract was analyzed by IC-CD. The LOQ was 10 μg/device (when puffed to battery exhaustion), and the estimated LOD was 0.52 μg/device.

#### 2.3.7. Benzo[a]pyrene in e-cigarette aerosol

This method was developed and validated to quantitatively determine the amounts of B[a]P (CAS #50-32-8) in mainstream cigarette smoke. The method of analysis was adapted for e-cigarette aerosol from CRM N$^{o}$ 58 (CORESTA, 2014b) and ISO standard 22634:2008 (ISO, 2008) for cigarette smoke analysis. The method was adapted for e-cigarette aerosol by implementation of the aerosol collection discussed in Section 2.3.1 and the following procedure. B[a]P was purchased from Sigma–Aldrich (St. Louis, MO) and all other reagents were purchased from Fisher Scientific. E-cigarette aerosol was collected on a CFP using a 20-port Cerulean smoking machine. The CFP was extracted with 5 mL hexanes. The e-cigarette aerosol extract was filtered, re-constituted to 300 μL and analyzed by GC/MS. The LOQ for B[a]P was 10 ng/device, and the estimated LOD was 2.2 ng/device.

*2.3.8. Carbon monoxide in e-cigarette aerosol*

CO in e-cigarette aerosols was measured following CRM N° 5 (CORESTA, 1993) and ISO standard 8454:2007 (ISO, 2007) using a CO analyzer (infrared) with a Cerulean SM450. LOQ was 5.0 mg/device, and LOD was not determined.

*2.3.9. Nicotine in e-cigarette aerosol*

This method was developed and validated to quantitatively determine the concentration of nicotine (purchased from Acros) (CAS# 54-11-5) in e-cigarette aerosols. E-cigarette aerosol was collected on a CFP in 20 puff increments using a 20-port Cerulean smoking machine by implementation of the aerosol collection discussed in Section 2.3.1. The CFP was extracted with 20 mL n-propanol containing Quinoline (Arcos) as the internal standard and rotated for 30 min. The e-cigarette aerosol extract was analyzed by GC with a flame ionization detector. The LOQ was 10 μg/puff and LOD was not determined.

*2.4. Toxicological evaluation – risk assessment*

Quantitative risk assessment calculations were performed for all listed potential impurities and degradation products for which there were published exposure limits as established by U.S. Environmental Protection Agency (EPA) in the Integrated Risk Information System (IRIS), California Office of Environmental Health Hazard Assessment (OEHHA; Proposition 65), American Conference of Governmental Industrial Hygienists (ACGIH), Occupational Safety and Health Administration (OSHA), or Deutsche Forschungsgemeinschaft (DFG) (Table 6). Proposition 65 lists No Significant Risk Levels (NSRLs), which are defined as daily intake levels posing a $10^{-5}$ (i.e., 1 in 100,000) lifetime risk of cancer (OEHHA, 2013). EPA provides carcinogenic inhalation concentrations at specified risk levels (e.g., $10^{-4}$, $10^{-5}$, $10^{-6}$). These values provide risk assessors with daily exposure levels of chemicals below which no additional significant toxicological risk may be incurred in a population. For the calculations made in this assessment, EPA exposure values corresponding with a 1 in 1 million ($10^{-6}$) lifetime increased cancer risk were used. Additionally, EPA may also provide a reference concentration (RfC), which is an estimate of a continuous inhalation exposure to humans that is likely to be without an appreciable risk of deleterious (non-cancer) effects during a lifetime. In general, threshold limit values (TLVs) and maximum allowable concentrations provided by ACGIH, OSHA, and/or DFG specify the limiting exposure concentrations of chemicals for daily inhalation during an 8-h workday over the course of a working lifetime. These levels are based upon an expert review of the published scientific literature in the areas of medicine, toxicology, industrial hygiene, and epidemiology. These values represent a level of exposure that a typical healthy worker can experience repeatedly without adverse health effects. Using TLVs, the daily exposure to a chemical without expected adverse health effects can be calculated by using the time-weighted average for 8 h/day (TWA-8 h) and assuming a daily air inhalation rate of 28.8 $m^3$/day or 1.2 $m^3$/h. For e-cigarette liquid formulations, a conservative transfer rate of 100% was assumed.

The following is an example using the TLV TWA-8 h for arsenic and its salts as arsenic. The TWA is reported as 0.01 mg/$m^3$ (ACGIH, 2015). Equation (1) is used to convert the TWA to an equivalent daily exposure:

$$\begin{aligned}&\text{exposure concentration}\left(\frac{\text{mg}}{\text{m}^3}\right)\times\text{hourly inhalation rate}\left(\frac{\text{m}^3}{\text{h}}\right)\\&\times\text{length of workday}\left(\frac{8\,\text{h}}{\text{day}}\right)\\&=\text{estimated daily exposure}\left(\frac{\text{mg}}{\text{day}}\right)\end{aligned}\tag{1}$$

Such that,

$$\frac{0.01\,\text{mg}}{\text{m}^3}\times\frac{1.2\,\text{m}^3}{\text{h}}\times\frac{8\,\text{h}}{\text{day}}=0.096\ \frac{\text{mg}}{\text{day}}=96\frac{\mu\text{g}}{\text{day}}$$

To compare the equivalent daily exposure derived from

**Table 6**
Risk assessment values.

| Chemical class | Analyte | IRIS[a] (μg/$m^3$) | NSRL[b] (μg/day) | TLV[c] (mg/$m^3$) | PEL[d] (mg/$m^3$) | RfC[e] (mg/$m^3$) | DFG[c] (mg/$m^3$) | NIOSH[c] (mg/$m^3$) |
|---|---|---|---|---|---|---|---|---|
| Carbonyls | Acetaldehyde | 0.5 | 90 | N/A | 360 | 0.009 | 91 | N/A |
| | Acrolein | N/A | N/A | N/A | 0.25 | 0.00002 | N/A | 0.25 |
| | Crotonaldehyde | N/A | N/A | N/A | 6 | N/A | N/A | N/A |
| | Formaldehyde | 0.08 | 40 | N/A | N/A | N/A | 0.37 | N/A |
| Aromatic amines | 4-Aminobiphenyl | N/A | 0.03 | N/A | N/A | N/A | N/A | N/A |
| Volatile organic compounds | Acrylonitrile | 0.01 | 0.07 | 4.3 | N/A | 0.002 | N/A | N/A |
| | Benzene | 0.13 | 13 | 1.6 | 3 | 0.03 | N/A | N/A |
| | 1,3-Butadiene | 0.03 | 0.4 | 4.4 | 2.2 | 0.002 | N/A | N/A |
| | Isoprene | N/A | N/A | N/A | N/A | N/A | 8.5 | N/A |
| | Toluene | N/A | 13,000 | 75 | N/A | 5 | 190 | 375 |
| Tobacco specific nitrosamines | NNK | N/A | 0.014 | N/A | N/A | N/A | N/A | N/A |
| | NNN | N/A | 0.5 | N/A | N/A | N/A | N/A | N/A |
| Ammonia | Ammonia | N/A | N/A | 17 | 35 | 0.1 | 14 | 18 |
| Polyaromatic hydrocarbons | Benzo[a]pyrene | N/A | 0.06 | N/A | 0.2 | N/A | N/A | 0.1 |
| Carbon monoxide | Carbon monoxide | N/A | N/A | 29 | 55 | N/A | 35 | 40 |
| Metals | Arsenic | 0.0002 | 0.06 | 0.01 | 0.01 | N/A | N/A | N/A |
| | Cadmium | 0.0006 | 0.05 | N/A | 0.005 | N/A | N/A | N/A |

DFG, Deutsche Forschungsgemeinschaft; IRIS, Integrated Risk Information System; N/A, not available; NIOSH, National Institute of Occupational Safety and Health; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine; NSRL, no significant risk level; PEL, permissible exposure limit; RfC, reference concentration; TLV, threshold limit value.

[a] As reported by EPA (1998a, 1998b, 1998c, 2002, 2003a, 2003b, 2003c).
[b] As reported by OEHHA (2013) (Toluene is a maximum allowable daily limit).
[c] As reported by ACGIH (2015).
[d] As reported by OSHA (2003).
[e] As reported by EPA ( 1998b, 2002, 2003a, 2003b, 2003d, 2003e, 2005).

exposure to arsenic at the TWA to the calculated daily exposure from the e-cigarette using the arsenic LOQ assuming 100% transfer from e-liquid formulation to aerosol, Equation (2) may be used.

$$\mathrm{LOQ}\left(\frac{\text{ng arsenic}}{\text{g formula}}\right)\times\frac{\text{mg formula}}{\text{cartridge}}\times\frac{\text{1 cartridge}}{\text{day}}\times\frac{\text{1 g}}{\text{1000 mg}}\times 100\%\ \text{transfer}=\frac{\text{ng arsenic}}{\text{day}} \tag{2}$$

Such that,

$$\frac{\text{430 ng arsenic}}{\text{g formula}}\times\frac{\text{420 mg formula}}{\text{cartridge}}\times\frac{\text{1 cartridge}}{\text{day}}\times\frac{\text{1 g}}{\text{1000 mg}}\times 100\%\ \text{transfer}=\frac{\text{181 ng arsenic}}{\text{day}}$$

Unpublished data show that an average MarkTen® user consumes one cartridge per day, which contains 420 mg formula. Assuming this daily consumption and that 100% of the arsenic transfers to aerosol, an average user may be exposed to a maximum of 430 ng (the method LOQ) arsenic per gram formula (181 ng arsenic per day). This estimated daily exposure is more than 500 fold lower than the equivalent daily exposure derived from exposure to arsenic at the TWA.

Similarly, Equation (3) is used to convert the exposure concentration to an equivalent daily exposure that is associated with a cancer risk of $10^{-6}$ or for chronic exposure non-cancer effects (RfC). Using toluene, a non-carcinogenic neurotoxicant as an example, with an EPA RfC of 5 mg/m$^3$ (EPA, 2005) the equivalent daily exposure derived from the exposure to toluene at the RfC may be calculated as follows:

$$\text{exposure concentration}\left(\frac{\text{mg}}{\text{m}^3}\right)\times\text{daily inhalation rate}\left(\frac{\text{m}^3}{\text{day}}\right)=\text{estimated daily exposure}\left(\frac{\text{mg}}{\text{day}}\right) \tag{3}$$

$$\frac{\text{5 mg}}{\text{m}^3}\times\frac{\text{28.8 m}^3}{\text{day}}=144\frac{\text{mg}}{\text{day}}$$

Because toluene is measured on a per device basis and its LOD is 1.7 μg/device, the total daily exposure to toluene of an average MarkTen® e-cigarette user is estimated to be less than 1.7 μg/day (the LOD) on a daily basis. This exposure estimate is more than 80,000 times less than the equivalent daily exposure derived from exposure to toluene at the EPA RfC.

## 3. Results and discussion

Table 7 shows the chemical classes, analytes, range of average triplicate results, LOQs, and units reported for the e-cigarette formulations evaluated in this study. Because one of the objectives of this research was to conduct a risk assessment analysis of the e-liquid formulation impurities and aerosol chemicals detected, calculations were performed on all LODs (or LOQs if LODs were not available) to ensure that the methods could detect analytes at concentrations relevant for this risk assessment.

Because all the risk values are based on average daily exposures, average daily MarkTen® e-cigarette consumption must be estimated. However, although e-cigarette use is gaining popularity, it is still a dynamic market, and data on consumption patterns are not robust. Some investigators have used retrospective online survey tools to determine e-cigarette use in puffs per day (Etter, 2010; Etter and Bullen, 2014). Puffs per day may be a difficult parameter for e-vapor product users to remember and quantify. These surveys may also fail to categorize whether the user is using a cartridge based product such as MarkTen® or an open or tank system. The patterns of use may differ substantially within the overall e-vapor category. Other investigators have reported mean cartridge or cartridge use per day of approximately 1 cartridge/day (Caponnetto et al., 2013; Farsalinos and Polosa, 2014) or 3 cartridges over a 7-day period (Wagener et al., 2014). These estimates of cartridge use per day closely mirror the manufacturer's unpublished data on MarkTen® e-cigarette consumption, so an assumption of one cartridge per day is considered to be the daily consumption rate.

EPA reports the *de minimus* risk level (at $10^{-6}$) for exposures to arsenic ($2\times10^{-4}$ μg/m$^3$) and cadmium ($6\times10^{-4}$ μg/m$^3$) as well as a RfC for ammonia (0.1 mg/m$^3$) (EPA, 1998c, 2003c, 2003e). OEHHA (2013) reports NSRLs for arsenic (0.06 μg/day), cadmium (0.05 μg/day), NNK (0.014 μg/day), and NNN (0.5 μg/day). ACGIH reports limits for arsenic (0.01 mg/m$^3$), while OSHA reports a permissible exposure limit (PEL) of 0.005 mg/m$^3$ for cadmium (ACGIH, 2015). Using the risk assessment procedure described in Section 2.4, the LODs for NNK, NNN and ammonia are sufficient with which to conduct quantitative risk assessments. The LODs are low enough to detect the presence of these constituents at levels that regulatory bodies consider to be relevant to human exposure. Assuming that 100% of each chemical from the liquid transfers to the aerosol and that the liquid contains the analyte at its LOD/LOQ, the daily exposure to each of these three analytes is well below the OEHHA NSRL (NNN and NNK) or the EPA RfC (ammonia). The analytical methods are not sufficiently sensitive to detect such low levels of arsenic and cadmium at EPA *de minimus* and OEHHA risk levels for some metals in e-liquid formulations, but the methods are sufficiently sensitive to show that the potential daily exposures to these metals fall below the calculated daily exposures derived from the ACGIH TLV (arsenic) and the OSHA PEL (cadmium). It is also unlikely that the metals will transfer with 100% efficiency from the e-liquid formulation to the aerosol. Nonetheless, additional work should be undertaken to improve the sensitivity of the metal analyses.

For the nicotine-related impurities of which risk assessment values were not available, the ICH guideline “Impurities in New Drug Products” Q3B(R2) was employed (ICH, 2006). The guideline describes three thresholds. First, the reporting threshold is a “limit above which a degradation product should be reported” (ICH, 2006). Next, the identification threshold is a “limit above which a degradation product should be identified” (ICH, 2006). Lastly, the qualification threshold is a “limit above which a degradation product should be qualified” where qualification involves a “process of acquiring and evaluating data that establishes the biological safety of an individual degradation product” (ICH, 2006). While NNN was not detectable, NNK was below the LOQ in the e-cigarette formulations investigated in this study (Table 7). The LOQ levels are less than 0.002% of the total nicotine concentration (based upon total nicotine concentration). While the e-cigarettes in this study are not pharmaceutical products, these values would be within acceptable limits for unspecified impurities in pharmaceutical grade nicotine. The LOQ for NNK is well below the reporting thresholds proposed in the ICH guideline Q3B(R2) for a maximum daily dose of 1 g or less of nicotine (reporting threshold = 0.1%) (ICH, 2006).

Nicotine-related impurities specified in the European Pharmacopeia were both below LOQ and within quantifiable ranges of the

**Table 7**
Range of average results and limits of quantitation for e-cigarette formulation analysis.

| Chemical class | Analyte | Range of average results (N = 3) | LOQ | Units |
|---|---|---|---|---|
| Metals | Arsenic | ND | 430 | ng/g |
| | Cadmium | ND | 220 | ng/g |
| Tobacco specific nitrosamines | NNK | ND–BLOQ | 90 | ng/g |
| | NNN | ND | 90 | ng/g |
| Ammonia | Ammonia | ND | 40 | μg/g |
| Nicotine-related impurities | Nicotine-N-oxides | 11–19 | 4.8 | μg/g |
| | Cotinine | BLOQ–9.4 | 4.8 | μg/g |
| | Nornicotine | 14–31 | 4.8 | μg/g |
| | Anatabine | ND–BLOQ | 4.8 | μg/g |
| | Myosmine | 7.4–13 | 4.8 | μg/g |
| | Anabasine | BLOQ | 4.8 | μg/g |
| | β-Nicotyrine | BLOQ | 4.8 | μg/g |

BLOQ, below the limit of quantitation; ND, not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine.

analytical methodology used (Table 7) (Council of Europe, 2012). All specified nicotine impurities were less than 0.3% of the nicotine concentration. Therefore, the specified impurities levels were well below the identification and qualification thresholds proposed in the ICH guideline Q3B(R2) for maximum daily dose of 1 mg–10 mg, respectively (ICH, 2006).

As shown in Table 8, most potentially harmful chemicals in the MarkTen® e-cigarette aerosols were not detectable using 4-s puff duration, 55-mL puff volume, and square wave puff profile. Under these puffing conditions, the average aerosol nicotine concentration was 29 μg/puff. Calculations were performed on all LODs (LOQs were used when LODs were not available) for the potential impurities and degradation products to ensure that the methods could measure the analytes in appropriate concentrations for our risk assessments. No exposure threshold information was available for 1-aminonaphthalene or 2-aminonaphthalene. Carcinogenic inhalation concentrations at $10^{-6}$ as defined by EPA were available for acetaldehyde (0.5 μg/m$^3$), formaldehyde (0.08 μg/m$^3$), acrylonitrile (0.01 μg/m$^3$), benzene (0.13 μg/m$^3$), 1,3-butadiene (0.002 μg/m$^3$), arsenic (0.0002 μg/m$^3$), and cadmium (0.0006 μg/m$^3$) (EPA, 1998a, 1998b, 1998c, 2002, 2003a, 2003b, 2003c). EPA also reports RfCs for toluene (5 mg/m$^3$), 1,3-butadiene (0.03 mg/m$^3$), benzene (0.03 mg/m$^3$), acrylonitrile (0.002 mg/m$^3$), acrolein (0.00002 mg/m$^3$), acetaldehyde (0.009 mg/m$^3$), and ammonia (0.1 mg/m$^3$) (EPA, 1998b, 2002, 2003a, 2003b, 2003d, 2003e, 2005). OSHA PELs were available for acetaldehyde (360 mg/m$^3$), acrolein (0.25 mg/m$^3$), crotonaldehyde (6 mg/m$^3$), 1,3-butadiene (2.2 mg/m$^3$), and cadmium (0.005 mg/m$^3$) (OSHA, 2003). ACGIH TLVs were available for benzene (1.6 mg/m$^3$), toluene (75 mg/m$^3$), CO (29 mg/m$^3$), and arsenic (0.01 mg/m$^3$) (ACGIH, 2015). Additionally, DFG reports occupational exposure limits for formaldehyde (0.37 mg/m$^3$), isoprene (85 mg/m$^3$), and ammonia (14 mg/m$^3$), while the National Institute of Occupational Safety and Health (NIOSH) issued a recommended exposure limit for B[a]P of 0.1 mg/m$^3$ (ACGIH, 2015). OEHHA (2013) has also published NSRLs for acetaldehyde (90 μg/day), formaldehyde (40 μg/day), 4-aminobiphenyl (0.03 μg/day), acrylonitrile (0.07 μg/day), benzene (13 μg/day), 1,3-butadiene (0.4 μg/day), toluene (13,000 μg/day), NNK (0.014 μg/day), NNN (0.5 μg/day), B[a]P (0.06 μg/day), arsenic (0.06 μg/day), and cadmium (0.05 μg/day) (Table 6). All LODs (LOQs where LODs were not available) were sufficient to perform a risk assessment as described in Section 2.4, compare with occupational exposure limits, and demonstrate that the potential daily exposure to each analyte is below published occupational exposure limits for each chemical. Additionally, with the exception of acrolein, the LODs (or LOQs) were sufficiently low to facilitate the comparison of exposure from MarkTen® e-cigarettes to published RfCs. Although acrolein was not detected in the aerosol from MarkTen® e-cigarettes, and the LOD is sufficient to demonstrate that acrolein exposure is below occupational exposure standards, additional efforts to lower the LOD of acrolein in aerosol should be made to demonstrate that potential exposure is also below the RfC.

**Table 8**
Range of average results and limits of quantitation for e-cigarette aerosol analysis.

| Chemical class | Analyte | Range of average results (N = 3) | LOQ | Units |
|---|---|---|---|---|
| Carbonyls | Acetaldehyde | BLOQ | 0.71 | μg/puff[a] |
| | Acrolein | ND | 0.36 | μg/puff[a] |
| | Crotonaldehyde | ND | 0.19 | μg/puff[a] |
| | Formaldehyde | 0.090 to 0.33 | 0.036 | μg/puff[a] |
| Aromatic amines | 4-Aminobiphenyl | ND | 0.50 | ng/device |
| | 1-Aminonaphthalene | ND | 2.0 | ng/device |
| | 2-Aminonaphthalene | ND | 1.0 | ng/device |
| Volatile organic compounds | Acrylonitrile | ND | 5.0 | μg/device |
| | Benzene | ND | 10 | μg/device |
| | 1,3-Butadiene | ND | 10 | μg/device |
| | Isoprene | ND | 50 | μg/device |
| | Toluene | ND | 20 | μg/device |
| Tobacco specific nitrosamines | NNK | BLOQ | 40 | ng/device |
| | NNN | BLOQ | 40 | ng/device |
| Ammonia | Ammonia | BLOQ | 10 | μg/device |
| Polyaromatic hydrocarbons | Benzo[a]pyrene | ND | 10 | ng/device |
| Carbon monoxide | Carbon monoxide | ND | 5.0 | mg/device[b] |

BLOQ, below the limit of quantitation; LOQ, limit of quantitation; ND = not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine.
[a] 20 puffs per collection.
[b] 50 puffs per collection.

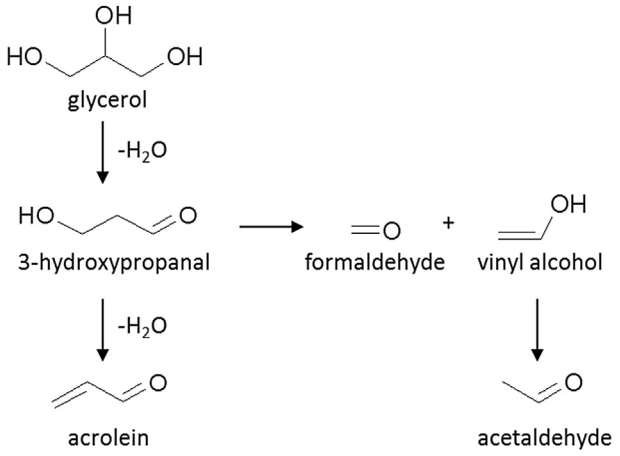


**Fig. 1.** Summarized mechanism for the (oxy)dehydration of glycerol (Deleplanque et al., 2010).

The LODs for acrylonitrile and 1,3-butadiene, while sufficient for comparison to published occupational exposure limits, are not sufficient for comparison to EPA risk assessment values or OEHHA NSRLs. Neither of these compounds was detected in MarkTen® e-cigarette aerosol, however, opportunities to increase the sensitivity of these methods should be taken. With the exception of formaldehyde, the LODs of other compounds measured in aerosol are sufficient for comparison with the EPA risk assessment values and the NSRLs.

Formaldehyde was detected at low levels in all devices and ranged from 1.8 to 6.5 μg/20-puff collection using the puffing parameters listed in Table 5. These levels can be estimated to average approximately 0.090–0.33 μg formaldehyde/puff (Table 8). The calculated potential daily exposure to formaldehyde resulting from this per-puff concentration range is approximately 2–5 times lower than the equivalent daily exposure derived from exposure to formaldehyde at the limit established by DFG. The NSRL for formaldehyde, however, is 40 μg/day. Assuming that one MarkTen® cartridge provides 80 puffs and that each puff contains 0.33 μg formaldehyde, the estimated daily exposure to formaldehyde is about 26 μg/day, which is less than the NSRL of 40 μg/day. This estimated exposure value is, however, more than 10 times the equivalent daily exposure derived from exposure to formaldehyde at the $10^{-6}$ risk value as defined by IRIS (2.3 μg/day; 0.08 μg/$m^3$ multiplied by 28.8 $m^3$/day).

Detection of low levels of carbonyl compounds in e-cigarette aerosols has been previously observed for commercial e-cigarette products (Goniewicz et al., 2014; Uchiyama et al., 2013; Lauterbach and Laugesen, 2012). In the study by Uchiyama et al. (2013), 9 of the 13 products tested showed detectable levels of carbonyls at variable levels. Goniewicz et al. (2014) reported that, of the 15 e-cigarettes in their study, formaldehyde and acetaldehyde were found in aerosols from all tested products, and acrolein was found in all but one. Goniewicz et al. (2014) also detected formaldehyde in medicinal inhalers "at levels that overlapped with those found in e-cigarette vapour." While formaldehyde is a Group I carcinogen according to the International Agency for Research on Cancer (IARC, 2006), it is also ubiquitous. The World Health Organization (WHO) estimates that the daily human exposure to formaldehyde from breathing is between 1 and 8 mg/day. These exposures result from formaldehyde in outside air as well as from homes and workspaces. WHO (2001) estimates that the formaldehyde exposure from a conventional home is 300–600 μg/day. Additionally, formaldehyde is a by-product of metabolic processes and is detectable in exhaled breath. The Agency for Toxic Substances and Disease Registry found that the median detected level of formaldehyde in breath is 4.263 ppb (ATSDR, 2010). Assuming a daily breath volume of 20 $m^3$/day, the average adult exhales 106 μg/day. This estimate is well above the NSRL of 40 μg/day and the IRIS cancer risk value of 2.3 μg/day. Based on the potential exposure to formaldehyde from sitting indoors or from exposure to human exhalate, the data suggest that the risk values reported by OEHHA and EPA are unattainable and may be overly conservative.

It is important to consider that puffing parameters and temperature of the e-cigarette heater coil can play a role in measured carbonyl levels. Kosmider et al. (2014) recently demonstrated that increasing the voltage of select commercially available refillable devices resulted in a 4- to 200-fold increase in select carbonyls. Therefore, under less-intense puffing parameters, carbonyl levels may be lower, and under more-intense parameters they may be higher. The mechanism for carbonyl formation has been previously discussed, and it was shown that glycerol and glycols can form carbonyls upon thermal degradation (Deleplanque et al., 2010; Kosmider et al., 2014; Laino et al., 2011; Paine III et al., 2007; Uchiyama et al., 2013).The mechanism is summarized for glycerol in Fig. 1.

It is difficult to compare reported concentrations of potentially harmful e-cigarette aerosol chemicals (e.g., carbonyls) across currently available e-cigarette studies because the puffing regimes are very different from study to study. Goniewicz et al. (2014) used a 1.8-s puff, 70-mL puff volume, and 10-s interval between puffs. In the study by Uchiyama et al. (2013), a 2-s puff, 55-mL puff volume, and 30-s interval, was used (Table 5). In this current study, a 4-s puff, 55-mL volume, and 30-s interval was used. Clearly, a consensus of appropriate puffing regimes for e-cigarette analysis is needed to best compare e-cigarette models. The CORESTA E-cigarette Task Force was recently formed to address this and other important e-cigarette issues. The puffing profile used in our study was selected based upon the following: it was a puffing regime being evaluated by the CORESTA task force at the time of this study; it was the maximum puff duration that the smoking machines could collect for carbonyl analysis (5-port linear KC Automation smoking machine with dual impingers); and it reflected observations of the average puffing topography of experienced e-cigarette users to the best of our knowledge at this time (Vansickel et al., 2014).

As stated by Goniewicz et al. (2014) and consistent with our observations, the levels of potentially harmful chemicals found in e-cigarette models are far less than those observed in conventional

**Table 9**
Standardized tobacco smoking regimes and e-cigarette puffing regime used in this study.

| Condition | Puff volume (mL) | Duration (s) | Approximate puff count | Interval (s) | Ventilation blocking (%) |
|---|---|---|---|---|---|
| ISO (conventional cigarettes) | 35 | 2 | 5–10 | 60 | 0 |
| MDPH (conventional cigarettes) | 45 | 2 | 8–15 | 30 | 50 |
| HCI (conventional cigarettes) | 55 | 2 | 6–14 | 30 | 100 |
| This study (e-cigarettes) | 55 | 4 | Up to battery exhaustion | 30 | NA |

HCI, Health Canada Intense; ISO, International Organization for Standardization; MDPH, Massachusetts Department of Public Health; NA, not applicable.

tobacco cigarettes (Counts et al., 2005). Nevertheless, it is again difficult to make a direct comparison between e-cigarette and conventional cigarette constituent yields because the puffing regimes are very different. Conventional cigarettes are typically tested under standardized conditions referred to as ISO, Massachusetts Department of Public Health (MDPH), and/or HCI conditions. Table 9 shows the differences in the puff volumes, puff durations, approximate puff count (for conventional cigarettes this depends on factors such rod length and filler density), intervals, and ventilation blocking percentage. It should be noted that machine smoking regimes for both conventional tobacco cigarettes and e-cigarettes are for product comparison purposes only and do not represent individual human exposure or how the tobacco product is actually used. It is well known that consumers of both conventional tobacco cigarettes and e-cigarettes vary greatly in how they use these products.

As shown in Table 8, TSNAs (NNN and NNK) were below the limit of quantitation (BLOQ) in the e-cigarette aerosols collected under the puffing parameters shown in Table 9. This, too, has been previously observed, and it was concluded that the levels found in e-cigarette models were far lower than those found in tobacco smoke (Goniewicz et al., 2014). Furthermore, these levels are well within acceptable limits for pharmaceutical grade nicotine (Council of Europe, 2012).

Ammonia was also BLOQ in the e-cigarette aerosols collected under the puffing parameters shown in Table 9 (the LOQ is 10 μg/device). It is well known that during protein metabolism in mammals, the urea cycle produces endogenous ammonia which is eliminated via the liver and the kidney. OSHA has established a PEL for inhaled ammonia in the workplace of approximately 167 mg/day (ACGIH, 2015). The LOQ for the ammonia method, based on the assumption discussed in Section 2.4, is more than 8000 times lower than the OSHA PEL.

## 4. Conclusions

Most potential impurities investigated in this study were not detectable or were well below LOQs in the commercially available MarkTen® e-cigarettes analyzed. The levels of potential e-liquid formulation impurities and potentially harmful chemicals detected in the aerosol were determined to be below published occupational exposure limits using the aerosol collection regime discussed herein. More- or less-aggressive puffing parameters for e-cigarette aerosol collections however, may result in higher or lower levels of potentially harmful chemicals in the aerosol.

While this research is not a comprehensive survey of commercially available products, it does demonstrate that many of the HPHCs evaluated in conventional tobacco cigarettes (FDA, 2012b) may not be applicable to measure in e-cigarette formulations and aerosols. However, the constituents that were observed in this study might provide insights regarding potential impurities likely to be further investigated with other products in this emerging product category. This work also demonstrates the urgent need for a standardized puffing regime for e-vapor products in order to make reasonable comparisons among studies. It is also imperative that standardized analytical testing methodologies be established for e-cigarettes in order to reduce analytical variability and maximize sensitivity for reported values of potential impurities in these products. A comprehensive survey of commercially available e-cigarettes would then be of value to the scientific community and regulators; however, this, too, is a challenging endeavor as these unregulated products are often changing, and new product forms frequently emerge in the marketplace.

## Acknowledgments

The authors acknowledge the editorial assistance of Eileen Y. Ivasauskas of Accuwrit Inc.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2015.11.009.

## References

ACGIH, 2015. In: American Conference of Governmental Industrial Hygienists 2015 Guide to Occupational Exposure Values. ACGIH, Cincinnati, OH.

ATSDR, 2010. Agency for Toxic Substances and Disease Registry Addendum to the Toxicological Profile for Formaldehyde. <http://www.atsdr.cdc.gov/toxprofiles/formaldehyde_addendum.pdf> (accessed June 2015).

Caponnetto, P., Russo, C., Bruno, C.M., Alamo, A., Amaradio, M.D., Polosa, R., 2013. Electronic cigarette: a possible substitute for cigarette dependence. Monaldi Arch. Chest Dis. 79, 12–19.

Cheng, T., 2014. Chemical evaluation of electronic cigarettes. Tob. Control 23 (Suppl. 2), ii11–ii17.

Cobb, N.K., Byron, M.J., Abrams, D.B., Shields, P.G., 2010. Novel nicotine delivery systems and public health: the rise of the "e-cigarette". Am. J. Public Health 100, 2340–2342.

CORESTA, 1993. CORESTA Recommended Method N° 5. In: Determination of Carbon Monoxide in the Mainstream Smoke of Cigarettes by Non-dispersive Infrared Analysis. <http://www.coresta.org/Recommended_Methods/CRM_05.pdf> (accessed June 2015).

CORESTA, 2011. CORESTA Recommended Method N° 73. In: Determination of Ammonia in Tobacco by Ion Chromatographic Analysis. <http://www.coresta.org/Recommended_Methods/CRM_73.pdf> (accessed June 2015).

CORESTA, 2012. CORESTA Recommended Method N° 75. In: Determination of Tobacco Specific Nitrosamines in Mainstream Cigarette Smoke by LC-MS/MS. <http://www.coresta.org/Recommended_Methods/CRM_75.pdf> (accessed October 2014).

CORESTA, 2013a. CORESTA Recommended Method N° 72. In: Determination of Tobacco Specific Nitrosamines in Smokeless Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry. <http://www.coresta.org/Recommended_Methods/CRM_72-update(July13. <http://www.coresta.org/Recommended_Methods/CRM_72-update(July13).pdf> (accessed June 2015).

CORESTA, 2013b. CORESTA Recommended Method N° 70. In: Determination of Selected Volatile Organic Compounds in the Mainstream Smoke of Cigarettes - Gas Chromatography-mass Spectrometry Method. <http://www.coresta.org/Recommended_Methods/CRM_70-update(March13. <http://www.coresta.org/Recommended_Methods/CRM_70-update(March13).pdf (accessed October 2014).

CORESTA, 2014a. CORESTA Recommended Method N° 74. In: Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC. <http://www.coresta.org/Recommended_Methods/CRM_74-update(July14. <http://www.coresta.org/Recommended_Methods/CRM_74-update(July14).pdf> (accessed June 2015).

CORESTA, 2014b. CORESTA Recommended Method N° 58. In: Determination of Benzo[a]pyrene in Mainstream Cigarette Smoke by GC-MS. <http://www.coresta.org/Recommended_Methods/CRM_58-update(July14. <http://www.coresta.org/Recommended_Methods/CRM_58-update(July14).pdf> (accessed June 2015).

CORESTA E-Cigarette Task Force, 2015. Technical Report. 2014 Electronic Cigarette Aerosol Parameters Study. March 2015. <http://www.coresta.org/Reports/ECIG-CTR_ECigAerosolParameters-2014Study_March2015.pdf> (accessed September 2015).

Council of Europe, 2012. European Pharmacopoeia 7.0. In: European Directorate for the Quality of Medicines and Healthcare (Strasbourg, France).

Counts, M.E., Morton, M.J., Laffoon, S.W., Cox, R.H., Lipowicz, P.J., 2005. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. Regul. Toxicol. Pharmacol. 41, 185–227.

Deleplanque, J., Dubois, J.L., Devaux, J.F., Ueda, W., 2010. Production of acrolein and acrylic acid through dehydration and oxydehydration of glycerol with mixed oxide catalysts. Catal. Today 157, 351–358.

EPA, 1998a. Formaldehyde (CASRN 50-00-0). <http://www.epa.gov/iris/subst/0419.htm> (accessed June 2015).

EPA, 1998b. Acetaldehyde (CASRN 75-07-0). <http://www.epa.gov/iris/subst/0290.htm> (accessed June 2015).

EPA, 1998c. Cadmium (CASRN 7440-43-9). <http://www.epa.gov/iris/subst/0141.htm> (accessed June 2015).

EPA, 2002. 1,3-Butadiene (CASRN 106-99-0). <http://www.epa.gov/iris/subst/0139.htm> (accessed June 2015).

EPA, 2003a. Benzene (CASRN 71-43-2). <http://www.epa.gov/iris/subst/0276.htm> (accessed June 2015).

EPA, 2003b. Acrylonitrile (CASRN 107-13-1). <http://www.epa.gov/iris/subst/0206.

htm> (accessed June 2015).
EPA, 2003c. Arsenic, Inorganic (CASRN 7440-38-2). <http://www.epa.gov/iris/subst/0278.htm> (accessed June 2015).
EPA, 2003d. Acrolein (CASRN 107-02-08). <http://www.epa.gov/iris/subst/0364.htm> (accessed June 2015).
EPA, 2003e. Ammonia (CASRN 7664-41-7). <http://www.epa.gov/iris/subst/0422.htm> (accessed June 2015).
EPA, 2005. Toluene (CASRN 108-88-3). <http://www.epa.gov/iris/subst/0118.htm> (accessed June 2015).
Etter, J.F., 2010. Electronic cigarettes: a survey of users. BMC Public Health 10, 231.
Etter, J.F., Zather, E., Svensson, S., 2013. Analysis of refill liquids for electronic cigarettes. Addiction 108, 1671–1679.
Etter, J.F., Bullen, C., 2014. A longitudinal study of electronic cigarette users. Addict. Behav. 39, 491–494.
Farsalinos, K.E., Polosa, R., 2014. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review. Ther. Adv. Drug Saf. 5, 67–86.
FDA, 2011. Guidance for Industry and FDA Staff. In: "Harmful and potentially harmful constituents" in tobacco products as used in Section 904(e) of the Federal Food, Drug, and Cosmetics Act. <http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM241352.pdf> (accessed June 2015).
FDA, 2012a. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed. Regist. 77, 20034–20037.
FDA, 2012b. Guidance for Industry. In: Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act. <http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297828.pdf> (accessed June 2015).
FDA, 2014. Deeming tobacco products to be subject to the Federal Food, Drug, and Cosmetic Act, as amended by the Family Smoking Prevention and Tobacco Control Act; Regulations on the sale and distribution of tobacco products and required warning statements for tobacco products. Fed. Regist. 79, 23142–23207.
Goniewicz, M.L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Jacob III, P., Benowitz, N., 2014. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tob. Control 23, 133–139.
Health Canada, 1999. Test Method T-115. In: Determination of "tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.
IARC, 2006. Monographs on the Evaluation of Carcinogenic Risks to Humans. In: Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol, vol. 88. <http://monographs.iarc.fr/ENG/Monographs/vol88/mono88.pdf> (accessed September 2015).
ICH, 2005. In: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline Q2(R1): Validation of Analytical Procedures: Text and Methodology Q2(R1). <http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf> (accessed June 2015).
ICH, 2006. In: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline Q3B(R2): Impurities in New Drug Products. <http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2__Guideline.pdf> (accessed June 2015).
ISO, 2007. Cigarettes – Determination of Carbon Monoxide in the Vapor Phase of Cigarette Smoke – NDIR Method. ISO Standard 8454. International Organization for Standardization.
ISO, 2008. Cigarettes – Determination of Benzo[a]pyrene in Cigarette Mainstream Smoke – Method Using Gas Chromatography/mass Spectrometry. ISO Standard 22634. International Organization for Standardization.
ISO, 2012. Routine Analytical Cigarette-smoking Machine – Definitions and Standard Conditions. ISO Standard 3308. International Organization for Standardization.
Kosmider, L., Sobczak, A., Fik, M., Knysak, J., Zaciera, M., Kurek, J., Goniewicz, M.L., 2014. Carbonyl compounds in electronic cigarette vapors: effects of nicotine solvent and battery output voltage. Nicotine Tob. Res. 16, 1319–1326.
Laino, T., Tuma, C., Curioni, A., Jochnowitz, E., Stolz, S., 2011. A revisited picture of the mechanism of glycerol dehydration. J. Phys. Chem. A 115, 3592–3595.
Lauterbach, J.H., Laugesen, M., 2012. Comparison of toxicant levels in mainstream aerosols generated by Ruyan® electronic nicotine delivery systems (ENDS) and conventional cigarette products. Toxicologist 126 (1) (Ref Type: Abstract).
OEHHA, 2013. Office of Environmental Health Hazard Assessment Proposition 65. In: No Significant Risk Levels (NSRLs) for Carcinogens and Maximum Allowable Dose Levels (MADLs) for Chemicals Causing Reproductive Toxicity. <http://www.oehha.ca.gov/prop65/pdf/safeharbor081513.pdf> (accessed June 2015).
OSHA, 2003. Occupational Safety and Health Standards. <www.osha.gov/dts/chemicalsampling/toc/chmn_A.html (accessed June 2015).
Paine III, J.B., Pithawalla, Y.B., Naworal, J.D., Thomas, J., 2007. Carbohydrate pyrolysis mechanisms from isotopic labeling: Part 1: the pyrolysis of glycerin: Discovery of competing fragmentation mechanisms affording acetaldehyde and formaldehyde and the implications for carbohydrate pyrolysis. J. Anal. Appl. Pyrol 80, 297–311.
Tayyarah, R., Long, G.A., 2014. Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air. Regul. Toxicol. Pharmacol. 70, 704–710.
Trehy, M.L., Ye, W., Hadwiger, M.E., Moore, T.W., Allgire, J.F., Woodruff, J.T., Ahadi, S.S., Black, J.C., Westenberger, B.J., 2011. Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. J. Liq. Chromatogr. Relat. Technol. 34, 1442–1459.
Uchiyama, S., Ohta, K., Inaba, Y., Kunugita, N., 2013. Determination of carbonyl compounds generated from the e-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. Anal. Sci. 29, 1219–1222.
Vansickel, A.R., Edmiston, J., Liang, Q., Duhon, C., Liu, J., Sarkar, M., 2014. Characterization of electronic cigarette prototype puff topography in adult exclusive cigarette smokers and adult exclusive electronic cigarette users. In: Presented at the 20th Annual Meeting of the Society for Research on Nicotine and Tobacco (SRNT), February 5-8, 2014, Seattle, WA. POS3-65.
Wagener, T.L., Meier, E., Hale, J.J., Oliver, E.R., Warner, M.L., Driskill, L.M., Gillaspy, S.R., Siegel, M.B., Foster, S., 2014. Pilot investigation of changes in readiness and confidence to quit smoking after E-cigarette experimentation and 1 week of use. Nicotine Tob. Res. 16, 108–114.
Westenberger, B., 2009. Evaluation of e-cigarettes. US Food and Drug Administration, Washington, DC. <http://www.fda.gov/downloads/drugs/scienceresearch/ucm173250.pdf> (accessed June 2015).
WHO, 2001. Formaldehyde. <http://www.euro.who.int/__data/assets/pdf_file/0014/123062/AQG2ndEd_5_8Formaldehyde.pdf> (accessed June 2015).
Williams, M., Villarreal, A., Bozhilov, K., Lin, S., Talbot, P., 2013. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. PLoS One 8, e57987.