A review of constituents identified in e-cigarette liquids and aerosols

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ABSTRACT

INTRODUCTION Identification of chemicals present in e-liquids and aerosols is a vital first step in assessing the human health effects of e-cigarettes. We aim to identify the qualitative and quantitative constituents present in e-cigarette liquids and aerosols.

METHODS A comprehensive search of scientific databases included literature up to July 2020. A total of 28 articles met inclusion criteria; 18 articles assessed e-liquid constituents and 15 articles assessed aerosol constituents. Of these, 5 assessed constituents present in both mediums. We included English-language publications that examine qualitative and/or quantitative constituents in e-cigarette liquids and aerosols.

RESULTS In total, articles identified 60 compounds in e-liquids and 47 compounds in aerosols. A total of 22 compounds were identified in both e-liquids and aerosols. These are: acenaphthylene, acetaldehyde, acetol, antimony, benzaldehyde, benzene, chromium, copper, diacetyl, formaldehyde, glycerol, lead, limonene, naphthalene, nickel, nicotine, nicotine-N'-oxides, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-Nitrosonornicotine (NNN), propylene glycol, toluene, and vegetable glycerin. Some of the identified chemicals have been labeled as harmful, toxic, or cancerous through human, animal, and cell line studies. A variety of laboratory methods were used for analyses, which made reported levels less consistent.

CONCLUSIONS E-liquids and aerosols contain a variety of chemicals with potential health effects from inhaling them. Further, secondhand health effects are unknown because of limited understanding of the dose of exposure by non-users. Identification of constituents in e-cigarettes is the first step to determine their risks to humans and support evidence-based regulations and health policies.

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INTRODUCTION

Although there is evidence of harms associated with e-cigarette (EC) exposure, EC use continues to rise rapidly[1-3]. The recent outbreak of e-cigarette or vaping product use-associated lung injury (EVALI) has been linked to vitamin E acetate, however, there is insufficient evidence to rule out the contribution of other chemicals of concern[4]. Without first identifying the chemicals present in e-liquids and aerosols, it is challenging to understand their short- and long-term effects on human health and implement EC regulations. Quantification of secondhand aerosol exposure is another challenge. Given the increasing prevalence of EC use, identifying and quantifying the chemical compounds present in e-liquids and aerosols is a vital first step in risk assessment and implementation of effective regulations.

E-liquid variability has made comprehensive assessment of e-liquid constituents difficult. Actual levels of constituents present in e-liquids may differ

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from the amounts listed on product labels[5]. It is unknown whether new products are formed upon mixture of unidentified chemicals with solvents, but studies indicate that chemical transformation can occur in e-liquids[6,7]. Further, aerosolization of e-liquid can result in formation of new chemicals, which are inhaled by the user.

Given the increasing popularity of EC use and the latest outbreak of serious respiratory illnesses of unknown causes among EC users, as well as the higher risk (close to 7-fold among teens and young adults) of COVID-19 complications among EC users[8], we synthesized the available literature to assess the qualitative and quantitative constituents present in e-liquids and aerosols. We aimed to generate lists of the identified chemicals in e-liquids and aerosols to aid in finding the mechanisms and causative constituents through further focused chemical analyses.

METHODS

Search strategy and study selection

The literature search for this review has been conducted according to PRISMA protocols. Searches of the following databases were conducted in July 2020: PubMed, FDA, Google Scholar, PsycINFO, and Journal of Institute of Medicine. The following limits were applied: published online and in the English language. The earliest relevant study was published in 2013. Appropriate synonyms and free terms were used in each database. A combination of the following search terms was used: e-cigarette and/or content and/or constituent(s) and/or of vape liquid and/or aerosol(s) and/or alkaloid and/or copper and/or electronic nicotine delivery system(s) and/or toxicity and/or HPHC and/or vape and/or biomarker and/or passive and/or secondhand and/or electronic cigarette and/or vapor and/or electronic and/or quantitative study and/or analysis and/or chemical(s) and/or exposure and/or article review.

After database searches, we compiled a total of 461 studies. Sixty-six duplicates were removed, leaving us with 395 studies to screen. We removed 233 studies upon review of titles and abstracts. A total of 162 full-text articles were assessed for eligibility. Upon review of full text, the authors eliminated an additional 134 studies based on invalid results (e.g. samples were contaminated due to laboratory errors, study was not reproducible), irrelevance to aims, and/or difficulty reporting data without a meta-analysis; reviews were also eliminated, as data were not condensable. This left 28 articles to include in this review.

Inclusion criteria

This review includes peer-reviewed studies that focus on qualitative and quantitative analysis of compounds present in e-liquids and aerosols.

Exclusion criteria

Studies were excluded from this review if one of the following exclusion criteria applied: lack of access to full text, invalid results, not available in English, focused only on vaporized tobacco or tobacco-derived products, did not contain original data or data that were difficult to condense without a meta-analysis, or aims of published manuscript were not relevant to aims of this review (e.g. articles assessed prevalence of e-cigarette use, flavor preferences among age groups, etc.).

Data extraction and categorization of included studies

At least one full-text review of included studies was performed and articles were assigned into one of three categories: e-liquids, aerosols, or both. Their categorization was based on the medium from which constituents were identified. Subsequently, data on analysis methodologies, identified constituents, sample sizes, range of constituents, and limits of detection (LOD) and quantitation (LOQ) were extracted from included articles.

Data synthesis

Data were extracted during full-text reviews of relevant articles and categorized accordingly to present information in Tables 1 and 2.

General study characteristics

The studies chosen for this literature review identify compounds present in e-liquids (Table 1) and aerosols (Table 2). For this review, we have gathered and presented information about identified constituents in e-liquids from 18 studies (Supplementary file Table S1) and identified constituents in aerosols from 15 studies (Supplementary file Table S2). Of the 28

Table 1. Constituents identified in e-cigarette liquids (for constituents to be listed, the chemical must have been present in 50% of samples in at least one study)

Constituent (Descriptor)	Studies indicating presence of constituent First author (year)	Frequency of constituent among samples per study (Identifying samples/total samples)	LOD or LOQ
Acenaphthene (PAH)	Beauval (2017)	3/6	0.20 ng/mL
	Han (2016)	7/55	N/A
Acenaphthylene* (PAH)	Beauval (2017)	4/6	0.02 ng/mL
	Han (2016)	4/55	N/A
Acetaldehyde* (aldehyde)	Farsalinos, Gillman (2015)	10/21	0.12 μg/mL
	Han (2016)	54/55	N/A
	LeBouf (2018)	89/146	106 ppb
	Sleiman (2016)	3/3	N/A
	Varlet (2015)	42/42	0.03 μg/g
Acetol* (alcohol)	Sleiman (2016)	3/3	N/A
Acetone (ketone)	Han (2016)	52/55	N/A
	LeBouf (2018)	74/146	275 ppb
	Sleiman (2016)	3/3	N/A
	Varlet (2015)	2/42	N/A
Aluminum (heavy metal)	Beauval (2017)	6/6	4.0 ng/mL
Anabasine (insecticide)	Famele (2017)	58/95	1.6 μg/m³
	Han (2016)	43/55	N/A
	Hutzler (2014)	1/28	N/A
	Lisko (2015)	30/36	N/A
Anatabine (alkaloid)	Famele (2017)	58/95	0.2 μg/m³
	Han (2016)	42/55	N/A
	Hutzler (2014)	2/28	N/A
	Lisko (2015)	30/36	N/A
Antimony* (heavy metal)	Beauval (2017)	6/6	0.1 ng/mL
Benzaldehyde* (aromatic aldehyde)	Czoli (2019)	36/166	N/A
	Han (2016)	3/55	N/A
	Hutzler (2014)	4/28	N/A
	LeBouf (2018)	18/146	N/A
	Tierney (2015)	3/30	N/A
	Varlet (2015)	30/42	0.035 μg/g
Benzene* (aromatic hydrocarbon)	Han (2016)	55/55	N/A
	LeBouf (2018)	20/146	102 ppb
	Wagner (2018)	0/13	0.7 ng/g
Caffeine (aromatic hydrocarbon)	Lisko (2017)	25/44	0.04 μg/g
Chlorpyrifos ethyl (pesticide)	Beauval (2017)	3/6	20 pg/mL
Chromium* (heavy metal)	Beauval (2017)	6/6	3.7 ng/mL
	Kamilari (2018)	21/22	N/A
Chrysene (PAH)	Beauval (2017)	3/6	0.02 ng/mL
	Han (2016)	13/55	N/A
Copper* (heavy metal)	Beauval (2017)	3/6	20 ng/mL
	Kamilari (2018)	22/22	N/A
Cotinine (alkaloid)	Famele (2017)	58/95	0.1 μg/m³
	Han (2016)	20/55	N/A
Diacetyl* (diketone)	Farsalinos, Kistler (2015)	110/159	N/A
	Lebouf (2018)	67/146	102 ppb
	Varlet (2015)	3/42	N/A

Continued

Table 1. Continued

Constituent (Descriptor)	*Studies indicating presence of constituent First author (year)*	*Frequency of constituent among samples per study (Identifying samples/total samples)*	*LOD or LOQ*
Ethanol (alcohol)	LeBouf (2018)	139/146	225 ppb
	Peace (2017)	3/3	N/A
	Sleiman (2016)	3/3	N/A
	Varlet (2015)	30/42	N/A
Ethyl benzene (aromatic hydrocarbon)	Han (2016)	43/55	N/A
	LeBouf (2018)	3/146	138 ppb
Ethyl butanoate (ether)	LeBouf (2018)	91/146	N/A
	Peace (2017)	1/3	N/A
Ethyl maltol (cyclic ketone)	Czoli (2019)	31/166	N/A
	Girvalaki (2018)	44/122	N/A
	Hutzler (2014)	16/28	N/A
	Peace (2017)	1/3	N/A
	Tierney (2015)	10/30	N/A
Ethyl vanillin (benzaldehyde)	Czoli (2019)	37/166	N/A
	Girvalaki (2018)	22/122	N/A
	Hahn (2014)	13/54	1.0 mg/L
	Hutzler (2014)	14/28	N/A
	Tierney (2015)	10/30	N/A
Ethylene glycol (hydrocarbon)	Hahn (2014)	N/A	0.17 mg/L
	Varlet (2015)	31/46	N/A
Fluoranthene (PAH)	Beauval (2017)	4/6	0.05 ng/mL
	Han (2016)	13/55	N/A
Fluorene (PAH)	Beauval (2017)	5/6	0.2 ng/mL
	Han (2016)	5/55	N/A
Formaldehyde* (aldehyde)	Farsalinos, Gillman (2015)	20/21	0.12 μg/mL
	Han (2016)	55/55	N/A
	Sleiman (2016)	3/3	N/A
	Varlet (2015)	42/42	0.06 μg/g
Glycerol* (alcohol)	Beauval (2017)	6/6	12.5 mg/mL
	Hahn (2014)	54/54	2.6 mg/L
	Han (2016)	55/55	N/A
	Peace (2017)	3/3	N/A
	Sleiman (2016)	3/3	N/A
Isonicoteine (pyridine derivative)	Lisko (2015)	30/36	N/A
Isopentyl alcohol (alcohol)	Sleiman (2016)	2/3	N/A
Isopropyl alcohol (alcohol)	LeBouf (2018)	75/146	189 ppb
Lead* (heavy metal)	Kamilari (2018)	22/22	N/A
Limonene* (hydrocarbon)	Hutzler (2014)	2/28	N/A
	LeBouf (2018)	79/146	275 ppb
MDMB-FUBINACA (psychoactive cannabinoid)	Peace (2017)	3/3	N/A
m,p-Xylene (aromatic hydrocarbon)	Han (2017)	55/55	N/A
	LeBouf (2018)	16/146	114 ppb
Myosmine (alkaloid)	Famele (2017)	58/95	0.1 μg/m^3
	Han (2016)	42/55	N/A
	Hutzler (2014)	2/28	N/A
	Lisko (2015)	30/36	N/A
NAB (alkaloid)	Han (2016)	43/55	N/A

Continued

Table 1. Continued

Constituent *(Descriptor)*	*Studies indicating presence of constituent First author (year)*	*Frequency of constituent among samples per study (Identifying samples/total samples)*	*LOD or LOQ*
Naphthalene* (PAH)	Beauval (2017)	5/6	0.2 ng/mL
	Han (2016)	12/55	N/A
Nickel* (heavy metal)	Kamilari (2018)	21/22	N/A
Nicotine* (alkaloid)	Beauval (2017)	3/6	2.0 mg/mL
	Hahn (2014)	34/54	1.6 mg/L
	Han (2016)	52/55	N/A
	Lisko (2015)	29/26	N/A
	Sleiman (2016)	3/3	N/A
Nicotine-N'-oxides* (alkaloid)	Famele (2017)	58/95	0.1 μg/m³
Nitrate (nitrogen ion)	Farsalinos, Gillman (2015)	11/21	2.5 μg/mL
NNK* (alkaloid)	Farsalinos, Gillman (2015)	21/21	1 ng/mL
	Han (2016)	2/55	N/A
NNN* (alkaloid)	Farsalinos, Gillman (2015)	12/21	1 ng/mL
Nornicotine (alkaloid)	Lisko (2015)	30/36	N/A
o-Xylene (aromatic hydrocarbon)	Han (2016)	51/55	N/A
	LeBouf (2018)	6/146	102 ppb
Propylene glycol* (alcohol)	Beauval (2017)	6/6	31.25 mg/mL
	Hahn (2014)	54/54	2.1 mg/L
	Han (2016)	55/55	N/A
	Peace (2017)	3/3	N/A
	Sleiman (2016)	3/3	N/A
Phenanthrene (PAH)	Beauval (2017)	6/6	0.2 ng/mL
	Han (2016)	7/55	N/A
Phenol (aromatic alcohol)	Farsalinos, Gillman (2015)	1/21	0.05 μg/mL
	Han (2016)	40/55	N/A
Propylene oxide (Cyclic ether)	Sleiman (2016)	3/3	N/A
Raspberry ketone (phenol)	Peace (2017)	2/3	N/A
Raspberry ketone PG (phenol)	Peace (2017)	3/3	N/A
Toluene* (aromatic hydrocarbon)	Han (2016)	32/55	N/A
	LeBouf (2016)	13/146	126 ppb
	Wagner (2018)	0/13	0.7 ng/g
Vanillin (phenolic aldehyde)	Czoli (2019)	36/166	N/A
	Hutlzer (2014)	22/28	N/A
	Sleiman (2016)	1/3	N/A
	Tierney (2015)	15/30	N/A
α-Isomethylionone (cyclic ketone)	Hutzler (2014)	1/28	N/A
	Sleiman (2016)	2/3	N/A
β-Damascone (cyclic ketone)	Girvalaki (2018)	23/122	N/A
	Tierney (2015)	1/30	N/A
β-Nicotyrine (alkaloid)	Famele (2017)	58/95	0.2 μg/m³
1-Methyl naphthalene (PAH)	Czoli (2019)	115/166	N/A
2-Methyl naphthalene (PAH)	Czoli (2019)	104/166	N/A
3-Hexen-1-ol (alcohol)	Hutzler (2014)	1/28	N/A
	Sleiman (2016)	1/3	N/A
	Tierney (2015)	1/30	N/A

*Constituent presence in liquid and aerosol. LOD: limit of detection. LOQ: limit of quantitation. NAB: N'-Nitrosoanabasine. NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. NNN: N-Nitrosonornicotine. PAH: polycyclic aromatic hydrocarbon. PG: propylene glycol. VG: vegetable glycerin.

Table 2. Constituents identified in e-cigarette aerosols (for constituents to be listed, the chemical must have been present in 50% of samples in at least one study)

Constituent *(Descriptor)*	*Studies indicating presence of constituent First author (year)*	*Frequency of constituent among samples per study (Found/total samples)*	*LOD or LOQ*
Acenaphthylene* (PAH)	Beauval (2017)	6/6	0.09 pg/mL puff
Acetaldehyde* (aldehyde)	Beauval (2017)	6/6	0.05 pg/mL puff
	Bekki (2014)	9/13	N/A
	Goniewicz (2013)	12/12	N/A
	Klager (2017)	26/26	27.3 μg/m^3
	Peace (2018)	1/4	N/A
	Sleiman (2016)	3/3	N/A
Acetoin (ketone)	Allen (2016)	46/51	0.05 μg/sample
	Klager (2017)	17/26	0.00 μg/m^3
Acetol* (alcohol)	Sleiman (2016)	3/3	N/A
Acetyl propionyl (diacetyl)	Farsalinos, Kistler (2015)	3/3	N/A
Acrolein (aldehyde)	Beauval (2017)	4/6	0.05 ng/mL puff
	Bekki (2014)	9/13	N/A
	Goniewicz (2013)	10/12	N/A
	Peace (2018)	1/4	N/A
	Sleiman (2016)	3/3	N/A
Antimony* (heavy metal)	Beauval (2017)	4/6	0.11 pg/mL puff
Benzaldehyde* (aromatic aldehyde)	Klager (2017)	17/26	9.81 μg/m^3
	Kosmider (2016)	108/145	0.025 μg/30 puffs
	Peace (2018)	1/4	N/A
	Sleiman (2016)	2/3	N/A
Benzene* (aromatic hydrocarbon)	Sleiman (2016)	3/3	N/A
	Wagner (2018)	0/19	3.2 μg/g
Butyraldehyde (aldehyde)	Sleiman (2016)	2/3	N/A
Cadmium (heavy metal)	Beauval (2017)	2/6	0.025 μg/30 puffs
	Goniewicz (2013)	11/12	N/A
Chromium* (heavy metal)	Beauval (2017)	3/6	2.1 pg/mL puff
	Halstead (2019)	9/17	0.125 ng/10 puffs
	Williams (2013)	N/A	N/A
Copper* (heavy metal)	Halstead (2019)	12/17	0.20 ng/10 puffs
	Williams (2013)	N/A	N/A
Crotonaldehyde (aldehyde)	Klager (2017)	4/26	0 μg/m^3
	Sleiman (2016)	3/3	N/A
Diacetin (diether)	Schripp (2013)	3/3	N/A
Diacetyl* (diketone)	Allen (2016)	39/51	0.05 μg/sample
	Farsalinos, Kistler (2015)	3/3	N/A
	Klager (2017)	16/26	0.00 μg/m^3
	Sleiman (2016)	3/3	N/A
Ethyl butyrate (ether)	Peace (2018)	4/4	N/A
Formaldehyde* (aldehyde)	Beauval (2017)	6/6	0.05 pg/mL puff
	Bekki (2014)	9/13	N/A
	Goniewicz (2013)	12/12	N/A
	Klager (2017)	24/26	5.77 μg/m^3
	Sleiman (2016)	3/3	N/A
Glycerol* (alcohol)	Beauval (2017)	6/6	3.4 μg/mL puff
	Peace (2018)	3/4	N/A
	Schripp (2013)	3/3	N/A

Continued

Table 2. Continued

Constituent *(Descriptor)*	*Studies indicating presence of constituent First author (year)*	*Frequency of constituent among samples per study (Found/total samples)*	*LOD or LOQ*
Glycidol (alcoholic epoxide)	Sleiman (2016)	3/3	N/A
Glyoxal (deladehyde)	Bekki (2014)	8/13	N/A
Hexaldehyde (aldehyde)	Sleiman (2016)	3/3	N/A
Isobutyraldehyde (aldehyde)	Klager (2017)	13/26	0.00 μg/m³
Lead* (heavy metal)	Beauval (2017)	4/6	0.23 pg/mL puff
	Goniewicz (2013)	12/12	N/A
	Halstead (2019)	8/17	0.05 ng/10 puffs
	Williams (2013)	N/A	N/A
Limonene* (hydrocarbon)	Peace (2018)	2/4	N/A
	Sleiman (2016)	2/3	N/A
Methacrolein (aldehyde)	Sleiman (2016)	3/3	N/A
Methyl ethyl ketone (ketone)	Sleiman (2016)	3/3	N/A
Methylglyoxal (aldehyde)	Bekki (2014)	8/13	N/A
	Sleiman (2016)	3/3	N/A
Naphthalene* (PAH)	Beauval (2017)	6/6	0.47 pg/mL puff
Nickel* (heavy metal)	Goniewicz (2013)	12/12	N/A
	Halstead (2019)	14/17	0.250 ng/10 puffs
	Williams (2013)	N/A	N/A
Nicotine* (alkaloid)	Beauval (2017)	3/6	0.0038 μg/mL puff
	Czogala (2013)	12/12	0.22 μg/m³
	Famele (2017)	7/13	0.1 μg/m³
	Peace (2018)	4/4	10 ng/mL
	Schripp (2013)	3/3	N/A
	Sleiman (2016)	3/3	N/A
Nicotine-N'-oxides* (alkaloid)	Famele (2017)	7/13	0.1 μg/m³
Nicotyrine (alkaloid)	Sleiman (2016)	3/3	N/A
NNK* (alkaloid)	Goniewicz (2013)	9/12	N/A
NNN* (alkaloid)	Goniewicz (2013)	9/12	N/A
o-methylbenzaldehyde (aromatic aldehyde)	Goniewicz (2013)	12/12	N/A
p-Tolualdehyde (aromatic aldehyde)	Sleiman (2016)	3/3	N/A
p,m-xylene (aromatic hydrocarbon)	Goniewicz (2013)	10/12	N/A
Propylene glycol* (alcohol)	Beauval (2017)	6/6	3.0 μg/mL puff
	Peace (2018)	4/4	N/A
	Schripp (2013)	3/3	N/A
Propanal (aldehyde)	Bekki (2014)	8/13	N/A
	Klager (2017)	23/26	1.2 μg/m³
	Sleiman (2016)	3/3	N/A
Stannum (heavy metal)	Halstead (2019)	10/17	0.10 ng/10 puffs
Toluene* (aromatic hydrocarbon)	Goniewicz (2013)	10/12	N/A
	Wagner (2018)	0/19	3.2 μg/g
Valderaldehyde (aldehyde)	Sleiman (2016)	3/3	N/A
2-Butanone (ketone)	Sleiman (2016)	3/3	N/A
2-Propen-1-ol (alcohol)	Sleiman (2016)	2/3	N/A
3-Ethenyl Pyridine (pyridine)	Sleiman (2016)	3/3	N/A
3-Methylbutyl-3-methylbutanoate (fatty acid ester)	Schripp (2013)	3/3	N/A

*Constituent presence in liquid and aerosol. LOD: limit of detection. LOQ: limit of quantitation. NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. NNN: N-Nitrosonornicotine. PAH: polycyclic aromatic hydrocarbon. PG: propylene glycol. VG: vegetable glycerin.

studies included in this review, 5 articles examined components of both e-liquids and aerosols[9-13].

Studies reporting chemicals present in e-liquids were published between 2014 and 2020; studies reporting chemicals present in EC aerosols were published between 2013 and 2020. If available, the LOD or LOQ is presented (Tables 1 and 2); if this information was unavailable, it was documented as 'N/A' in Tables 1 and 2 and in the Supplementary tables. For studies that included both the LOD and LOQ, the LOD is presented, since it is more reliable for determining whether an analyte is present or absent[14].

Constituents are documented in Table 1 or Table 2 if at least one study detects the specific constituents in ≥50% of e-liquid or aerosol samples. In order to allow comprehensiveness, the remainder of studies that detected the same constituent (i.e. in <50% of samples) are also included.

RESULTS

Constituents identified in both e-liquids and aerosols

Of all the constituents identified from the 28 articles in this review, a total of 22 chemicals were identified in both e-liquids and aerosols. The common chemicals present in both mediums are: acenaphthylene, acetaldehyde, acetol, antimony, benzaldehyde, benzene, chromium, copper, diacetyl, formaldehyde, glycerol, lead, limonene, naphthalene, nickel, nicotine, nicotine-N'-oxides, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-Nitrosonornicotine (NNN), propylene glycol, toluene, and vegetable glycerin.

Studies analyzing e-liquid constituents

Of the articles included in this review, 18 articles identified constituents present in e-liquids[9-13,15-25]. A total of 60 constituents were found among all studies. Out of the 18 studies that examined e-liquids, 17 articles identified at least one constituent in e-liquids; the remaining article was in search of various chemicals in e-liquids from combustion-related constituents that were designated by the U.S. Food and Drug Administration (FDA) as harmful or potentially harmful constituents[13]. Studies utilized refill e-liquids, concentrated flavors, natural extract of tobacco liquids, and/or cartridges. Sample sizes ranged from 3 to 166; these values do not include conventional cigarettes, if present. For six of the 18 studies, the primary aim was to test for validity and reliability of novel measurements for identification and quantitation of e-liquid constituents. We have chosen to include these studies because they compare their novel scale against measurement scales that have already been validated throughout the literature (i.e. GC-MS, NMR spectroscopy, etc.).

Studies analyzing e-cigarette aerosol constituents

Of the articles included in this review, 15 articles identified constituents present in EC aerosols[9-13,26-34]. A total of 47 constituents were found among all studies. Out of the 15 studies that examined e-cigarette aerosols, 14 articles identified at least one constituent in aerosols; the remaining article was in search of various chemicals in aerosols from combustion-related constituents that were designated by the FDA as harmful or potentially harmful constituents[13]. Sample sizes ranged from 2 to 159; these values do not include conventional cigarettes, if present. To identify constituents present in EC aerosols, studies either sampled air after a human subject used ECs or used a smoking machine to produce aerosols, which were captured and analyzed with valid and reliable analytic techniques. For five of the 15 studies, the primary aim was to test for validity and reliability of novel measurements for identification and quantitation of e-liquid constituents. We have chosen to include these studies because they compare their novel scale against measurement scales that have already been validated throughout the literature (i.e. GC-MS, NMR spectroscopy, etc.).

Analytical methods used for identification of e-liquid constituents

Various techniques were utilized to assess qualitative and quantitative e-liquid constituents (Table 3). Of the 18 studies that examined e-liquid constituents, the primary aim of six studies was to develop and test a novel analytical method for detection and quantitation of compounds.

Several studies utilized multiple methods in order to detect various types of compounds (i.e. metals, polycyclic aromatic compounds, etc.). Of the 18 articles, all but three used gas chromatography-mass

Table 3. Laboratory analysis methods for identification and quantitation of e-cigarette liquids

First author (year)	Method(s)
Beauval (2017)	GC-MS-MS GC-FID ICP-MS GC-MS-MS in electron impact ionization mode
Czoli (2019)	UPLC-MS-MS
Famele (2017)	LC-MS-MS
Farsalinos, Gillman (2015)	GC GC-MS GC-MS-MS
Farsalinos, Kistler (2015)	FID-GC GC-MS HPLC with electrochemical detector HPLC-FLD HPLC-UV
Girvalaki (2018)	GC-MS LC-MS
Hahn (2014)	NMR spectroscopy
Han (2016)	GC-FID GC-MS HPLC-MS-MS HPLC-DAD HPLC-FLD
Hutzler (2014)	GC-MS
Kamilari (2018)	Total reflection X-ray fluorescence spectrometry
LeBouf (2018)	HS-GC-MS
Lisko (2015)	GC-MS-MS
Lisko (2017)	GC-MS
Peace (2017)	DART-MS GC-MS HS-GC-FID
Sleiman (2016)	HS-GC-MS Thermal desorption-GC-MS
Tierney (2015)	GC-MS
Varlet (2015)	GC-MS HS-GC-MS LC-MS-MS LC-UV-MS
Wagner (2018)	GC-MS

DART-MS: direct analysis in real time mass spectroscopy. GC: gas chromatography. GC-FID: gas chromatography with flame ionization detector. GC-MS: gas chromatography-mass spectrometry. GC-MS-MS: gas chromatography with tandem mass spectrometry. HPLC: high performance liquid chromatography. HPLC-DAD: high performance liquid chromatography with diode-array detector. HPLC-FLD: high performance liquid chromatography with fluorescence detector. HPLC-MS-MS: high performance liquid chromatography with tandem mass spectrometry. HPLC-UV: high performance liquid chromatography with ultraviolet radiation. HS-GC-FID: headspace gas chromatography with flame ionization detector. ICP-MS: inductively coupled plasma mass spectrometry. HS-FID: headspace with flame ionization detector. HS-GC-MS: headspace gas chromatography with mass spectrometry. LC-MS: liquid chromatography-mass spectrometry. LC-MS-MS: liquid chromatography with tandem mass spectrometry. LC-UV-MS: liquid chromatography with ultraviolet radiation and mass spectrometry. NMR: nuclear magnetic resonance. UPLC: ultra-performance liquid chromatography.

spectrometry (GC-MS) or some form of GC-MS (e.g. headspace GC-MS) to analyze compounds present in e-liquids[10,11,16]. Nine studies used one analysis method only, while the remaining 8 studies used more than one.

Analytical methods used for identification of aerosol constituents

Various techniques were utilized to assess qualitative and quantitative aerosol constituents (Table 4). Several studies utilized multiple methods in order to detect various types of compounds (i.e. metals, polycyclic aromatic compounds, etc.). Of the 15 papers, seven studies utilized GC-MS or some form of GC-MS. High performance liquid chromatography (HPLC), or some form of it, was used in nine studies. Five studies used one analysis method only, while the remaining nine studies used more than one.

Table 4. Laboratory analysis methods for identification and quantitation of e-cigarette aerosols

First author (year)	Method(s)
Allen (2016)	GC-ECD
Beauval (2017)	GC-MS-MS UPLC-MS-MS ICP-MS HPLC-DAD
Bekki (2014)	HPLC
Czogala (2013)	GC-NPD GC-MS
Famele (2017)	LC-MS-MS
Farsalinos, Kistler (2015)	HPLC
Goniewicz (2013)	HPLC-DAD GC-MS UPLC-MS ICP-MS
Halstead (2019)	MS-MS
Klager (2017)	HPLC-UV GC-ECD
Kosmider (2016)	HPLC
Peace (2018)	HPLC-MS DART-MS GC-MS
Schripp (2013)	Thermal desorption GC-MS HPLC coupled with variable wavelength detector
Sleiman (2016)	HS-GC-MS HPLC-UV TD-GC-MS

Continued

Table 4. Continued

First author (year)	Method(s)
Wagner (2018)	GC-NCI MS GC-MS with electron ionization
Williams (2013)	ICP-OES SEM EDXS

DART-MS: data analysis in real time-mass spectrometry. EDXS: energy dispersive x-ray spectroscopy. GC: gas chromatography. GC-ECD: gas chromatography with electron capture detector. GC-FID: gas chromatography with flame ionization detector. GC-MS: gas chromatography-mass spectrometry. GC-MS-MS: gas chromatography with tandem mass spectroscopy. GC-NCI MS: gas chromatography-negative chemical ionization mass spectrometry. GC-NPD: gas chromatography with nitrogen phosphorous detector. HPLC: high performance liquid chromatography. HPLC-DAD: high performance liquid chromatography with diode-array detector. HPLC-UV: high performance liquid chromatography with ultraviolet radiation. HS-GC-MS: headspace gas chromatography with mass spectrometry. ICP-MS: inductively coupled plasma mass spectrometry. ICP-OES: inductively coupled plasma atomic emission spectroscopy. ICP-UV-MS: inductively coupled plasma with ultraviolet radiation and mass spectrometry. LC-MS-MS: liquid chromatography with tandem mass spectrometry. OSHA: Occupational Safety and Health Administration. SEM: scanning electron microscope. TD-GS-MS: thermal desorption coupled with gas chromatography mass spectrometry. UPLC-MS: ultra-performance liquid chromatography-mass spectrometry. UPLC-MS-MS: ultra-performance liquid chromatography with tandem mass spectrometry.

DISCUSSION

Main constituents present in e-liquids and aerosols

A total of 22 chemicals were identified in both e-liquids and aerosols. These chemicals are known carcinogens, heavy metals, and nicotine. We notice a variation in constituents and their respective levels among various e-cigarette products; it is not known whether these are byproducts of heating the e-liquid. Further, it is important to note that several of these constituents have been identified in tobacco smoke and are listed by the FDA as harmful or potentially harmful constituents (HPHC)[35].

Acenaphthylene is present in a majority of samples among studies that identified this chemical. Acetaldehyde was detected in five e-liquid studies and six aerosol studies; another study showed that acetaldehyde was present in 100% of e-liquid and 100% of aerosol samples[12]. Antimony was present in 6 of 6 samples in e-liquids, but only 4 of 6 samples in aerosols in one study[9]. Of the studies that detected diacetyl, it was present in all of or a majority of e-liquid or aerosol samples. Other than in one article, formaldehyde was present in 100% of all e-liquids that identified it; the same is true for formaldehyde detected in aerosol samples. Both glycerol and propylene glycol, known components of e-liquids, are present in all samples in studies that identified them. Lead was present in 100% of e-liquid samples and in all or a majority of aerosol samples that reported sample size values. Nickel was present in all but one sample of e-liquids and all samples of aerosols that reported sample size values. Of the constituents discussed in the current section, acetaldehyde, formaldehyde, lead, and nickel have been identified in conventional cigarette smoke and are classified as HPHCs by the FDA. Several other constituents identified in this review, such as nicotine, ethyl benzene, crotonaldehyde, chromium, and anabasine, are also HPHCs as classified by the FDA[35]. The presence of such chemicals in e-liquids and their aerosols invite concern regarding the effects of secondhand exposure, which may have negative impacts on population health.

Constituents identified in e-liquids

Many studies identified the presence of minor tobacco alkaloids, flavorings, metals, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs) in e-liquids; other studies found pesticides[9], psychoactive drugs[24], and caffeine[23] present. One study found microorganisms present in e-liquids[36]. Four articles identified the presence of anabasine, anatabine, and myosomine, which are common minor tobacco alkaloids, in e-liquids[10,18,19,22]. Multiple articles identified constituents related to flavorings, such as diacetyl and vanillin, in e-liquid samples. Diacetyl was also identified in aerosols[11,12,26,30]. Presence of metals in aerosols has been hypothesized to be a result of liquid heating on the metal surface of the atomizer[37], but trace elements of metals are detected in e-liquids, which undermines such a hypothesis. Some elements found in e-liquids include aluminum, antimony, chromium, copper, and lead[9,20,32,38].

Constituents identified in e-cigarette aerosols

Similar constituents were identified in EC aerosols. Some articles indicate that different levels of chemicals are present in EC aerosols compared to liquids. One study found unsafe levels of formaldehyde in aerosols, indicating that the median concentrations in EC aerosols exceed the limits set by the National Institute for Occupational Safety and the American Conference of Governmental Industrial Hygienists[30].The presence of nicotine in EC aerosols may characterize it as a potential secondhand exposure[9,10,12,28,32,33]. One study indicates the presence of benzaldehyde in 108 of 145 samples of aerosols, with higher levels in aerosols of flavored e-liquids compared to that of conventional cigarettes; specifically, cherry-flavored liquids were shown to have the highest emissions of benzaldehyde[31]. Another study indicates that ECs are a source of particulate matter[33], while another shows that indoor use of ECs does not cause exposure to fine particles[28].

Identification of harms in humans

In total, 91 constituents have been identified in e-liquids and aerosols (Tables 1 and 2). Exposure to several of the constituents have been shown to cause harmful effects in humans. Diacetyl, which was identified in a majority of aerosol samples, is a flavoring agent that has been shown to cause bronchiolitis obliterans, also known as popcorn lung, when inhaled[39]. This is a chronic, irreversible pulmonary condition that causes a rapid decline in lung function; prognosis is usually poor and non-transplant related treatments are insufficient. Carcinogens, such as NNN and NNK, were identified in e-liquids and aerosols. Activated NNK and NNN induce mutations in oncogenes and tumor suppressor genes, which may be an indication of tumor initiation[40]. Formaldehyde can increase the risk of asthma[41], squamous cell carcinoma, nasopharyngeal cancer, Hodgkin's lymphoma, and leukemia[42]. Some chemicals, such as acetaldehyde, are suspected to contribute to abuse liability of ECs. Acetaldehyde, a known toxicant identified in several e-liquids and aerosols, increases the reinforcing effects of nicotine and has been shown to have reinforcing effects itself. It also can alter the oral microbiome, which may result in poor oral hygiene and downstream negative health impacts[43]. Nicotine increases the risks of cardiovascular, respiratory, and gastrointestinal disorders, lowers the human immune response, can harm reproductive health, and can lead to cancer. Secondhand exposure to nicotine results in substantial occupancy of α4β2 nicotinic acetylcholine receptors in the brain[44]. Though solvents used in e-liquids are typically safe for ingestion at low doses, their effects on human health when inhaled are unknown. One case study indicates that inhalation of vegetable glycerin may be a cause of lipoid pneumonia seen in patients presenting with respiratory illnesses[45].

Although there is evidence regarding the harms of several constituents present in EC products, the health effects of EC use are largely unknown. It is unclear whether many of the identified constituents are safe for inhalation at any level. The effects of such constituents in combination with nicotine and other chemicals, upon mixture, and upon aerosolization are also unknown and may be a culprit for the recent outbreaks of pulmonary disease. Most recently in the light of COVID-19, EC use has been shown to substantially increase the risk of developing COVID-19[8], which might be mediated by damage of EC constituents to lung tissue or decreasing the immune system function, resulting in higher risk of disease among users.

Identification of harms in animal models

Several studies indicate the potential harms associated with EC use in animal models. E-liquids have been shown to significantly reduce energy intake and induce hyperglycemia[46]. EC exposure was associated with an increase in respiratory symptoms and changes in respiratory functioning and host defences, such as airway irritation, mucus hypersecretion, and inflammatory responses[46]. Several constituents listed in this review, such as diacetyl, acrolein, formaldehyde, and acetaldehyde, are known respiratory irritants[47]. E-liquids may be nephrotoxic, as they alter the antioxidant defences present in renal collecting ducts and promote minor changes in renal function[48]. Early exposure to e-liquids may lead to chronic neuropathology, hindering proper central nervous system development[49]. There were greater changes in gene expression among animals exposed to aerosols without nicotine compared to those exposed to nicotine, suggesting that non-nicotinic constituents in ECs lead to neuropathological changes[49]. E-liquids have shown toxicity in the liver; one study shows that injection of e-liquids gave rise to more histopathological injuries compared to injection of nicotine alone[50]. Exposure to e-liquids has also been shown to alter testicular function in male rats[51]. Though there is no consensus regarding which constituent is associated with the aforementioned harms, the authors indicate that nicotine and flavoring compounds (i.e. diacetin), may be viable culprits in altering liver and testicular function[50,51]. We speculate that harmful outcomes are a result of multi-constituent interaction.

Identification of harms in cell lines

Several in vitro studies have found e-liquids and aerosols to be cytotoxic[52,53]. The exposure of human lung cell lines to EC aerosols results in damage to bronchial epithelial cells[54]. It is unclear whether this damage is linked to flavoring agents or aerosol nanoparticles. There is agreement that the cytotoxicity of e-liquids is highly dependent on their flavoring chemicals[55]. Not surprisingly, considering the myriad of flavoring chemicals used, various cellular physiological responses have been documented. Exposure to aerosols is shown to induce cell shape modification and promotion of cell apoptosis[56,57]. Flavoring agents cause high levels of cytotoxicity in human embryonic cells and mouse neural stem cells[58]. Other studies have found that cell lines exposed to EC aerosols have similar responses as those exposed to conventional tobacco smoke[59].

Implications for regulation

The FDA issued regulatory authority over all EC forms in 2016, prohibiting sale of EC devices and products to minors and requiring new products to be approved by the FDA before being marketed[60,61]. In response to the outbreak of EVALI, the FDA was under scrutiny and criticism for insufficient efforts to determine the health risks from these products. The lack of clear health impacts associated with EC exposure is a result of limited data regarding the identity of chemical constituents present in e-cigarettes and their health impacts[60].

Regulation and its implementation have been hindered by the wide presence of thousands of products that are not regulated and the incorrect perception that e-cigarettes are harmless. Though there is some sparse data that indicate the use of ECs as potential nicotine replacement therapies, the EVALI outbreak is a clear indication that ECs have adverse effects and require regulation. To allow for safe EC use, quantifying the health effects of ECs is a vital step in promotion of effective EC regulations.

Our study provides a list of multiple chemicals that are present in e-liquids and/or their corresponding aerosols. This evidence can inform regulations regarding EC diversity and reduce the risk among current EC users.

Strengths and limitations

A limitation of this study is that it is not a systematic review. The studies in this review did not systematically look for the same chemicals; the articles had varying aims and chose to analyze different constituents among e-liquids or aerosols. This may result in a large number of constituents being unreported in our review. Although not all studies looked for every single constituent, or even the same constituent, this may be caused by difficulty in prioritizing which chemicals require urgent investigation. This review partially contributes to fill those gaps by providing an overview of the chemicals present in a majority of e-liquids and aerosols.

Another limitation is the fact that the studies used in this review used various techniques for sample preparation and analysis. Sample preparation and sample analysis methods can impact the chemicals and LOD/LOQ values that are detected in studies, which may cause differences in reporting. In this study, we focus on qualitative results and the proportions to which they are present in collected studies to provide a gauge for which constituents should be studied with urgency. This review allows those classifications by identifying what chemicals are present across studies in large quantities.

Despite the above limitations and considering the recent outbreaks and the current vaping epidemic among youth, this review provides a good starting point to identify constituents that are of risk to humans. A list is provided of several constituents that may guide researchers in determining what constituents to examine when studying EC liquids and/or aerosols, which in turn will allow scientists, clinicians, policymakers, and public health practitioners towards better understanding the health effects of such chemicals upon inhalation.

CONCLUSIONS

This review gathers identified components present in e-liquids and aerosols from 28 articles. E-liquids and aerosols contain a variety of unidentified chemical constituents. It is unknown whether such chemicals are added during the manufacturing process, upon mixture with other constituents, or via another route. Although some of the chemicals reported in this review have been deemed safe for ingestion, the outcomes associated with inhalation of these chemicals are unknown. Inhalation of such chemicals justifies classification as a different route of exposure and warrants further toxicity assessment. This review allows researchers to initiate systematic analyses of e-liquids and aerosols to allow for a better understanding of the effects of such products.

Use of ECs as a safer alternative to conventional cigarettes is not supported as an official method. The constituents found in aerosols may lead to secondhand exposure risk. In this review, we focus on a list of constituents that are frequently found among e-cigarettes and e-liquids. Regulation is overdue in this industry and has been exposing millions of people to unknown chemicals with unknown health effects. Though some of the chemicals used in e-liquids have been deemed safe for ingestion, there is little investigation on their health effects upon inhalation. Exposure to such chemicals via inhalation is likely associated with different outcomes. This exposure route warrants classification of such chemicals as unique exposures and needs urgent investigation. In order to remain cautious and refrain from propagating use of such products, which have caused an epidemic of nicotine addiction among youth and are associated with the recent outbreak of respiratory illnesses, we recommend the completion of further systematic analyses in identification and quantitation of these constituents throughout all e-liquids and their respective aerosols.

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Both authors were involved in the study concept, design, and interpretations of the results. EAE gathered the articles, extracted the data, and wrote the main draft of the manuscript. Both authors provided critical revisions of the manuscript and were involved in the final approval of the manuscript.

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