Original Research Article

Carcinogenic Potential of E-cigarettes: Vapor Profile and Cellular Effects

Abstract

E-cigarettes are devices that vaporize a liquid made of polyglycerol, glycol, flavorings, and nicotine, for inhalation. Initially created for smoking cessation, the health risks of these devices are still not clear. This literature review compiles data on the chemical profile of e-vapor and cell exposure studies to formulate conclusions regarding cancer risk and provide suggestions for future research. The reviewed studies identified a large range of potentially harmful compounds, namely formaldehyde, acrolein, and acetaldehyde, which were found in all studies. Metabolites of these compounds were then identified in exposed patients, showing bodily absorption. *In vitro* studies found evidence for cellular damage, including DNA mutations, reduced cell viability, and differentiated protein expression which may increase user's cancer risk. Though the evidence is inconclusive given the heterogeneity of the field. Future studies should focus on the human effects of vaping, testing bronchial brushings and lavage fluid from users to determine the *in vivo* effects of exposure. Closely monitoring e-cigarette users for early warning signs of cancer would also help us understand future risk and answer questions about the safety of these devices.

Keywords: Electronic Cigarette Use, Electronic Cigarettes, Lung Cancer Risk, Lung Cancer Prevention, Chemical Profile

Abbreviations	
A549	Adenocarcinoma Human Alveolar Basal Epithelial Cells
AHR	Aryl Hydrocarbon Receptor
BEAS-2B*	Bronchial Epithelial Cell Line
BIRC5	Baculoviral Inhibitor of Apoptosis Repeat Containing 5
CYP1A1	Cytochrome P450 Family 1 Subfamily A Member 1
CYP1B1	Cytochrome P450 Family 1 Subfamily B Member 1
CYP2A5	Cytochrome P450 Family 2 Subfamily A Member 5
CYP2A6	Cytochrome P450 Family 2 Subfamily A Member
CYP450	Cytochrome P450
GC/MS	Gas Chromatography/Mass Spectrometry
HaCaT	Human Epidermal Keratinocyte Line
iNOS	Nitric Oxide Synthase
M&P-xylene	Meta-xylene, Para-xylene
MMP-9	Matrix Metalloproteinase 9
MMP-12	Matric Metalloproteinase 12
MUC5AC	Mucin 5AC
NNK	Nicotine-derived Nitrosamine Ketone
NNN	N-nitrosonornicotine
O6-medG adducts	O6-methylguanine
OGG1/2	8-Oxoguanine glycosylase 1 and 2
WNT	Wingless-related integration site
XPC	Xeroderma Pigmentosum

Introduction

E-cigarettes have been growing in popularity among North Americans since their introduction in the late 2000s and have risen in popularity since (especially among young people[1]).

The process of smoking an e-cigarette involves vaporizing a liquid with a heating coil so it can be inhaled into the lungs[2]. The liquid vaporized in an e-cigarette (e-liquids) are typically a mixture of propylene, glycol, glycerin, nicotine, tetrahydrocannabinol (THC), and flavorings[3]. There are also many different types of devices, with different rates of air flow, heating coils, and materials, and many different types of liquids, with a variety of flavors, ratios, and nicotine levels [4,5]. This variety has made it complicated to study e-cigarettes, as it is difficult to pinpoint specific issues or components of concern. This was especially true in the 2019 E-cigarette and Vaping Associated Lung Injury (EVALI) outbreak, where it took several months for the dangerous component to be isolated, as patients used an incredible variety of products[4].

Currently there is limited data on the carcinogenic effects of e-cigarettes in humans, due in part to their relative novelty. The link between cigarettes and lung cancer took several decades to be identified, and several more to broadly accepted. and the fact that a rise in cancer rates takes years to decades to be detected in the population [6]. This mistake has been learned from, and already there are studies determining the chemical profile of e-cigarette vapor to identify aerosol compositions and potential for chronic toxic exposure. There also is some data on the effects of vapor on mouse lungs, human explant tissue, or *in vitro* cells. In this review we collect and synthesize this data on chemical composition and *in vitro* effects to formulate conclusions about cancer risk from e-cigarette use.

Methods

Google Scholar database was reviewed for studies containing information on the chemical profile of E-cigarettes and cellular effects.

Table 1: Search terms by category		
Chemical Profile	**E-cigarette**	**Tested Item**
Chemical Profile	e-cigarette	DNA
Chemical*	Electronic cigarette	Lung
Toxic*	e-cig*	Human
		Epithelial

Each search category was combined using an AND operator, and all possible search term combinations were used.

Upon obtaining search results, titles were screened for inclusion, and saved for abstract screening. Abstract screening was then completed, looking for papers that specifically offered data on the chemical composition of e-cigarettes or effect on cells. No literature reviews or grey literature was used, and studies included were only published in English and past the year 2000. Studies were then full text reviewed for final acceptance, meeting the above criteria. Finally, data analysis and synthesis was carried out using the chart shown below.

Table 2: Effects of E-cigarette vapor on Mice and Human Cells				
Study	**Exposed Material**	**Vapor Type/Device**	**Cellular Changes**	**DNA Changes**
Smith et al	Mice 4sec puff duration, 30sec puff intervals, exposure chamber of 1m3. 3 hours a day, 3 days a week, 12 total weeks.	1.6-10mL nicotine with 50/50 propylene glycol/vegetable glycerin	N/A	Increased α-methyl adducts No Change in O6-medG adducts Lowered viability

(example study, data not valid)

After extraction, data was written up and presented in the report shown below.

Results

Table 3: Compounds identified in devices in reviewed studies										
Author -> Compound	Conklin et al**	Hecht et al[7**]	Uchiyama et al[8]	Goniewicz et al[9]	Geiss et al[10]	Ooi et al[11]	Rankin et al[12]	Zervas et al[13]*	Saffari et al[14]*	Gray et al[15]*
Acetaldehyde			X	X	X	X	X			
Acrolein	X	X	X	X	X	X	X			
Acrylonitrile	X									
Benzaldehyde				T		X				
Benzene	X	X		T		X				
Crotonaldehyde		X		T						
Cyanide	X									
Diphenyl ether						X				
Ethyl benzene	X			T		X				
Formaldehyde			X	X	X	X	X			
Glyoxal			X							
M&P-xylene				X						
Methylglyoxal			X							
Naphthalene						X				
N,N-dimethylformamide	X									
NNK		X		X						
NNN				X						
Propanal					X		X			
Propylene Oxide		X								
Styrene	X									
Toluene				X		X				
Xylene	X					X				
PAH			--------	-------	-----	----	----	-----	----	----
1-Methylphenanthrene				T			X			
Benz(α)anthracene							X			
Chrysene							X			
Benzo–(k) Fluoranthene Benzo–(b) Fluoranthene							X			
Phenanthrene							X			
Pyrene		X					X			
Metals			-------	-------	-------	----	--------	-------	-------	------
Cadmium				^X						
Chromium										X
Copper								X		
Iron								X		
Lead				^X				X		X
Nickel								X	X	X

Silver									X	
Tin										X
Zinc									X	X

The following table outlines the chemical profile of e-cigarettes from reviewed studies.

X – compound was identified in 50% of devices *- These studies only tested for metals.
^Found in 25% of devices

**Conklin et al and Hecht et al did not test for formaldehyde or acetaldehyde metabolites

Few compounds were identified in all studies, and a large variation in the compounds was identified in e-cigarettes with most being found in only one study, and not in all e-cigarettes. For a compound to be included in the table, it had to be found in over 50% of devices and there was significant variation in chemical profiles found within the same study. Showing not only interstudy variation but also interstudy differences. The only compounds consistently found were formaldehyde, acrolein, and acetaldehyde.

To understand the potential for inter-study confounding, Table 3 shows study methods and materials. Studies employed similar methods to analyze the vapors, though there were differences in the preparation of samples that may have affected outcomes. There also was no overlap in the types of devices and liquids used.

Table 4: Exposure scenario and vapor types used in reviewed exposure-based studies			
Study	Exposure Scenario	Vapor Type	Analytical Method
Uchiyama et al[16]	55mL puff volume at 2sec duration, 10 puffs total	Tested 9 brands	GC/MS
Goniewicz et al[17]	70mL puff volume, 1.8sec puff duration, 15 puffs total, 10sec puff interval,	16-18 mg nicotine (with one at 4, 8, and 11), cartridge and cartomizer type devices, Marlboro, Camel, Tobacco, Regular, Trendy, and Menthol flavor	GC/MS
Geiss et al[18]	35mL puff volume, 4sec puff duration, 13 puffs total	Atomizer and cartomizer device type, Tobacco, and mint flavor, 0, 0.9, and 0.18 mg/mL nicotine.	Liquid chromatography
Ooi et al[19]	3sec puff duration, 12 puffs total	Hangsen Menthol E-liquid, 18mg/mL and 0mg/mL, propylene glycol and glycerol mixtures	GC/MS
Zervas et al[20]	20 mL of liquid, boiled through commercial heating elements	Pure propylene glycol, pure glycerol, 50/50, 33.3/33.3/33.3 PG, VG, Water, a3 nicotine contents = 0, 0.04, 0.08%.	Total Reflection X-Ray Fluorescence spectrometry
Saffari et al[21]	Smoked *ad libitum*, average 1 puff/minute, total 7 minutes. Approx. 1.3mL per hour.	0 – 0.16mg/mL nicotine. 1.5mL volume commercial liquids (Propylene glycol, glycerol, aroma, water)	Time-integrated particle matter sampler
Gray et al[22]	Tested liquids	Different brands and flavors, variety of devices of origin	Plasma mass spectrometry
Hecht et al[23]	Median use duration = 9 months (3-36 range) Time quitting smoking = 9 months (2-36 range) Average use = 1 use /day (0.3-5 range).	Average nicotine concentrations = 12.5 +/- 7mg/mL. Popular brands included eGo, Itazte, Aqua, and Aspire.	Urinary Biomarkers
Conklin et al[24]	Tobacco abstention for 48 hours. 48 users, 12 non-users	NJOY King Menthol E-cigarette, 3% nicotine ad libitum, no longer than 15 min and no less than 15 puffs	Urinary Biomarkers

In general, e-cigarettes had lower levels of harmful compounds compared to combustion cigarettes. Though certain compounds may be higher in e-cigarettes, due to the nature of these devices and their liquids. One study found aldehydes (including formaldehyde) in higher concentrations in e-cigarette vapor compared to cigarette smoke[25]. Notably, several studies found

metals in E-cigarettes. Unlike traditional cigarettes, the metal components of e-cigarettes provide sources for metal contamination. While nicotine was consistently higher in combustion cigarettes, there is some evidence to show that e-cigarettes may be able to produce similar levels. With one study finding a 1.8mg/mL liquid to a half nicotine cigarette[26].

Even when compounds were at lower concentrations, they still raised concerns. The Geiss study[18] found that concentrations of identified compounds exceeded the World Health Organization's short term exposure limits. They also have health concerns with cancer, skin, and respiratory specificity, as shown in Table 5.

Table 5: IARC Carcinogenicity and EPA Health Classifications for compounds identified in EC vapor in reviewed studies

Compound	IARC Classification[27]	EPA Classification[28]
Acetaldehyde	2B	Respiratory irritation (W), Germ cell mutagenicity (W), Carcinogenicity (D)
Acrolein	3	Acute inhalation toxicity (D), Skin corrosion/irritation (D), Acute dermal toxicity (D)
Acrylonitrile	2B	Acute dermal toxicity (D), Acute inhalation toxicity (D), Respiratory irritation (D)
Benzaldehyde	N/A	N/A
Benzene	N/A	Aspiration hazard (D), Skin irritation (W), organ damage through prolonged exposure (D), carcinogenicity (D)
Butyraldehyde	N/A	N/A
Crotonaldehyde	2B	Evidence for Acute inhalation toxicity in rats
Cyanide	N/A	N/A
Diphenyl ether	N/A	Evidence for Irritation of the upper respiratory tract
Ethyl benzene	2B	Acute inhalation toxicity (W), organ damage after prolonged exposure (W)
Formaldehyde	1	Acute inhalation toxicity (D), germ cell mutagenicity (W), carcinogenicity (D)
Glyoxal	N/A	Skin irritation (W), acute inhalation toxicity (W), germ cell mutagenicity (W)
M&P-xylene	3	N/A
Methylglyoxal	3	Skin irritation (W), respiratory tract irritation (W), germ cell mutagenicity (W)
Naphthalene	2B	Carcinogenicity (W)
N,N-	2A	Acute dermal toxicity (W), Acute inhalation toxicity (W)

dimethylformamide		
NNK	1	Carcinogenicity (W
NNN	1	N/A
Propionaldehyde	N/A	Respiratory irritation (W) skin irritation (W)
Propylene Oxide	2B	Dermal toxicity (D), Respiratory irritant (W), germ cell mutagenicity (D), Carcinogenicity (D)
Styrene	2A	
Toluene	3	Skin irritation (W), organ damage: chronic exposure (W)
Xylene	3	N/A
PAH	-----	-----
1-Methylphenanthrene	3	Carcinogenicity (W)
Benz(alpha)anthracene	2B	N/A
Chrysene	2B	N/A
Benzo–(b) Fluoranthene Benzo–(k) Fluoranthene	2B	Organ toxicity: single exposure
Phenanthrene	3	N/A
Pyrene	3	Skin irritation (W), respiratory irritation (W)
Metals	--------	--------
Cadmium	1	Germ cell mutagenicity (W) carcinogenicity (D) organ damage: prolonged exposure (danger)
Chromium	3	Skin irritation (W), respiratory sensitization (asthma symptoms, breathing difficulties, danger)
Copper	N/A	N/A
Iron	1	N/A
Lead	2B	N/A
Nickel	2B	Skin sensitization (W), carcinogenicity (D) organ damage through prolonged exposure (D)
Silver	N/A	N/A
Tin	N/A	Respiratory irritation (W)
Zinc	N/A	N/A

W – Warning (moderate risk) D – Danger (high risk) N/A – no effects reported

This table demonstrates that several compounds found in vapor have potentially carcinogenic and toxic effects. This table is not exhaustive, and additional health risks may be present.

Cascade impactor data has shown that nicotine and menthol particles could be deposited in the oropharynx, trachea, bronchioles, and alveoli[19]. This may help us understand how bioavailable these compounds are. As the greatest limitation of these studies is their inability to provide concrete answers to questions about human risk.

To further understand this, a study from Hecht et al[7] analyzed urine samples from twenty-eight e-cigarette users. When e-cigarette user's metabolite levels were compared to combustion cigarette user's[29,30, 31, 23] levels of nicotine and cotinine in e-cigarette users were similar to or lower, while all other compounds were lower in E-cigarette users.

Conklin et al[24] exposed mice to commercial e-cigarette liquids and tested for urinary metabolites of aldehydes. Metabolites of formaldehyde, acetaldehyde, and acrolein all increased after e-cigarette and combustion cigarette exposure[32]. Menthol flavored e-cigarettes resulted in acrolein and nicotine levels equivalent to a tobacco flavored e-cigarette, demonstrating differences in flavors and user exposure[32].

Cellular Damage

Several studies have exposed human cells to vapor to understand their effects on cellular activities.

Table 6: Effects of Vapor Exposure in *In Vitro* Cell Studies

Study	Exposed Material	Exposure Scenario	Cellular Changes
Rankin et al[25]	A549, BEAS-2B Lung explant tissue	24 h	O/-Viability (A549, Tissue/BEAS-2B) + DNA strand breaks
Lee et al[33]	Mice	12-week, vaper type	+ α-methyl-γ-OH-1,N2-PdG adducts -O6-medG adducts -Nucleotide/base excision repair -XPC and OGG1/2 repair proteins

Cervellati et al[34]	A549	50min vaper type	-Viability + LDH
Yu et al[35]	HaCaT cells	1 week (1% conc)	-Viability +cell death +DNA damage (strand breaks)
Cirillo et al[36]	H1299 lung carcinoma cells	15 min vaper type	-Viability (24h after exposure)
Al-Saleh et al[37]	TK6 cells	1% conc	-Viability =/< 75% (in 13/30 liquids) +DNA damage (strand breaks)
Gerloff et al[38]	BEAS-2B, H292, HFL-1	24h exposure (100µM- 1mM)	oViability
Serpa et al[39]	BEAS-2B	4min vaper type	+apoptosis +necrosis
Tang et al[40]	Mice	54w vaper type	+lung adenocarcinoma
Marshall et al[41]	Lung tissue from exposed mice	8month vaper type	+CYP1A1/2A5 protein +AhR +SOD1 +BCL-XL -E-cadherin -CRM1
Pinkston et al[42]	BEAS-2B, H292 cells	1h vaper type	o/-Viability (H292/BEAS-2B) +CYP1A1 +iNOS -MMP-9 +MMP-12 -AHR
Herr et al[43]	Calu-3, H292, HBEC	15min vaper type	+CYP2A6 (1.37x increase)
Czekala et al	In vitro epithelial tissue model (EpiAirway)	Vaper type (80 puffs)	oViability oDNA damage
Ghosh et al[44]	Human bronchial epithelia from users	Vaper type	+CYP1B1 +MUC5AC
Xue et al[45]	A549, HBEC	Not available	+Cell proliferation (12%) +MMP9 + BIRC5 -WNT inhibitory factor 1
Stacy et al[46]	HBECs with silenced p53 and activated KRAS (H3mut-P53/KRAS)	10-day exposure	O anti-proliferative effects (low nic) O cell invasion +colony growth (high nic)

Viability loss was found in 2/3 of studies. The two lung tissue studies did not find lowered viability, showing the potential for there to be limited in vivo viability decrease. Given

that the Czekala et al study provides the closest approximation to human exposure, given the 3D tissue model used, it is possible viability loss will not be present in more complex human tissues.

An increase in CYP450 enzymes was identified in addition to an increase in xenobiotic metabolism. Xue et al found that e-cigarette exposure led to 191 differentially expressed proteins compared to air controls[45]. Several of which have pro-carcinogenic outcomes. There also were significant findings of DNA damage, namely DNA strand breaks. Which may point to an increased risk for cancer development.

Liquids containing nicotine and flavorings were found to have the greatest effect on cells while humectants (propylene glycol/glycerol) alone had little to no effect[47].

There was a wide variation in the exposure scenarios employed and the devices/liquids used were found as with the chemical profile studies listed above. There also were variations in the exposed cell types which can affect outcomes.

Discussion

TSNAs are contested compounds of particular concern, as they pose significant lung cancer risk due to their pulmonary organ specificity[48]. TSNAs (such as NNN and NNK) have been in some studies[49, 50, 51] while being absent in others[52]. Small 2 or 3 ring PAHs were also found in one reviewed study[25], though any presence is of concern given their carcinogenic potential.

Study Designs

Our findings demonstrate a pervasive issue in e-cigarette research, the heterogeneity of device design and liquid composition. This is likely the main source of the profile variation

identified across and within our studies. The huge variation in devices/liquids makes it impossible to predict the safety of each device. The volatility of the heating process can also affect compound production, adding to the complexity.

This variation begs the question, are there devices that do not expose users to harmful compounds? The Uchiyama et al study found no carbonyl compounds in 4/13 devices, with others containing 60mg/mL of formaldehyde. It is likely that patterns in device/liquid composition can account for a significant portion of this variation. The lack of crossover in devices/liquids used in studies of both vapor and cellular exposure makes analysis of this impossible. Future studies should analyze liquids in-depth to draw conclusions between specific liquid components and their vapor outcomes, and differences in chemical profile and cellular effects.

Another challenge to analyzing the current literature is the significant difference in employed study methods. Two main groups of study designs were identified in both cell and profile studies; “short-term exposure” that utilized a short but intense period of exposure, and “vaper-type” groups that modeled exposure after user behavior. Though there was significant heterogeneity within these classifications; with short term exposure times ranging from 24 or 48h in one study, to 50 minutes in another.

Despite this heterogeneity in design and materials, there were still trends in the summarized studies. Specifically, significant increases in formaldehyde, acetaldehyde, and acrolein. As well as some changes in DNA, though the full evidence on this was not extensively reviewed. It is interesting then, that conclusions were still identified when exposures were so varied. Potentially pointing to the intensity of the effects of e-cigarettes.

One of outcomes of this review, is evidence on the effects of glycol on vapor profile. Several studies found that as glycerol percentage increased, so did the device's toxic profile. This provides an opportunity to restrict the amount of glycol in e-liquids for harm-reduction purposes. Several studies also tested how glycol/glycerol ratios would affect toxic profiles. A study by Ooi et al[19] liquids with different ratios of propylene glycol and glycerol and found the presence of aldehydes in vapor were related to liquids with higher glycol ratios. This is corroborated by Conklin et al and Wang et al[53, 54]. Another identified that the glycerol percentage in liquids had a positive correlation with metal concentration[19].

Chemical Profile and Cellular Effects

The presence of TSNAs and PAHs in e-cigarettes is contested and cannot be concluded here. Given the carcinogenicity of these compounds, their presence or absence would greatly affect cancer risk. Evidence would point to the possibility of TSNAs and PAHs in at least some e-cigarettes, given the heterogeneity of device profiles seen. The production of these compounds is also heavily reliant on tobacco content and other specific conditions that vary in devices. A focused study testing or TSNA's and PAHs may provide insight into this issue.

Metals found in E-cigarettes correlated to device composition, and thus likely originate from the devices themselves. Though others have proposed that e-cigarettes become contaminated with metals during manufacturing. Our studies identified several device factors that increased metal transfer: a high liquid boiling temperature, high nicotine content, and increased device airflow. This poses an opportunity for design changes to protect users by reducing these factors. It may also be prudent to sell liquids separately from devices, as liquids purchased as "refills" did not contain significant amounts of metal in a study that tested both[22].

More research on this would confirm if liquids contained less metals if purchased independently from the device.

Flavoring limitations could also pose an option for regulatory protection. Studies have identified that different flavor types produce different vapor emission profiles. Many of our reviewed studies found that flavorings contributed significantly to cellular harm, and that unflavored liquids had little to no effects[32,19]. As such, further studies should analyze different flavors from the same brands and in the same devices to identify differences between toxicological profile and flavoring type. This could help us understand what flavoring chemicals pose the greatest threat to users and thus should be removed or regulated.

The results of our cellular exposure review offered mixed results. Safety of e-cigarettes cannot be confirmed given the evidence for DNA damage, pro-carcinogenic changes, and viability loss shown. Though the inconclusive and heterogenous nature of the data makes any further conclusion impossible. The cells used for exposure provide another area for variation. BEAS-2B cells consistently lost viability after exposure, while A549 and lung tissues did not (though only 2 studies tested tissues). The inclusion of several different cell lines makes it difficult to ascertain the exact level of harm users experience. As well as the difficulty of interpreting *in vitro* to *in vivo* studies.

Future studies should focus on the effects of e-cigarettes on a select group of cell lines to identify links between device type, cell type, and biomarkers for DNA damage, viability, and pro-cancer protein expression. An analysis of the effects these devices have on human cells, respiratory functioning and symptoms, and respiratory disease prevalence is needed also needed to draw conclusions about the effects of the exposures stated here while offering the opportunity to protect users through concrete understanding and health regulations.

Conclusion

From the current review, e-cigarette vapor is confirmed to contain harmful compounds. Formaldehyde, acetaldehyde, acrolein, and metals were consistently present in most e-cigarettes. There was significant variation in the compounds identified in chemical profiles, making further conclusions impossible. There were no commonalities in the devices and liquids used in our reviewed studies and significant differences in the exposure levels used for analysis, which makes comparison difficult. Future studies should focus on providing analysis of the laboratory methods of similar studies and conducting large scale analysis of liquids and vapors. While variations in chemical profiles were between studies, there was also variation within studies, showing that these variations mostly likely originate from the liquids and devices, not study errors. Even with this variation, every study found potentially harmful and carcinogenic compounds, showing no liquid or device can be considered safe.

E-cigarettes contain lower levels of harmful compounds compared to combustion cigarettes, but in concentrations significantly above non-smoking exposure. These lower concentrations still pose health risks, as shown by *in vitro* studies that identified changes in cell viability, increased DNA mutations, and altered protein expression. Urine metabolites of these compounds have been found in users at significant levels, demonstrating the potential for bio-absorption. Pointing to the possibility that e-cigarette uses impacts cellular functioning and may harm human health.

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