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The effects of electronic cigarette smoking in adolescents

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THE EFFECTS OF ELECTRONIC CIGARETTE SMOKING IN ADOLESCENTS

by

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ABSTRACT

Electronic nicotine delivery systems (ENDS), or electronic cigarettes (e-cigarettes), are battery-powered handheld devices designed to aerosolize a solution of nicotine and other chemicals for inhalation. The specific mechanical and chemical features of different ENDS affect the systemic exposure and bioavailability of the different chemicals in e-juices. E-cigarettes can come with different cartridge sizes, power outputs, e-liquid constituents, and nicotine delivery. User puff topography also contributes to varying toxicant exposure.

ENDS have demonstrated potential as a cessation tool or alternative cigarette product due to its safety profile relative to the combustible cigarette. E-cigarettes have significantly lower concentrations of biomarkers of tobacco-related toxicant exposure and produce less and less harmful second-hand smoke compared to CC. However, ENDS users have significantly greater concentrations of those same biomarkers, highlighting that e-cigarettes do pose a harm to users' health, even if that may be lower than CC. The same is observed in e-cigarette second-hand smoke as nicotine and aerosol particles were detected in statistically significant amounts. Its toxicity is only amplified by the misconception that they are safer than CC and thus pose no absolute risk, misleading users to use without caution. Therefore, although ENDS do have the potential in reducing smoking in adults who are already addicted to nicotine, it comes with the risk of

dual use of conventional and electronic cigarettes and of attracting non-smokers, especially as seen in the youth.

The rise in adolescent e-cigarette use can be attributed to its appeal, of both its flavored e-liquids and its image amongst youth that is perpetuated through the intentional marketing of e-cigarette manufacturers. The latent consequences of e-cigarettes are compounded in adolescents, who are in critical stages of brain development, habit formation, and social development.

Youth report having experienced short-term clinical symptoms such as cough, lightheadedness, headache, and shortness of breath. Physiologically, vaping has been found to affect the pulmonary and cardiovascular systems. Vaping alters the equilibrium of the mucociliary clearance system in the pulmonary system and increases the risk of chronic bronchitis, cough, and phlegm. There is increased in pro-inflammatory cytokine secretions, increased alveolar macrophage apoptosis, impairment of phagocytosis, decreased ciliary beating, inhibition of the CFTR channel, and increased mucin expression. In the cardiovascular system, e-cigarette aerosol extract alters angiogenesis, oxidative stress, endothelial dysfunction, sympathetic nerve system activation, platelet activation and anticoagulation inhibition, and cardiac remodeling. E-cigarettes and e-cigarette smoke have also been associated with carcinogenesis in lung epithelium and possibly urothelium.

Although e-cigarettes have, on average, less nicotine compared to CC, the significant risk for adolescents to graduate to combustible cigarettes renders this moot. The factors influencing this graduation is modeled through the catalyst model, which

details the transition from ‘no use’ to ‘e-cigarette use’ and the transition from ‘e-cigarette use’ to ‘tobacco use.’ Schneider and Diehl hypothesized that the first transition is facilitated through a variety of factors, including flavor, health, price, role models, concealment, and acceptance. The subsequent transition can be attributed to the addiction hypothesis, accessibility hypothesis, and the experience hypothesis. It is clear from the numerous studies conducted, which show students who used e-cigarettes were 4-7 times more likely to report CC use, that e-cigarettes play a catalytic role in enabling the transition to conventional cigarettes. And with increased nicotine exposure, adolescents are subject to impairments in working and verbal memory during abstinence, changes in drug sensitivity and reward-related manifestations in adulthood, more severe dependence during adolescence, and deficits in attentional performance, impaired serial pattern learning, impaired context conditioning and increased anxiety and depressive-like behaviors in adults. They also have reduced control of motivation, reward, and pleasure. This culminates to the gateway hypothesis which states that nicotine can serve as a gateway drug that lowers the youths’ threshold for addiction to opioids, alcohol, and other agents.

Recently, there has been a dramatic increase in cases of EVALI or e-cigarette or vaping associated lung injury, particularly in the adolescent population that is more likely to use illicit e-cigarettes than their adult counterparts. EVALI presents with a wide range of respiratory, gastrointestinal, and constitutional symptoms and is characterized as a sterile exogenous pneumonitis-like reaction with variable degrees of diffuse alveolar damage. Vitamin E acetate, common in illicit products, is strongly linked to this outbreak

due to its presence in a vast majority of bronchoalveolar lavage fluid samples of confirmed EVALI cases.

As ENDS use has increased amongst adolescents, so have its latent consequences. A coordinated effort from policy makers, public health agencies, healthcare providers, researchers, and especially parents and educators is essential for successful protection of this vulnerable population.

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AEC …….……. Aiway epithelial cells

BAL …….…….. Bronchoalveolar lavage

CBD ……...…….. Cannabinoid

CC …….……...……. Combustible cigarette

CFTR ……..… Cystic fibrosis transmembrane conductance regulator

CO …….…….. Carbon monoxide

COPD ……..…….. Chronic obstructive pulmonary disease

eCAE …….. E-cigarette aerosol extract

ECS ……...…….. E-cigarette smoke

e-cigarette ……..……... Electronic cigarette

ENDS ……..……...…… Electronic nicotine delivery systems

EVALI ……...…… E-cigarette or vaping associated lung injury

FA …….…….. Filtered air

H&E …… Hematoxylin and eosin

HPHC ……..…. Harmful and potentially harmful constituents

nAChRs ……..…. Nicotinic acetylcholine receptors

NRT ……..…… Nicotine replacement therapy

OR ……..…….…… Odds ratio

PAH ……..…….. Polycyclic aromatic hydrocarbon

PEG …….. Polyethylene glycol

PG ……. Propylene glycol

PMN ……..…….. Polymorphonuclear cell

PND …….…….. Post-natal day

ROS ……..…… Reactive oxygen species

THC …….……. Tetrahydrocannabinol

TSNA …….. Tobacco-specific nitrosamines

VA …..……. Volatile aldehyde

VEA ……. Vitamin E acetate

VG ……Vegetable glycerin or glycerol

VOC ……..... Volatile organic compounds

INTRODUCTION OF ELECTRONIC CIGARETTES

Composition & Use

Electronic nicotine delivery systems (ENDS), also called vapes, mods, or electronic cigarettes (e-cigarettes) are battery-powered handheld devices designed to aerosolize a solution of nicotine and other chemicals for inhalation. E-cigarettes come in varying shapes and sizes, but most are structurally consistent with four main components, including:

1. Cartridge or reservoir that holds the e-liquid, an aqueous solution of flavorings, nicotine or another drug, and additives [18];
2. Heating element or atomizer;
3. Battery as a power source and
4. Mouthpiece from where the aerosolized e-liquid is inhaled [44].

The particular constituents of the e-liquid also vary depending on manufacturer. E-liquids are generally a solution of concentrated flavorings, variable concentrations of nicotine, or tetrahydrocannabinol (THC) and cannabinoid (CBD) oils, and additives [18]. Common additives, that serve as solvents, include vegetable glycerol (VG), propylene glycol (PG), ethylene glycol, and polyethylene glycol (PEG) [18, 46].

Agent	Positive samples	Mean	Median	Standard deviation	Best fitting risk function for concentration of agent in the beverage[a]
Nicotine (mg/ml)	65%	11	6.8	13	*RiskNormal*(11.023;13.134;*RiskTruncate*(0;))
Glycerol (g/100 g)	94%	37	35	23	*RiskWeibull(1.8104;44.812;RiskShift(−2.8327);RiskTruncate(0;))*
1,2-Propanediol (g/100 g)	94%	57	64	30	*RiskTriang(−18.939;91.8;100.45;RiskTruncate(0;))*
Ethylene glycol (g/100 g)	91%	10	5	18	*RiskLoglogistic(−0.40204;5.15;1.8215;RiskTruncate(0;))*
1,3-Propanediol (g/100 g)	13%	0.6	0	1.7	*RiskResample(2;[all measurements])*
Thujone (mg/L)	4%	6.7	0	34	*RiskResample(2;[all measurements])*
Ethyl vanillin (mg/L)	26%	30	0	68	*RiskResample(2;[all measurements])*

[a]The best fit distributions were selected based on Kolmogorov-Smirnov statistics. For 1,3-propanediol, thujone and ethyl vanillin, distribution fitting was not possible due to the low incidence. Random resampling is used for these agents from a data table with all measurements where all samples with not detectable concentrations were treated as zero.

Table 1: Overview about constituents in electronic cigarettes with descriptive statistics and best fit distributions [18]

When using ENDS, the battery powered atomizer heats and aerosolizes the solution, also known as the e-liquid or e-juice, into an aerosol that the consumer can inhale through the mouthpiece. The ENDS are activated via a power button or through the act of inhaling which triggers an airflow sensor. The use of such products is often referred to as vaping, dabbing, or JUULing (a reference to JUUL, a specific brand of e-cigarette).

Figure 1: Different types of e-cigarette products in the market, ranging from cigalikes to mods and pens [46]

Variability in ENDS

Although the general structure of ENDS are comparable, individual models vary in their specific mechanical and chemical features.

The cartridge sizes and refill capability differ between brands and e-cigarette models. Some ENDS, known as first-generation e-cigarettes or cigalikes, are shaped similar to conventional cigarettes and adopt a closed system model where its cartridges are not refillable. Others, like mods and tanks, have an open system with larger cartridges that hold more e-liquid and can be refilled. These differences translate to the ease of use of the e-cigarette, as consumers need not continually repurchase the product or refill their cartridge as frequently, and influences the frequency of use by the consumer.

The different power outputs of available e-cigarettes, determined by atomizer resistance and battery voltage ($P=V^2/R$), govern the heating of the atomizer and the amount of solution aerosolized. Therefore, the nicotine yield, or the amount of nicotine that emerges from the mouthpiece of the e-cigarette, and the nicotine inhaled per standard breath increases with greater power output, when the degree of activation is normalized, and nicotine delivery is more efficient. Open system ENDS, compared to closed systems, are less restricted in size and therefore have larger batteries that can provide power longer and with less resistance. Resistance in commonly marketed e-cigarettes ranges from 1.0 to 6.5 Ω, with some ENDS having a resistance less than 1 [6]. The resistance of the atomizer is intrinsic to the metal it is made of, usually nichrome wire, which is 80% nickel and 20% chrome, but can also be made from kanthal, an alloy made from iron, chromium, and aluminum. Battery voltage ranges from 3 to 6 V and has been trending

upward with every new generation of the e-cigarette [6]. In some models of e-cigarettes, this feature can be adjusted by the user, personalizing the nicotine yield to their needs.

The specific constituents and their concentrations in solution vary because the exact composition of e-liquids is not uniform across and within manufacturers. E-liquids can include the intended flavorings, nicotine or a drug, solvents, and other toxicants such as carcinogens, heavy metals like nickel, tin, and led, and volatile organic compounds (VOC) ‘’. Propylene glycol (PG) and glycerol (VG), and their ratio in different e-liquids, alter the delivery of nicotine and its other constituents due to their different boiling points and particle size. VG’s boiling point is at 290ºC while PG vaporizes at 188ºC [6]. The higher boiling point of VG allows the solution to reach higher temperatures and may influence toxicant emissions. PG yields smaller particles than VG and can penetrate and deposit nicotine and toxicants deeper into the alveoli of the lungs [5]. The different flavorings also affect the toxicity of the e-liquids, with greater concentrations of vanillin and cinnamaldehyde positively correlated to the overall toxicity [24].

Thermal breakdown of the liquid ingredients also affects the specific toxicants that users are exposed to in the inhaled aerosol. Studies have found variable concentrations of formaldehyde, acetaldehyde, acetone, VOCs, polycyclic aromatic hydrocarbons (PAH), and reactive oxygen species (ROS) in e-cigarette aerosols, depending on battery voltage and solvent [6]. Kosmider et al determined that PG-based e-liquids generated significantly higher yield of carbonyls (formaldehyde, acetaldehyde, and acetone) than VG-based liquids and increased battery voltage resulted in higher concentrations of these carbonyls. When increasing the voltage from 3.2 to 4.8V,

carbonyl yield increased by 4-200, which is in the reported range of 1.6-52 µg/cigarette [28]. Flavorings were found to have no influence on the products of thermal decomposition [28]. While these compounds are present at lower than or similar ranges as those observed in combustible cigarette (CC) vapor and present a less than or equal toxicity potential, some compounds, like glyoxal and methyl glyoxal, are found in e-cigarette aerosols but not cigarette smoke [6].

	Levels in EC vapor (ng/15 puffs)												
	VG based				VG:PG based				PG based				
Carbonyl compounds	A1	A2	A3	C1	A4	A5	A6[a]	C2	A7	A8	A9	A10	C3
Formaldehyde	BLQ	49 ± 2	BLQ	BLQ	51 ± 28	55 ± 7	ND	ND	BLQ	BLQ	59 ± 6	BLQ	BLQ
Acetaldehyde	BLQ	20 ± 4	27 ± 5	ND	104 ± 74	107 ± 24	ND	ND	60 ± 12	40 ± 5	41 ± 9	54 ± 11	BLQ
Acetone	59 ± 12	62 ± 5	64 ± 4	BLQ	181 ± 50	296 ± 91	ND	ND	213 ± 193	181 ± 31	ND	127 ± 34	ND
Butanal	15 ± 4	35 ± 28	49 ± 7	16 ± 4	35 ± 1	41 ± 16	104 ± 96	222 ± 85	ND	15 ± 5	BLQ	185 ± 77	152 ± 185
Propanal	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acrolein	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	BLQ	ND
Benzaldehyde	ND	ND	ND	ND	21 ± 9	BLQ	ND	ND	BLQ	BLQ	46 ± 15	27 ± 6	ND
Crotonaldehyde	ND	BLQ	BLQ	ND	BLQ	BLQ	ND	ND	ND	ND	ND	53	ND
Valeric aldehyde	BLQ	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	BLQ	BLQ	ND
Isovaleric aldehyde	ND	ND	ND	ND	47 ± 17	40 ± 3	ND	ND	33 ± 10	ND	ND	ND	ND
m-Methylbenzaldehyde	BLQ	BLQ	BLQ	BLQ	94 ± 51	78 ± 25	BLQ	BLQ	39 ± 19	39 ± 18	54 ± 14	BLQ	BLQ
o-Methylbenzaldehyde	ND	ND	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	BLQ	ND

Note. VG = vegetable glycerin; PG = propylene glycol; BLQ = below limit of quantitation; ND = not detected.
[a]In addition to VG (13.8%) and PG (30%), solution A6 contained PEG (polyethylene glycol; 40%).

Table 2: Levels of carbonyl compounds in vapors generated from EC refilled with commercially available (A1–A10) and control (C1–C3) nicotine solutions (ng/15 puffs; mean ± SD; N = 3) [28]

Even the chemical structure of the nicotine delivered differs amongst brands. With two basic nitrogen groups, nicotine delivery can be in the form of free base nicotine or nicotine lactate salts, determining its rate of delivery. The free base form of nicotine is volatile and more likely to off gas from the aerosolized droplets early on, depositing in the oral cavity and upper respiratory tract. Absorption of nicotine here is slower than at the respiratory zone of the lungs. Nicotine salts, on the other hand, are less volatile and remain associated with the droplets until they reach the alveoli. At the alveoli, nicotine salts dissociate, and the non-polar, lipid-soluble free base can absorb quickly into the

pulmonary epithelium. The pharmacokinetics of nicotine salts more closely resemble that of conventional cigarettes, resulting in comparable rates of nicotine delivery [36].

All these factors and more contribute to the variable systemic exposure and bioavailability of the different chemicals in the e-juices. This is not accounting for user puff topography, that describes the individual patterns and behaviors of the user including the length of inhalation, puff volume, successive puffs, the frequency of use, and user experience, which would greatly influence the safety profile of ENDS. With each successive puff, the temperature reached by the atomizer increases and the e-liquid is heated at higher temperatures, which has been shown to be directly correlated with nicotine delivery and thermal decomposition [42]. User's experience also affects the blood nicotine levels achieved. More experienced users can achieve comparable or greater levels to that of CC's as they are able to puff longer and larger volumes [36]. Another example of how individual consumer use affects systemic exposure is the direct dripping method of use, where users drip the e-liquid directly onto the exposed heating coil system, which can reach higher temperatures than e-cigarettes that use an automatic wick system. This method reportedly provides a better "throat hit" and greater vapor production for the user. However, by using this method and aerosolizing the e-juice at higher temperatures, there was lower nicotine yield and greater emission of volatile aldehydes (VA) like formaldehyde [42].

History & Proliferation

The appearance of the modern e-cigarette can be approximated to 1965 when the first patent was submitted by Herbert Gilbert in the US [34]. The current e-cigarette, with its characteristic atomizer, however, was developed in 2003 by Hon Lik, a Chinese pharmacist [34]. Since its spread into the U.S. market in the mid-2000s, the popularity of ENDS has exploded with 466 e-cigarette brands being sold by January 2014 and a net increase of 10.5 brands per month seen during the review period [51]. The market value of e-cigarettes, as of 2019, was estimated to be approximately $2.5 billion [16].

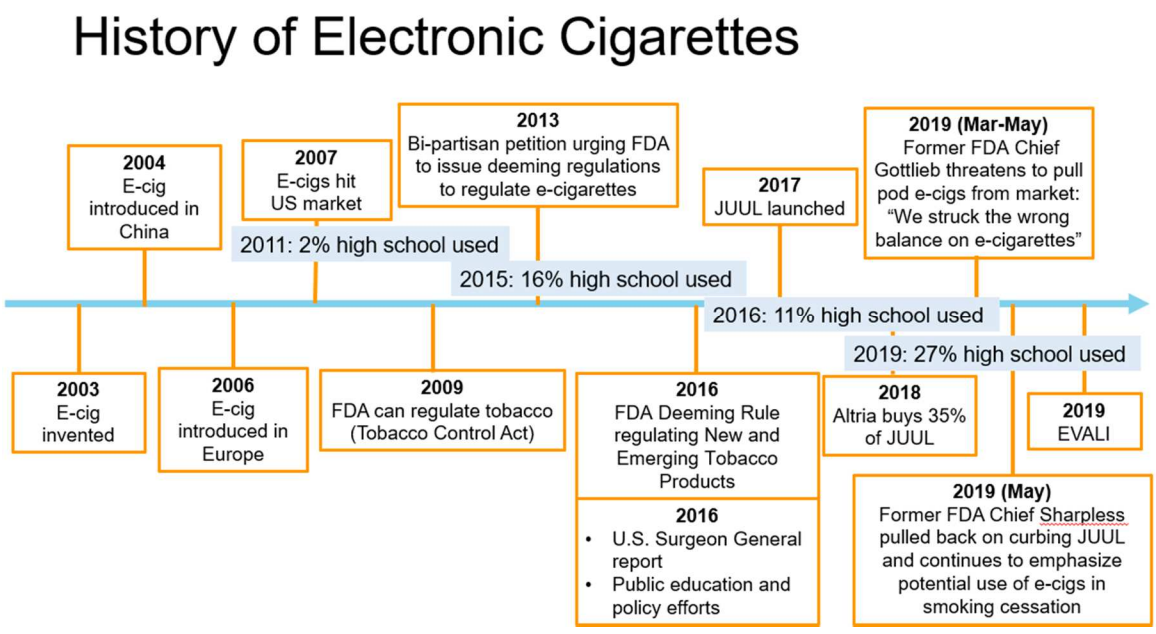

Figure 2: History of Electronic Cigarettes, juxtaposed with the proliferation of ENDS use among high school students [25]

POTENTIAL BENEFITS OF THE E-CIGARETTE

Since its inception, ENDS have been touted as a safer alternative to smoking combustible cigarettes, demonstrating its potential as a cessation tool or alternative cigarette product. A study funded by Ruyan, the company which released Lik's electronic atomizing cigarette, reported that the "Ruyan® e-cigarette is designed to be a safe alternative to smoking. It is very safe relative to cigarettes, and also safe in absolute terms on all measurements we have applied" [30]. The question that now presents itself is, are ENDS really safer than conventional cigarettes and safe in absolute terms, without the bias of funding by e-cigarette companies?

Toxicity of the e-cigarette

Toxicant Exposure in Users

Combustion of tobacco, the method of nicotine delivery of traditional cigarettes, releases harmful and potentially harmful constituents (HPHC) including carbon monoxide (CO), hydrogen cyanide, ammonia, and more than 6,000 other chemicals [20]. These chemicals in tobacco smoke are linked to cardiovascular disease, lung disease, cancer, and much more. But how do e-cigarettes, which removes combustion from the equation, fair in terms of HPHCs that are released and taken in by users?

Goniewicz et al assessed groups of biomarkers of tobacco-related toxicant exposure, including the level of urinary nicotine metabolites, tobacco-specific

nitrosamines (TSNA), metals, PAHs, and VOCs in a study of 5105 participants, categorized into current exclusive e-cigarette users, current exclusive cigarette smokers, dual users, and never tobacco users [17]. They found that compared with cigarette-only smokers, e-cigarette-only users had significantly lower concentrations of all urinary nicotine metabolites, all TSNAs, cadmium, all PAHs, and 17 VOCs of those that were measured. Nicotine metabolite mean concentrations were approximately 93% lower, cadmium concentrations were 30% lower, naphthalene was 62% lower, pyrene was 47% lower, acrolein was 60% lower, acrylonitrile was 97% lower, and acrylamide was 59% lower in e-cigarette-only users [17]. The lower concentration of biomarkers translates to lower biological responses to diseases associated with smoking, such as cardiovascular diseases [41].

However, these values depend on the individual users' puff topography and the specific ENDS that is used. For example, those who use nicotine salt-containing e-juices have a greater systemic exposure to nicotine due to the more efficient absorption mechanisms of nicotine salts versus free base nicotine. A review found that nicotine levels in e-juices vary considerably, with a range of 0–87.2 mg/ml [35], and with consumers, reporting they use 1-4 ml of nicotine salt e-juice and upwards of 10-30ml of free base nicotine e-liquid per day. There is an average of 22 to 36 mg of nicotine per pack of cigarettes. Although nicotine concentrations do not solely determine nicotine yield, it is positively correlated to it. And the great variability in ENDS reveals that, though toxicity exposure can be lower than that from combustible cigarettes, it too can vary.

Its independent toxicity should also not be ignored when assessing toxicity exposure in users. In the Goniewicz et al study, nicotine-related toxicant biomarkers of e-cigarette users were also compared to never users. The never users had significantly lower mean concentrations of all major nicotine metabolites and total nicotine equivalents, all TSNAs, 4 metals such as lead and cadmium, 1 PAH (pyrene), and 4 VOCs including acrylonitrile [17]. This study, and many more, have shown that using e-cigarettes does pose a harm to users' health, even if that may be lower than combustible cigarettes.

Second-hand e-cigarette Aerosol

The use of e-cigarettes is commonly known as "vaping," but the inhaled product is more accurately an aerosol composed of atomized liquid droplets. These droplets are then exhaled and is the only source of second-hand smoke. Combustible cigarette smoke, on the other hand, is made up of solid and semi-solid particles that deposit on surfaces and progressively become more toxic with time. Second-hand exposure to CC smoke is due to the exhaled smoke by the user, known as mainstream smoke, and largely the smoke emitted from the burning cigarette, or sidestream smoke [35]. The differences in the sources and composition of the second-hand smoke from combustible and electronic cigarettes helps explain the differing safety profiles. Compared to e-cigarettes, second-hand CC smoke is given off in larger volumes and remains in the environment longer, exposing bystanders to progressively more toxic chemicals.

A study by Czogala et al found that the air concentration of nicotine released by e-cigarettes was ten times less potent than those associated with traditional cigarettes [12]. The study involved 5 male participants who were dual users of e-cigarettes and conventional tobacco cigarettes with a nicotine dependence of 5.8 ±2.1, determined by the Fagerström Test for Nicotine Dependence. During the experiment, each participant first used their e-cigarette twice for 5 minutes each time and with a 30-minute interval, after which the room was decontaminated and ventilated for 5 minutes, and then smoked two cigarettes with a 30-minute interval between each cigarette. One-hour average air concentrations of nicotine, aerosol particles, carbon monoxide, and VOCs were recorded in the exposure chamber and calculated for each experimental condition and a baseline. The average nicotine concentration was 10 times higher and average aerosol particle concentration was 7 times higher after smoking CC than after -cigarettes[12]. No changes were observed in CO and toluene concentrations with ENDS use while cigarette smoking resulted in an average increase of 2 to 3 ppm of CO and a 3.5-fold increase of toluene [12]. Other VOCs detected after tobacco cigarette use were ethylbenzene, m,p-xylene, and 0-xylene [12]. These observations suggest that second-hand aerosol exposure from e-cigarettes may be less harmful than second-hand exposure from traditional cigarettes.

Czogala et al's study also revealed that there were significant increases in air nicotine and aerosol particle concentrations after e-cigarette use [12]. Nicotine levels were observed at 3.32±2.49 $\mu g/m^3$, while at baseline nicotine was below the level of detection. Aerosol particle levels were detected at 151.7±86.8 $\mu g/m^3$ versus 32.4±30.0 $\mu g/m^3$ at baseline. The statistically significant increases in these compounds demonstrates

the present potential risk of second-hand smoke from ENDS. The pleasant smell from flavorings, which starkly contrasts the burning of a cigarette, may mislead the public to believe ENDS pose no second-hand smoke risk but this is shown to be false. Currently, e-cigarettes are legally allowed to be used in indoor public places such as vaping conventions and e-cigarette shops [35]. At these conventions, cloud competitions are held where the density and thickness of the exhaled smoke is ranked and rewarded but by doing so, exposing convention goers to the toxicity of secondhand smoke at levels even greater than observed in Czogala's study, which measured the air toxicant concentrations from one user rather than hundreds. ENDS also pose a threat to indoor air quality because the discreetness and pleasant smell of the aerosol enables consumers to use them in areas where smoking is banned.

		Nicotine (μg/m^3)		$PM_{2.5}$ (μg/m^3)			CO (ppm vol/vol)		
		Baseline	E-cigarette	Tobacco cigarette	Baseline	E-cigarette	Tobacco cigarette	Baseline	E-cigarette	Tobacco cigarette
Experiments with dual product users (Study 2)										
13	Smoker 1	BLD	0.65	26.9	80.0	63.3	690.0	1	1	3
14	Smoker 2	BLD	0.85	38.1	8.0	123.0	726.0	2	2	5
15	Smoker 3	BLD	5.02	25.6	13.3	91.3	661.3	2	2	3
16	Smoker 4	BLD	3.87	25.6	44.0	208.3	802.0	1	1	4
17	Smoker 5	BLD	6.23	41.8	16.7	272.7	1217.3	1	1	4
Average (Experiments 13–17)		BLD	3.32±2.49[a,b]	31.6±7.8	32.4±30.0	151.7±86.8[a,b]	819.3±228.6	1.40±0.55	1.40±0.55[b]	3.80±0.84

Note. BLD = below limit of detection (0.22 μg/m^3).
[a]Significant difference with baseline ($p < .05$).
[b]Significant difference with tobacco cigarette ($p < .05$).

Table 3: Changes in nicotine, aerosol particles (PM2.5), and carbon monoxide air concentration inside exposure chamber after use of e-cigarette [12]

Role in Smoking Cessation

As was marketed in the first ENDS products, e-cigarettes continue to be sold and used as a tool for smoking cessation. It is seen by the public as a safer product that can be used to taper the nicotine concentration users are addicted to until they are no longer dependent on nicotine, from either combustible or electronic cigarettes. The popularity of this use for e-cigarettes has grown substantially. In theory, ENDS are a promising tool for cessation because of its lower toxicity, on average lower concentrations of nicotine, and the behavioral and sensory aspects of smoking addiction, like the hand to mouth motion, that they satisfy. Possibly used in conjunction with FDA-approved NRTs, true cessation rates can be achieved.

However, there is lack of concrete evidence supporting the real-world application of ENDS for smoking cessation and it is not currently approved by the FDA as a smoking cessation aid. Of the then current randomized controlled trials and observational studies assessing ENDS role in cessation, Dib et al concluded in their meta-analysis that no credible inferences can be made due to the studies' low certainty [13]. More recent studies do reveal that 1-year combustible cigarette abstinence rates are greater with e-cigarette use compared to NRT utilization, 18.0% versus 9.9% respectively [19]. Use of nicotine salts could potentially increase abstinence rates because the rate of nicotine delivery is similar to that of traditional cigarettes, providing the same satisfaction and quenching their cravings better. However, 80% of participants using ENDS as a cessation tool were found to be using the product at the 1-year follow up [19]. This suggests that e-

cigarette use may help smokers quit smoking traditional cigarettes, but they transfer their nicotine addiction to e-cigarettes. The lower concentrations of nicotine in e-cigarettes also pose the risk of compensatory behavior as users puff more deeply, more frequently, and for longer to satisfy their nicotine cravings and reduces their desire to smoke [3]. Rather than as smoking cessation tool, ENDS may have a role as a harm-reduced nicotine product. It also poses the possibility of dual use, where consumers smoke combustible cigarettes and use e-cigarettes. There is evidence to suggest that many e-cigarette users continue to smoke cigarettes [8]; exposure to dual use of combustible tobacco products and e-cigarette aerosols may be more deleterious to the respiratory system than either product alone [30]. Dual use of cigarettes and ENDS result in the greatest degree of toxicant exposure [17]. Thus, ENDS do have the potential in reducing smoking in adults who are already addicted to nicotine, though it comes with risks itself, but they have also been shown in recent trends to attract non-smokers, especially as seen in the youth.

Comparative vs. Absolute Risk

Although e-cigarettes have been shown to be equivalent to or safer than traditional combustible cigarettes in terms of overall toxicity, the safety profile of ENDS cannot be reviewed without consideration of its independent addictive potential and toxicity. There is an absolute risk of e-cigarette use. The primary function of an ENDS is to deliver nicotine and other drugs, and with that comes their addictive nature. With its accessibility and perception as safer than CCs and thus deemed safe overall, the effects of

nicotine and other toxicants are compounded, making users and bystanders, especially adolescents, susceptible to the known and largely unknown effects of the multitude of toxicants present in the E-liquid. Given e-cigarettes relatively recent introduction to the marketplace, further research is needed to evaluate the risks associated with e-cigarette use, especially contemporary patterns that include dual use of e-cigarettes and CC. The greatest absolute risk with e-cigarettes currently is the narrow focus on the comparative risk to combustible cigarettes that overshadows all else.

ADOLESCENT EXPOSURE TO E-CIGARETTES

Rise of e-cigarette Use

Current e-cigarette use, which is defined as use at least one day in the past 30 days, in middle school and high school students has gained significant traction and continues to rise. According to the National Youth Tobacco Survey in 2011, use among U.S. high school students was estimated to be at 1.5% [34]. Rates, which increased to 11.7% of high school students who then currently vaped in 2017, has grown dramatically to 27.5% among high school students and 10.5% among middle school students in 2019 [10]. In just eight years, the percent of current adolescent ENDS use has increased more than 1,800%. This translates to over 5 million current middle and high school student ENDS users in 2019 and almost 1 million adolescent every day e-cigarette users [46]. Not only are the number of adolescent users increasing, but the frequency at which they are vaping is also rising substantially because of its addictive qualities.

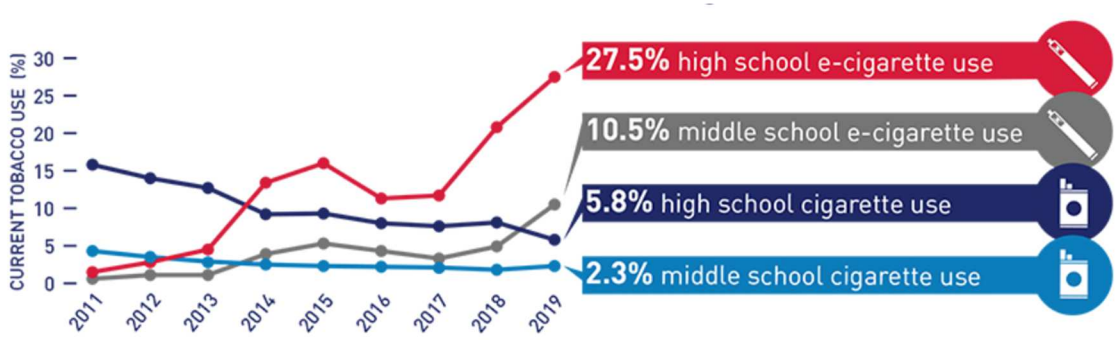

Figure 3: Trends of cigarette and e-cigarette use by middle school and high school students from 2011 to 2019 [50]

From its design and features to its image amongst youth, the appeal of e-cigarettes to the adolescent can be seen as a major culprit for the rise of ENDS use in this population. First and foremost, e-cigarettes are easy to obtain and to keep for adolescents. They are easily accessible to the youth, with their affordable price and the abundance of online retailers, and, once bought, can be kept in secret from school staff and parents because of the unsuspecting scents and compact form. But the main appeal to adolescents that the e-cigarette has is its role in social dynamics, which is a predominant concern in the youth. Users are associated with popularity and e-cigarettes facilitate social gains. Barrington-Trimis et al found that psychosocial factors, such as home use, friends' use of and positive attitudes toward e-cigarette, were strongly associated adolescent use of e-cigarettes [2]. For example, users able to cloud chase, which involves performing tricks from exhaled e-cigarette smoke, gain social standing and status from peers. And its sleek and modern design only contributes more to this image. Coupled with the flavored e-juices and the directed marketing done by e-cigarette companies, there is no question as to why ENDS use has proliferated in the youth population.

Flavored E-liquids

A clear and leading factor in the rise in adolescent tobacco use in recent years is the flavors of e-liquids that directly facilitates the first use by adolescents. Of the adolescents who have vaped, most start with menthol or flavored products. Over 7700 unique flavors are available to attract adolescents with their novelty [52] and a

palatability that masks the flavor and harshness of tobacco. Ambrose et al's study in 2015 of 13,651 youth supports this. They showed that the first ENDS product used by 81% of ever-user youth participants was flavored and 85.3% of product use in past 30-day e-cigarette youth use was of flavored products [1]. Youth and young adults continue to use e-cigarettes because "they come in flavors I like," as stated by 81.5% of youth, 12 to 17 years old. Only 66.4% of adults 25 years and older in the study, in comparison, contributed the flavor of the e-liquid to their use of e-cigarettes. This discrepancy can possibly be explained through the dissonance between the sweet and fun flavors with the toxicity of nicotine. It can be hard to imagine that such flavors are bad for the body. 37.5% of middle school and high school students believe that intermittent e-cigarette smoking causes little to no harm [47]. E-cigarette companies have been able to capitalize on these flavors while flavors other than menthol in combustible cigarettes have been banned to limit appeal to adolescents. The false security of flavors coupled with the lack of accurate information regarding ENDS predictably promotes abuse of ENDS by minors.

Reasons for Use	% (95% CI) e-Cigarettes (n = 418)[c]
I use [product] because they come in flavors I like	81.5 (77.9-85.0)
I use [product] because they are affordable	47.8 (42.9-52.6)
I use [product] because I can smoke/use them at times when or in places where smoking cigarettes isn't allowed	58.9 (54.1-63.7)
I use [product] because I like socializing while using them	40.3 (34.9-45.8)
I use [product] because it doesn't bother non-tobacco users	53.9 (48.1-59.8)
I use [product] because they might be less harmful to me than cigarettes	79.1 (75.2-83.0)
I use [product] because they might be less harmful to people around me than cigarettes	78.1 (74.3-81.8)
I use [product] because they don't smell	58.7 (54.2-63.2)
I use [product] because they help people to quit smoking cigarettes	59.5 (54.6-64.5)
I use [product] because people who are important to me use them	34.9 (30.6-39.2)
I use [product] because people in the media or other public figures use them	36.1 (31.5-40.7)

Table 4: Leading reasons for non-cigarette tobacco product use among past 30-day tobacco users, by product—population assessment of tobacco and health study youth respondents aged 12-17 years, 2013-2014 [1]

Marketing Towards Teens

Targeted marketing to teens is a serious issue that directly influences the prevalence of illicit teen e-cigarette use. Companies have invested the bulk of their resources in marketing in mainstream media and on the internet. Such channels include magazines, retailer point-of-sale ads, product placement, and television commercials, which is prohibited of cigarette manufacturers. Online direct-to-consumer marketing that allows brands to interact directly with targeted audiences and potential customers is mostly done through social media platforms such as Twitter, Facebook, Instagram,

Reddit, and YouTube, where adolescents frequent most regularly [34]. In 2016, 78.2% or 20.5 million middle school and high school students were estimated to have been exposed to e-cigarette advertisements from any source, with 68.0% having seen an ad at a retail store, 40.6% on the internet, and 37.7% on television [31]. Through such marketing, e-cigarettes in the adolescent population are socially accepted and are even admired. This is a testament to the effectiveness and reach of these tactics to large and targeted audiences.

JUUL

A pioneer and leader of the e-cigarette market is the company JUUL Labs, which held 73.4% of the e-cigarette market share in July 2019 [21]. According to the CDC 2019 National Youth Tobacco Survey, more than 59% of high school ENDS users reported that JUUL Labs was their brand of choice. JUUL Labs exemplifies the dangerous potential of e-cigarettes.

JUUL Labs revolutionized the e-cigarette market through its innovative use of nicotine salts rather than free base nicotine that was the norm at the time. Nicotine salts, JUUL Labs reports, are less harsh upon inhalation because nicotine salts are closer to physiologic pH and do not activate alkaline sensitive protective mechanisms in the lungs. This allows for the 0.7 ml of nicotine per pod, which is equivalent to 20 combustible cigarettes, providing the same satisfaction as smoking a CC. JUUL pods are also absorbed with similar efficiency to nicotine in combustible cigarettes, adding to this satisfaction. Although this has been linked to greater adherence to cessation, it also

appeals to immature smokers who do not have a tolerance to inhaling anything other than air. They are able to vape JUUL e-cigarettes with less coughing and harshness but become dependent on the greater concentration of nicotine. This also perpetuates the pervasive misbelief that e-cigarettes are harmless because users do not respond to the JUUL as they do combustible cigarettes, thus dissociating e-cigarettes and traditional cigarettes and toxicity even more.

The marketing tactics of JUUL Labs also demonstrates the harm potential of the current trajectory of e-cigarettes. JUUL Labs spent $2.1 million on marketing between 2015 and 2017, more than $1 million of which was used to market its products online through campaigns on Twitter, Instagram, YouTube, and social media influencers [22]. Themes of its campaigns included pleasure/relaxation, socialization/romance, flavors, cost savings and discounts, holidays/seasons, style/identity, and satisfaction [23]. Rarely its purported function as a smoking cessation tool and harm-reduced nicotine product is at the forefront of its advertisements. Consequently, nearly two thirds of current JUUL users 15 to 21 years old are unaware that JUUL products always contain nicotine [49]. The intentions of JUUL Labs can be interpreted through its marketing campaigns; that sales are the top priority, no matter to who.

CONSEQUENCES OF E-CIGARETTE USE

With the growing numbers of adolescent e-cigarette users worldwide, it is essential that the consequences, both short and long term, of vaping be understood in the context of the adolescent. Adolescence is a pivotal time for growth and development and the effects of e-cigarettes, its constituents, and its aerosolized nature on this development are damaging. The latent consequences of e-cigarettes are compounded in adolescents, who are in critical stages of brain development, habit formation, and social development.

Effects of E-cigarette Aerosol and its Constituents

The research done on the effects of e-cigarettes have been conducted through mouse models, in vitro human samples, and in vivo and applied to the general population, including adolescents.

Reported Symptoms of E-cigarette Use

A cross-sectional telephone survey was conducted of U.S. youth ages 13 to 17 to assess the symptoms experienced by ever-user adolescents: 42.3% reported cough, 31.5% had experienced dizziness or lightheadedness, 25.4% had headaches or migraines, 14.9% reported dry or irritated mouth and throat, 13.7% complained of shortness of breath, 3.5% experienced a change in or loss of taste, and 5.7% also experienced other symptoms including nausea, dry eyes, earache, and chest tightness [27].

Pulmonary System

Significant research has been done to ascertain the physiologic effects that vaping has on the pulmonary system. Symptomatically, the risk of chronic bronchitis including chronic cough and phlegm, asthma, chronic obstructive pulmonary disease (COPD), and dyspnea increases with vaping [24]. These symptoms can be attributed to the altered physiological equilibrium of the pulmonary system. The e-cigarette aerosol causes erythematous and irritable airway mucosa due to an increase in pro-inflammatory cytokine secretions, induced alveolar macrophage apoptosis, and impairment of neutrophil and macrophage phagocytosis [24]. Impaired phagocytosis, coupled with decreased ciliary beating as a result of exposure to e-cigarette vapor, leaves the lungs susceptible to pathogens as the lung's immune system is unable to clear them out [24]. There is also altered expression of genes involved in oxidative and xenobiotic stress pathways that can contribute to the inflammation of the airways [24].

Vaping has also been found to inhibit the cystic fibrosis transmembrane conductance regulator (CFTR) channel and its mediated chloride secretion, inducing airway epithelial dehydration, in vitro [24]. Although its effects on the CFTR channel in vivo are not yet confirmed, the humectants, PG and VG, of the e-liquid do increase mucin expression and decreased membrane fluidity in the primary airway epithelia after vaping [24]. These observations are consistent with each other and the effects on endocytosis that have been observed, as decreased membrane fluidity may affect endocytosis. Increased mucin and dehydration only amplify the inhibition of the mucociliary clearance system, contributing again to the increased risk of chronic bronchitis, cough, and phlegm.

Cardiovascular System

Research on the effects of e-cigarette aerosol extract (eCAE) on the cardiovascular system are expansive, linking eCAE to angiogenesis, oxidative stress, endothelial dysfunction, sympathetic nerve system activation, platelet activation and anticoagulation inhibition, and cardiac remodeling. Kennedy et al conducted a systematic review of these experimental studies. They found that eCAE exposure in human subjects leads to increases in heart rate and blood pressure and abnormalities in heart rate variability, suggesting sympathetic nerve activation by eCAE [26]. E-cigarette exposure also increases atherosclerotic risk in users due to the increase in ROS production and reduction of antioxidants after exposure [26]. Endothelial dysfunction, which also adds to atherosclerotic risk, was observed through disordered endothelial cellular structure, function and interactions in in vitro studies, vascular inflammatory markers and angiogenesis in mouse models, and increased arterial stiffness in human subjects [26]. Platelet homeostasis was also disrupted by eCAE exposure in all studies, suggesting an increase in thrombotic risk for those who use ENDS. Increases in endothelial (c)1q deposition, reactive hyperemia and murine left ventricular mass were also observed as a consequence of vaping but have not been identified or linked to cigarette smoking [26]. A table summarizing all effects observed in the studies of interest is provided below.

Proposed pathogenic mechanisms

	Angiogenesis	Oxidative stress	Endothelial dysfunction	Sympathetic nerve system activation	Platelet activation / anticoagulation inhibition	Cardiac remodelling
Biomarkers	● ↑ CD31 immunostaining (Shi et al., 2019) ● ↑ CD34 immunostaining (Shi et al., 2019) ● ↑ endothelial cell tube formation(Lee et al., 2019)	● ↑ reactive oxygen species (ROS) - $H_2O_{2(Biondi-Zoccai\ et\ al.,\ 2019)}$ - sNox2-dp (Carnevale et al., 2016) - 8-isoPGF2a (Biondi-Zoccai et al., 2019) - Plasma myeloperoxidase (Chaumont et al., 2018) - Malondialdehyde(Espinoza-Derout et al., 2019) - ↑ circulating CD40L (activates endothelial cells to release ROS) (Biondi-Zoccai et al., 2019) - ↑ serum C-reactive Protein (Chatterjee et al., 2019) ● ↓ antioxidant activity - Vitamin E levels(Biondi-Zoccai et al., 2019) - NO bioavailability - Nitric Oxide(Carnevale et al., 2016) metabolites(Chatterjee et al., 2019) - HBA%(Biondi-Zoccai et al., 2019)	● ↑ arterial stiffness - ↓ flow-mediated dilatation (Biondi-Zoccai et al., 2019) - ↑ pulse-wave velocity (Antoniewicz et al., 2019) - ↑ augmentation index x 75 (Chaumont et al., 2018) ● ↓ vasodilatory response to acetylcholine(Chaumont et al., 2018) ● ↓ vasodilatory response to methacholine(Olfert et al., 2017) ● ↑ vasoconstrictive response to phenylephrine(Olfert et al., 2017) ● ↑ endothelial progenitor cells (Antoniewicz et al., 2016) ● ↑ endothelial complement deposition(Barber et al., 2017) ● ↑ endothelial complement inhibitor expression(Barber et al., 2017) ● Endothelial barrier disruption (Schweitzer et al., 2015) ● ↑ reactive hyperaemia index (Kerr et al., 2018) ● ↑ vascular inflammatory markers - PECAM-1 (Kaisar et al., 2017) - VCAM-1 (Kaisar et al., 2017) - ICAM-1 (Chatterjee et al., 2019) ● ↑ endothelial cell: - Morphological alterations (Putzhammer et al., 2016) - DNA damage(Anderson et al., 2016) - Inhibition of migration(Lee et al., 2019) ● ↓ endothelial cell: - Proliferation(Putzhammer et al., 2016) - Cell density(Barber et al., 2017) - Metabolic activity(Barber et al., 2017) - Viability(Barber et al., 2017)	● ↑ heart rate(Antoniewicz et al., 2019) ● ↑ (exercising)(Fogt et al., 2016) systolic blood pressure(Biondi-Zoccai et al., 2019) ● ↑ (exercising)(Fogt et al., 2016) diastolic blood pressure(Biondi-Zoccai et al., 2019) ● Abnormal heart rate variability - Cardiac vagal tone(Moheimani et al., 2017a) - Sympathetic tone(Moheimani et al., 2017a) ● Peripheral vasoconstriction (Pywell et al., 2018)	● ↑ platelet: - Aggregation(Noella et al., 2018) - Adhesion(Hom et al., 2016) - Complement deposition (Hom et al., 2016) - Alpha particle secretion (Qasim et al., 2018) - Dense particle secretion (Qasim et al., 2018) - Integrin activation(Qasim et al., 2018) - Resistance to prostacyclin inhibition(Qasim et al., 2018) - ↓ thrombomodulin(Kaisar et al., 2017) - ↓ bleeding time(Qasim et al., 2018) - ↓ occlusion time(Qasim et al., 2018)	● Altered cardiac structure: - ↑ left ventricular mass(Olfert et al., 2017) ● Altered cardiac function: - ↓ left ventricular ejection Fracture (Espinoza-Derout et al., 2019) - ↓ left ventricular fractional Shortening (Espinoza-Derout et al., 2019) - ↓ velocity of circumferential fibre Shortening(Espinoza-Derout et al., 2019) ● Cardiac mutagens: - O^6-methyldeoxyguanosines(Lee et al., 2018) - γ-Hydroxy-1,N2-propano-deoxyguanosines(Lee et al., 2018)

Table 5: Summary of findings: the proposed complex pathogenic mechanisms of e-cigarettes' effect on the heart [26]

Cancer

The relative novelty of ENDS, which first gained widespread popularity in the mid-2000s, restricts the availability of studies assessing the long-term consequences of e-cigarette use, especially in vivo. Still, some e-cigarette smoke (ECS) studies with mouse models are being done, like the Tang et al study, to determine the correlation, if any, between ECS and cancer.

In the Tang et al study, mouse models were exposed four hours per day, 5 days a week, for 54 weeks to one of three conditions [43]:

1. ECS generated from e-juice with isopolypropylene glycol (PG) and vegetable glycerin (VG) at a 1:1 ratio (Veh) as the solvent; particulate matter concentration was maintained at 130 mg/m^3 and the aerosol nicotine concentration at 0.196 mg/m^3 in the chamber
2. Veh generated with a e-cigarette aerosol generator set at a constant power (1.9 A, 4.0 V)
3. Ambient filtered air (FA).

The lungs, heart, liver, kidneys, intestine, pancreas, brain, spleen, and bladder of all surviving mouse models were prepared and stained with hematoxylin and eosin (H&E) to be reviewed by three separate pathologists. Bladder tissue slides were also stained with antibodies for the proliferation markers MCM-2 and PCNA and the basal cell marker KRT5. Upon gross review and histologic examination, they found that 22.5% of ECS mice, no Veh mice, and 5.6% of FA-exposed mice developed lung adenocarcinomas [43]. The bladder urothelium of 57.5% of ECS-exposed mice and 6.3%

of Veh mice had hyperplastic lesions, characterized by a significant increase of urothelial layers, expansion of KRT5-positive basal urothelial cells, and a distinct elevation of the cell proliferation markers MCM-2 and PCNA, while no control mice displayed such pathology [43]. The statistically significant observations of this study support the role ECS on carcinogenesis in lung epithelium and possibly urothelium.

Tang et al speculate that long-term exposure to ECS allows the accumulation of DNA damage and transformation of epithelium into cancerous cells. This conclusion builds on their previous findings that short-term exposure to ECS and nicotine and nicotine-derived nitrosamine ketone induces γ-OHPdG and O6-methyl-dG mutations, in lung and bladder epithelium, and inhibit DNA repair in lung tissues of mice.

There is controversy regarding the carcinogenic properties of nicotine and ECS due to inconsistent results of the research currently available. The prevailing thought is that nicotine is non-carcinogenic. The researchers in this study acknowledge the conflicting studies and highlight that their experimental data in model systems simply warrants deeper study. Studies assessed nicotine's carcinogenic potential through delivery via drinking water, subcutaneous injections, and air-vaporized, whereas Tang et al studied ECS. Possibly, the method of nicotine delivery afforded by the e-cigarette, which aerosolizes nicotine into smaller particles compared to tobacco smoke and allows nicotine to deposit deeper into the alveoli of the lungs, is responsible for the opposing results. Nicotine from tobacco smoke mainly settles in the upper aerodigestive tract where plentiful antioxidants neutralize nitrosamines.

Exposure	Mice with tumor*	Mice without tumor*	No. of dead mice before final killing†	Total	Tumor incidence, %
FA	1	17	2	20	5.6
Veh	0	18	2	20	0
ECS	9	31	5	45	22.5

*Surviving mice with tumor or tumor-free up to 54 wk.
†All dead mice were lung tumor-free.

Table 6: Lung adenocarcinoma incidence in ECS-, Veh-, and FA-exposed mice [43]

Graduation from E-cigarettes: Catalyst Model

The catalyst model was proposed by Schneider and Diehl in 2016 as a model for the relationship and the influence of e-cigarette use on the initiation of cigarette smoking in adolescents. The model consists of two stages: the transition from 'no use' to 'e-cigarette use' and the transition from 'e-cigarette use' to 'tobacco use.' Schneider and Diehl hypothesized that the first transition is facilitated through a variety of factors, including flavor, health, price, role models, concealment, and acceptance [39]. The subsequent transition can be attributed to the addiction hypothesis, accessibility hypothesis, and the experience hypothesis [39]. The addiction hypothesis attributes the transition to the addictive nature of nicotine in e-cigarettes and the natural progression to seek out greater concentrations of nicotine and a more satisfying "hit" that is available with combustible cigarettes. The accessibility hypothesis explains that e-cigarettes and traditional cigarettes are often available through the same commercial and social sources and access to e-cigarettes would mean access to cigarettes, encouraging the transition. The experience hypothesis highlights the similar habitual and ritual processes between ENDS and combustible cigarettes in facilitating the transition.

Every adolescent navigates through these stages influenced by different factors that determine their trajectory. But those adolescents who transition through the first stage are at greater risk for tobacco use because e-cigarette use acts as a catalyst that facilitates the transition from 'no use' to 'tobacco use.' Even an isolated incidence of e-cigarette use can encourage smoking uptake in this model because the experience hypothesis attributes experience, even minimal, with the habitual and ritual procedures of smoking to ease the initial transition to combustible cigarettes. Thus, it is important to address and regulate the different potential initiation factors.

The catalyst model has been repeatedly supported through various longitudinal studies that assess the relationship between e-cigarette use and tobacco smoking. Bold et al conducted a survey in Connecticut high schools over a three-year period, with each year referred to as a wave, where students would anonymously report their use [5]. Students who used e-cigarettes in the past month at wave 1 had an odds ratio (OR) of 7.08, which means that they are 7 times more likely to report combustible cigarette use at wave 2 than students who were not vaping. With an OR of 3.87, individuals who vaped e-cigarettes in the past month at wave 2 were almost 4 times more likely to have smoked cigarettes than those who did not use e-cigarettes. The study also revealed that e-cigarette use and cigarette smoking frequency increased over time, demonstrated by the 26% of cigarette users and 20.5% of e-cigarette users who reported using their respective nicotine products 21–30 days out of the past month in wave 3 compared to 15.3% and 10.3% in wave 1, respectively. This supports the growing dependency of adolescents on the nicotine products that they use.

Another study by Berry et al conducted a similar survey study using data from the Population Assessment of Tobacco and Health Study (2013-2016) [3]. They found that prior e-cigarette use was associated with an OR of 4.09 or a more than 4 times the odds of ever cigarette use compared to no prior tobacco use. It also revealed an OR of 2.75 for current cigarette use. The study estimated that e-cigarettes were likely responsible for 22% of new ever cigarette use and 15.3% of current cigarette use, which amounts to nearly 180,000 new cigarette users.

Whatever the influences that facilitate the progression from e-cigarette to combustible cigarette use and whatever model represents this transition, it is clear from the numerous studies conducted that e-cigarettes play a catalytic role in enabling it. This graduation from e-cigarettes to combustible cigarettes would only increase the toxicity to which they are exposed.

Nicotine on the Developing Adolescent Brain

Nicotine and its effects have been studied at great depths due to the prevalence of combustible cigarettes. However, with the proliferation of e-cigarettes, especially within the adolescent population, nicotine's toxicity has a greater reach. It is therefore important to highlight the effects that nicotine can have on the developing brain.

Adolescence is marked by the reorganization and maturation of regions of the brain necessary for mature cognitive and executive function, working memory, reward processing, emotional regulation, and motivated behavior through the regulation of

nicotinic acetylcholine receptors (nAChRs) [51]. Nicotine exposure, which alters the cholinergic system, during adolescence induces acute and long-term effects in these regions, such as the prefrontal cortex, nucleus accumbens (NAc), and amygdala [38]. These changes translate to impairments in working memory and verbal memory during periods of abstinence, changes in drug sensitivity and reward-related manifestations in adulthood, more severe dependence during adolescence, and deficits in attentional performance, impaired serial pattern learning, impaired context conditioning and increased anxiety and depressive-like behaviors in adults [38]. One study demonstrated the cognitive effects of nicotine on adolescence via mouse models. Adolescent mice were exposed to nicotine for 12 days, from post-natal day (PND) 38 to 50, and, after 30 days of abstinence, exhibited deficits in contextual but not cued fear conditioning. Contextual fear conditioning is hippocampus-dependent and such deficits highlights the learning deficits from nicotine administration. They also showed the learning deficits are isolated to adolescence because the same effects were not observed in adult mice when receiving the same treatment [38].

Other studies suggest nicotine induces brain plasticity. Plasticity, possibly dependent D1 dopamine receptors, has been observed in the NAc shell. There is also altered expression of genes involved in neuroplasticity, leading to structural changes such as decreases in apical dendrite length in the hippocampal CA1 region and increases in dendritic length in medium spiny neurons from the NAc shell [38]. Long-term nicotine use also leads to the suppression of nicotine-induced elevation of striatal dopamine and

norepinephrine levels, which initially affects the brain's control of motivation, reward, and pleasure [38].

Gateway hypothesis

Not only would e-cigarettes drive adolescents to smoke combustible cigarettes, nicotine can serve as a gateway drug that lowers the youths' threshold for addiction to opioids, alcohol, and other agents. This is the Gateway Hypothesis. Epidemiological data supports this as smokers above the age of 12 are 5 times more likely to use illicit drugs and marijuana use in adulthood is over 3 times more likely in smokers who began smoking before the age of 13 compared to non-smokers [38].

Mojica et al demonstrated that brief nicotine exposure during early adolescence enhances drug-related learning [33]. In the study, rats were administered nicotine, two intravenous injections of 0.03 mg/kg/0.1 ml, or saline for four days during early adolescence or adulthood, PND 28-31 and PND 86-89 respectively. The rats were then placed in operant self-administration conditioning chambers with nose pokes reinforced by cocaine daily for 2-hour cocaine self-administration sessions for at least 12 days or until acquisition requirements were met. All adolescent rats pretreated with nicotine met the acquisition requirements while 13 of 18 adult rats met the requirements, supporting that nicotine pretreatment significantly enhanced drug-related learning in adolescents but not in adults.

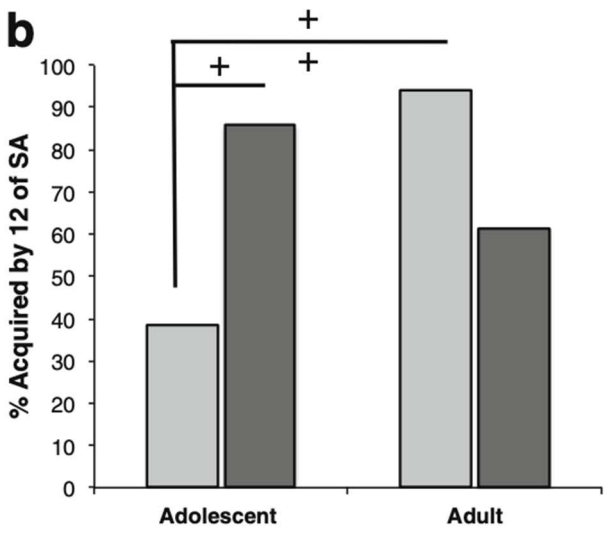

Figure 4: Age differences in the effect of nicotine pretreatment on the acquisition of cocaine self-administration [33]

Larraga et al demonstrated that nicotine administration in adolescence increased alcohol intake, suggesting that adolescent nicotine exposure increased sensitivity to alcohol reward in adulthood, which supports the gateway hypothesis [29]. In the study, adolescent and adult mice self-administered alcohol, nicotine, or a combination of both in an intravenous self-administration paradigm. Then, the same mice, after maturing to adulthood, were evaluated for alcohol drinking via an in-the-dark (DID), 2-bottle choice test, with water and alcohol. The male rats that self-administered nicotine, with or in combination with alcohol, as adolescents drank more alcohol in adulthood than rats in the saline control group in all three test doses. Adult rats that self-administered alcohol and alcohol did not demonstrate increased alcohol intake in the 2-bottle choice test. Notably, subsequent alcohol intake by female rats were unaffected by self-administration of nicotine with alcohol.

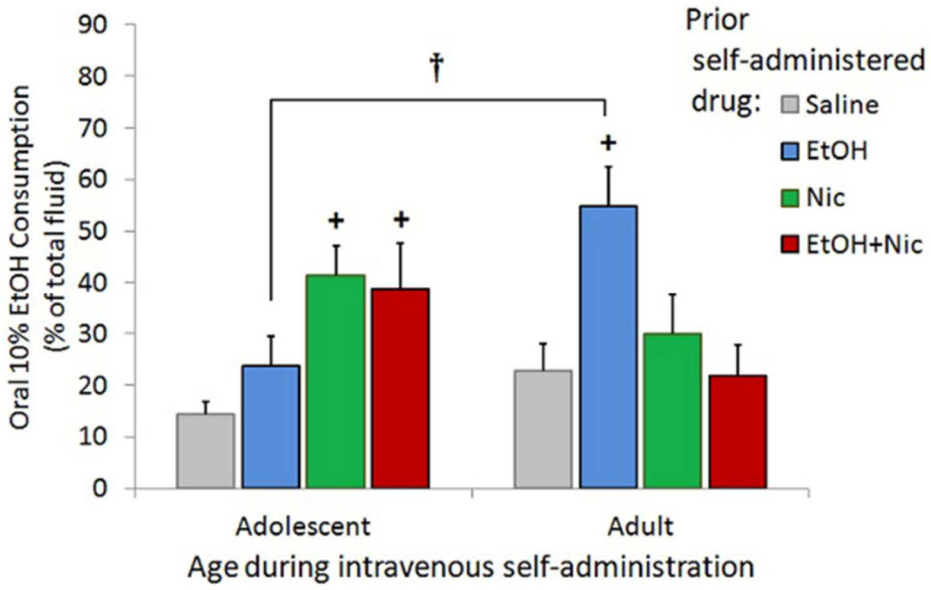

Figure 5: Nicotine self-administration during adolescence increases subsequent alcohol drinking in adulthood [29]

EVALI

Presentation

EVALI, or e-cigarette or vaping associated lung injury, is a diagnosis of exclusion where diagnosis is contingent on other potential etiologies being ruled out, absence of pulmonary infection, a recent history of vaping within 90 days, and abnormal chest imaging findings of pulmonary infiltrates [7]. Symptoms usually present gradually, first with mild shortness of breath and/or chest pain that progresses to severe difficulty breathing. It presents with a wide range of respiratory, gastrointestinal, and constitutional symptoms, including cough, dyspnea, chest pain, nausea, vomiting and diarrhea, fatigue, fever, and weight loss [4, 7]

Potential Cellular and Molecular Mechanisms

This acute lung injury is characterized as a sterile exogenous pneumonitis-like reaction with variable degrees of diffuse alveolar damage, indicated by increased levels of club cell protein 16 (CC16), and substantial involvement of innate immune mechanisms [7]. In a healthy lung, the innate immune system functions in maintaining airway homeostasis, through lung surfactants, mucociliary clearance, and phagocytosis of inhaled particles. The immune cells that drive these physiological functions, and thus are the first responders following ENDS aerosol exposure, include airway epithelial cells (AECs), alveolar macrophages, and polymorphonuclear cells (PMNs) [7].

E-cigarette aerosol exposure, as previously discussed, increases the user's susceptibility to infection due to dysfunction of the innate immune system. Users were found to have reduced levels of proteins CSF-1, CCL26, and eotaxin-3 that are essential for mucosal host defense [7]. PMN function and its neutrophil-derived extracellular traps (NETosis) are also disrupted as a result of e-cigarette aerosols and inhibit the appropriate immune response from the lungs. The compromised immune system results in more inflammation from increased rates of infection. The chemical assault from e-cigarette aerosols also contribute to pneumonitis. Higher concentrations of C-reactive protein and inflammasome complex proteins which promote cellular pyroptosis have been observed [7]. However, this increased inflammation is not resolved, also as a consequence of ENDS use. Exposure to ENDS changes the phenotype and function of alveolar macrophages, suppressing their phagocytic activity that helps clear the insult, both apoptotic cells and harmful irritants, and thus resolve inflammation. The chronic

inflammation can lead to alveolar damage and potentially EVALI. However, the exact mechanism and the culprit is unknown at this time.

Role of Vitamin E Acetate

There is currently no single substance that is conclusively implicated to cause EVALI. Recent histopathological reports showed the presence of blackened lungs, which can suggest the involvement of PAHs in EVALI [7]. However, prevailing thought supports that vitamin E acetate, an additive most notably in THC-containing vaping products, is strongly linked to the EVALI outbreak [37, 45], particularly in products that are distributed through illicit channels. Vitamin E acetate, available as a vitamin supplement and in many cosmetic products, does not cause harm when ingested or applied to the skin. When inhaled, however, vitamin E acetate has been suggested to interfere with normal lung function.

Blount et al performed isotope dilution mass spectrometry using bronchoalveolar lavage (BAL) fluid samples collected from 51 EVALI patients, of which 25 patients were confirmed and 26 were suspected, and 99 healthy participants to measure levels of several priority toxicants, such as vitamin E acetate, diluent terpenes, and medium-chain triglyceride oils [4]. They found vitamin E acetate in 48 of the 51 (94%) EVALI patients' BAL fluid samples and no other toxicants from either group, except coconut oil and limonene in 1 EVALI patient each. 47 of the 50 (94%) EVALI patients for whom laboratory or epidemiologic data was available either had THC or its metabolites detected in their BAL fluid or reported using THC vaping products in the 90 days prior to

symptom onset. 30 of 47 (64%) case patients had detectable nicotine or its metabolites in their BAL fluid [4].

The presence of vitamin E acetate in the vast majority of BAL fluid samples in EVALI cases highlights the strong link between vitamin E acetate and EVALI, though no causal claims can be made at this point. Also, the prevalence of THC-product vaping in affected EVALI patients supports its role in EVALI onset.

Incidence

There was a sharp rise of EVALI cases in August 2019, which peaked in September 2019. Since then, there has been a gradual but consistent decline in cases possibly due to increased awareness, removal of vitamin E acetate in some products, and law enforcement actions related to some illicit products [37].

As of January 14, 2020, there has been a total of 2,688 hospitalized EVALI cases and 60 confirmed deaths in the US reported to the CDC [37]. The median age of the deceased patient was 51 years, with patients ranging from 15 to 75 years old. Among the hospitalized cases reported as of January 14, 2020, 66% of patients were male and the median age was 24 years and ranged from 13 to 85 years old. 15% of those patients were under 18 years old, 37% of patients were 18 to 24 years old, 24% were 25 to 34 years old, and 24% of patients were older than 35 years old [37]. 2,022 of the 2,688 patients provided data on substance use, with 82% reporting using THC-containing products, 33% using THC-containing products exclusively, 57% vaping nicotine-containing products, and 14% reporting exclusive use of nicotine-containing products [37]. Of the hospitalized

EVALI patients, younger patients, aged 13 to 17 years old, were significantly more likely to obtain ENDS from informal sources. 94% of patients in this age range had acquired their THC-containing products only from informal sources compared to 62% of those aged 45 years or older. 42% of EVALI patients aged 13 to 17 years acquired nicotine-containing ENDS from only informal sources versus 12% of the patients 45 years or older.

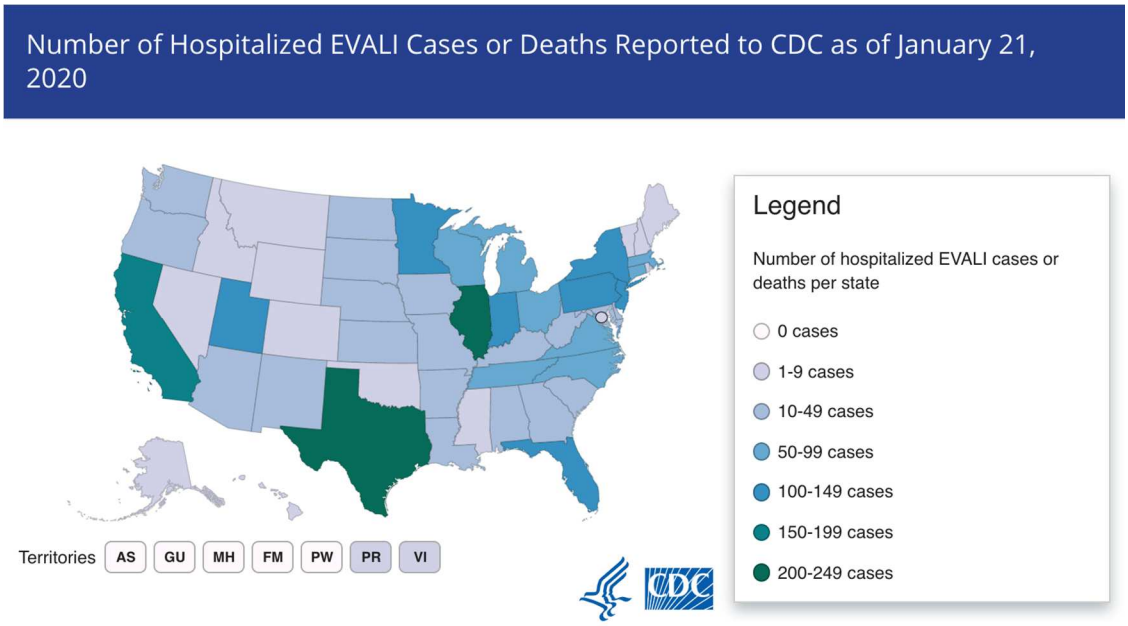

Figure 6: Number of Hospitalized EVALI Cases or Deaths Reported to CDC as of January 21, 2020 [37]

Implications

The severity of EVALI and its prevalence in adolescent populations demonstrates the potential harm that e-cigarettes pose to adolescents, who are more likely to use e-cigarettes than their adult counterparts. EVALI cases are also more strongly linked to products obtained through illicit channels, which was the most common method of

acquisition in the patients aged 13 to 17 years. This can be attributed to the age restrictions, rightly so, of ENDS sales that force youth to obtain e-cigarettes from illegal sources. These correlations highlight the targeted susceptibility of adolescents to EVALI, demonstrating the necessity to regulate ENDS.

CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

ENDS have pervaded the adolescent demographic, integrating itself as a staple in youth culture through its accessibility, concealability, status, and flavors. The intentional marketing of e-cigarettes, that targets teens, only tightens its grip on youth, as exemplified by JUUL Labs. And as ENDS use has increased amongst adolescents, so have its consequences. Particularly of concern in the youth population is the graduation to combustible cigarettes that compounds the effects of nicotine on the developing brain in this critical stage and deepens the Gateway hypothesis. ENDS do have the potential for smoking cessation or as an alternative smoking product for already combustible cigarette smokers able to make a complete switch to e-cigarettes. But in the end, however, the long-term health consequences of ENDS are still unknown and so e-cigarettes should be used with caution and definitely not by minors. Its pervasiveness in youth culture emphasizes the necessity of regulation to control adolescent exposure. A coordinated effort from policy makers, public health agencies, healthcare providers, researchers, and especially parents and educators is essential for successful protection of this vulnerable population.

Policy Changes

With policy changes, the issue presents itself to make potentially lower risk nicotine products intended for smokers accessible, while simultaneously discouraging use by non-smokers, especially youth.

Current Policies

FDA regulation of the tobacco industry first began with the Family Smoking Prevention and Tobacco Control gave, which allowed the FDA to regulate the manufacture, import, packaging, labeling, advertising, promotion, sale, and distribution of ENDS, including components and parts of ENDS but excluding accessories [46]. In 2016, the Child Nicotine Poisoning Prevention Act of 2016 was passed in response to the more than 8,000 accidental e-liquid exposures in children younger than 6 years old reported by U.S. poison control centers between 2012 to 2017 [15] and requires child-resistant packaging of E-juices. By August 2018, all covered tobacco products were required to bear tobacco labeling and warning statements on packaging and advertisements [9, 46]. And finally in January 2020, the FDA announced that it will ban all flavored e-cigarette pods except tobacco and menthol. This announcement was much less stringent than their previous announcement in September 2019 to "clear the market" of flavored e-cigarette and vape products. Nearly 50% of study participants in Du et al's study reported that they would find a method to buy their preferred E-liquid flavor or add flavoring agents themselves if flavors were banned [14].

In response to this new regulation and JUUL Labs halting sales of some flavors after scrutiny for its leading role in the youth e-cigarette epidemic, Puff Bars have grown in popularity. Puff Bar is a relatively new e-cigarette product that is similar in design and functionality to the popular JUUL but is disposable. They are able to sell flavors like O.M.G (orange, mango, guava) because its design for one-time-use allows it to bypass federal policy that regulates flavored e-cigarettes [48]. Currently, such regulation only applies to closed system e-liquid cartridges and not to refillable cartridges or disposable products. Puff Bar is only one brand of tens that has taken advantage of this loophole.

Recently the U.S. House of Representatives passed the Protecting American Lungs and reversing the Youth Tobacco Epidemic Act of 2020 (H.R. 2339) that would ban the use of characterizing flavors in all tobacco products, include vape and e-cigarette products, but has not been brought to a vote in the Senate.

Standardization

Much of the regulation bypassing is possible because ENDS are currently heterogeneous, coming in many styles, constituents and their concentrations, mechanical features, and more. On top of that, the e-cigarette industry is evolving quickly. By standardizing ENDS and the E-liquids, there can be more efficient and consistent regulation of ENDS. Standardization can include battery power, constituents, nicotine concentrations, marketing, and safety requirements. In England, for example, where the quality, safety, and marketing of ENDS are regulated and nicotine concentrations are capped at less than half of that permitted in the U.S., fewer 11-18 year olds have tried e-

cigarettes (15.4%) and adults most commonly use ENDS products to stop smoking [11, 32, 45]. Regulating and standardizing ENDS will also benefit research, providing internal and external validity of the studies that can then be a source of reliable and applicable information for health workers, teachers, parents, and adolescents. Routine screening procedures can be developed and appropriate care given with such information.

Age Regulation

Age regulation is also an important step that is necessary to mitigate the harms of e-cigarettes. The new federal law to ban the sale of all tobacco products, including e-cigarettes, to persons under 21 years of age will not only legally inhibit a larger population of youth from obtaining tobacco products, but it will also deter the accessibility of illicit ENDS for adolescents. This increase in age gap between the susceptible adolescent and the adult who has access to e-cigarettes distances the connection a teen has to a peer who can buy it for them, making it much harder and less likely for adolescents to vape.

Call to Action

The unknown consequences, especially long-term, and harm potential of ENDS highlights the unchartered territory that we still have to uncover through research. The current knowledge, that demonstrates the pulmonary, cardiac, neurologic, and addictive effects of e-cigarettes, emphasizes the urgency of this research, especially due to the

projected growth of the industry. Future studies should not only examine the physiological consequences of vaping itself but also its epidemiologic effects and the efficacy of current regulation, in order to guide the adoption of future policies. With new research much also come equal efforts in teaching and communicating actionable recommendations to health professionals, public health officials, and the general public about safe ENDS use and the potential harms. We must dispel the widespread misconception that e-cigarettes are without harm.

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