Interdisciplinary OS State of the Science on Electronic Nicotine Delivery Systems (ENDS)

Includes literature through March 31, 2020

Contents

PURPOSE AND OVERVIEW

This document provides an overview of scientific literature pertaining to electronic nicotine delivery systems (ENDS) as of March 31, 2020. This overview encompasses information presented in the consensus study report "Public Health Consequences of E-cigarettes" published in 2018 by the National Academies of Sciences, Engineering and Medicine (the NASEM Report)[1] and supplements this information with findings from literature published through March 31, 2020. This document also discusses gaps in the NASEM Report and identifies remaining and new knowledge gaps. This document is not intended to be an exhaustive catalogue of information relevant to ENDS and there may be additional literature that can be cited in support of reviews.

The content of this document is arranged similarly to the NASEM Report. Section 1 describes the general operation, design, components, common ingredients, and common product characteristics of ENDS products. Section 2 discusses the individual health risks posed by ENDS and how different product characteristics may affect these risks. Section 3 discusses the population health aspects of ENDS use including prevalence and patterns of ENDS use, how product characteristics and marketing influence the appeal of ENDS, and the risk perceptions and population health risks associated with ENDS use.

It is intended for this document to:

- help address general questions for ENDS,
- highlight information or testing needed to answer these questions related to ENDS, and
- provide a basis for the comparison of ENDS to other tobacco products (including other ENDS).

This document is an information resource for reviewers to consider when performing their review of a specific application. However, the document itself is not a primary resource and should not be cited as part of a final decisional document within reviews. However, reviewers may cite specific references from within the document or if deemed appropriate, may use the text provided for a specific reference, as applicable to their review. The determination of how the science is relevant to a specific review is the decision of the reviewer, TPL, and secondary or tertiary reviewers.

DATA SOURCES AND METHODS

The NASEM report was the primary data source for literature published prior to 8/31/2017. The ENDS Work Group literature database was the primary source for data published between 8/31/2017 and 3/31/2020. Literature from the NASEM report was used without qualification. Literature not included in the NASEM report was selected and scored according to criteria developed by each discipline within OS. In general, each discipline first identified a pool of peer reviewed articles from which to select relevant studies, and then screened these articles according to discipline specific inclusion and exclusion criteria.

Compared to other tobacco products, ENDS are still relatively new in the academic literature. As a result, information from potentially non-peer reviewed sources, such as patents and conference presentations, are sometimes included for additional insight to the extent appropriate in this document.

LIST OF ABBREVIATIONS

α_{fb} = ratio of nicotine present in freebase form versus protonated form (between 0 and 1)
1-OHP = 1-hydroxypyrene
1,3-DCP = 1,3-dichloropropan-2-ol
2-MHA = 2-Methyl Hippuric Acid
3-HC = Trans-3'-hydroxycotinine
3-HPMA = N-Acetyl-S-(3-hydroxypropyl)cysteine
3-MCPD = 3-monochloropropane-1,2-diol
3-MHA+4-MHA = 3-Methyl Hippuric Acid + 4-Methyl Hippuric Acid
ADD = attention deficit disorder
AE = Adverse Experiences
AMCC = N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine
aOR = adjusted odds ratio
API = Application Programming Interface
aPR = adjusted prevalence ratio
AUC_{0-t} = area under the "concentration vs. time" curve which assesses the total nicotine exposure
a_w = water activity
BAL = Bronchoalveolar Lavage
B[a]P = Benzo[a]pyrene
BMS = battery management system
BOE = biomarkers of exposure
bp = boiling point
BRFSS = Behavioral Risk Factor Surveillance System
CBD = cannabidiol
CEMA = 2-cyanoethylmercapturic acid
CI = confidence interval
C_{max} = maximum nicotine concentration levels reached
CO = carbon monoxide
CPD = cigarettes smoked per day
CReSS = Clinical Research Support System
CYMA = N-Acetyl-S-(2-cyanoethyl)-L-cysteine
DEHP = di(2-ethylhexyl) phthalate
DEP = diethyl phthalate
DMP = dimethyl phthalate
DTPA = diethylenetriaminepentaacetic acid
e-CPT = e-cigarette purchase task
EASI = ENDS Addiction Severity Index
EDS = E-cigarette Dependence Scale
EDTA = ethylenediaminetetraacetic acid
ENDS = Electronic Nicotine Delivery Systems
e-NDSS = Nicotine Dependence Syndrome Scale
eTOP = E-cigarette Topography Instrument
EVALI = E-cigarette or vaping use-associated lung injury
e-WISDM = Wisconsin Inventory of Smoking Dependence Motives
FTCD = Fagerström Test for Cigarette Dependence

GC-MS = gas chromatography-mass spectrometry
GRAS = generally recognized as safe
HBEC = Human Bronchial Epithelial Cells
HINTS = Health Information National Trends Survey
HMPMA = 3-hydroxy-1-ethylpropylmercapturic acid
HONC = Hooked on Nicotine Checklist
HPHCs = harmful or potentially harmful compounds
HPMMA = N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine
ITC = International Tobacco Control Policy Evaluation Project
LC-UV = liquid chromatography-ultraviolet detection
LDH = Lactate Dehydrogenase
LGBTQ = Lesbian, Gay, Bisexual, Transgender, Queer/Questioning
LOD = limit of detection
LOQ = limit of quantification
MCP = Multiple Choice Procedure
MHBMA = Monohydroxy-3-butenyl mercapturic acid
MHBMA3 = N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine
MMAD = mass median aerodynamic diameter
MMP = Matrix Metalloprotease
MTF = Monitoring the Future Study
MTT = Dimethylthiazol-Diphenyltetrazolium Bromide
MTurk = Amazon Mechanical Turk
NAB = N-nitrosoanabasine
NAT = N'-nitrosoanatabine
NEISS = National Electronic Injury Surveillance System
NESARC = National Epidemiologic Survey on Alcohol and Related Conditions
NFDC = National Fire data center
NHANES = National Health and Nutrition Examination Survey
NHB = Non-Hispanic Black
NHIS = National Health Interview Survey
NHW = Non-Hispanic White
NMR = Nuclear Magnetic Resonance
NNAL = (4-(methylnitrosamino)-1–(3-pyridyl)-1-butanol
NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-butanone
NNN = N'-nitrosonornicotine
NRT = nicotine replacement therapy
NTA = nitrilotriacetic acid
NYTS = National Youth Tobacco Survey
OV% = percentage of oven volatiles (determination of moisture content)
PAH = Polyaromatic Hydrocarbon
PATH = Population Assessment of Tobacco and Health
PBPK = Physiologically Based Pharmacokinetic
PG:VG = propylene glycol: vegetable glycerin
PK = pharmacokinetic
$PM_{0.1}$ = Particulate Matter with a Mean Aerodynamic Diameter of 0.1 µm or Less
$PM_{2.5}$ = Particulate Matter with a Mean Aerodynamic Diameter of 2.5 µm or Less

PROMIS = Patient-Reported Outcomes Measurement Information System
PROMIS-E = Patient-Reported Outcomes Measurement Information System for ENDS
PS-ECDI = Penn State Electronic Cigarette Dependence Index
QVC = Questionnaire of Vaping Craving
RDTAs = rebuildable dripping tank atomizers
ROS = Reactive Oxygen Species
SD = standard deviation
SPA = Smoking Puff Analyzer (SPA-M is the mobile version)
S-PMA = S-phenylmercapturic acid
TAMC = total aerobic microbial count
THC = Tetrahydrocannabinol
T_{max} = time it takes to reach maximum nicotine concentration levels
TNE = total nicotine equivalents
TNE3 = Nicotine, Cotinine, Trans-3'-hydroxycotinine
TNE6 = nicotine, cotinine, trans-3-hydroxycotinine, nicotine- glucuronide, cotinine- glucuronide, trans-3'-hydroxycotinine- glucuronide
TPM = Total Particulate Matter
TPRPS = Tobacco Products and Risk Perceptions surveys
TSNA = Tobacco Specific Nitrosamine
TYMC = total yeast and mold count
UK = United Kingdom
USB = Universal Serial Bus
VAS = Visual analogue scale
VOCs = Volatile Organic Compounds
wPUM = Wireless Personal Use Monitor
YRBS = Youth Risk Behavior Surveillance System

LIST OF UNITS

Ω = ohms (unit of electrical resistance)
cfu = colony forming unit
mAh = milliampere Hour (measures electric current over time, energy capacity of a battery)
mg/mL = milligrams per milliliter (generally shows concentration of a compound in a liquid)
µm = micrometer (unit of length; in this document refers primarily to diameter of aerosol droplets)
ng = nanograms
s = seconds
V = volts (unit of electric potential difference)
W = watts (unit of power)

SECTION 1. PRODUCT DESIGN AND CHARACTERISTICS

A. PRINCIPLE OF OPERATION

Electronic nicotine delivery systems (ENDS) generate an inhalable aerosol from a bulk solution called an e-liquid. Although the specific design and complexity of these products vary widely, generally ENDS produce aerosol using a common set of components. Specifically, these components include:

- An atomizer to generate the aerosol
- A reservoir to hold e-liquid prior to atomization
- A power source for the atomizer
- A mechanical switch or electronics to control the flow of power to the atomizer.

Several different methods have been used to aerosolize the e-liquid aerosol including heat, ultrasound, and mechanical nebulization. Currently, heat-based atomizers are used in the majority of ENDS. In typical heat-based ENDS, the atomizer is prepared for use by allowing a small amount of e-liquid to flow from the reservoir into a porous matrix (e.g., a cotton wick or mesh) adjacent to a heating element (e.g., coil). The amount of e-liquid that fills the matrix is generally controlled by a balance between capillary action, fluid pressure from the reservoir, and flow resistance through the pores or channels of the matrix. In some products, priming of the porous matrix occurs immediately prior to use, while other atomizers remain primed continuously. To initiate aerosol production, electrical power is supplied to the heating element. The heating element converts electrical energy into radiated thermal energy, which raises the temperature of the e-liquid contained in the porous matrix. As the e-liquid heats, higher quantities of e-liquid constituents enter the vapor phase near the heating element. When the user inhales through the product, the vaporized e-liquid constituents are drawn away from the heating element and cool. As the vapor cools, the e-liquid constituents spontaneously return to the liquid phase and produce an aerosol. Fresh e-liquid from the reservoir replenishes e-liquid lost from the porous matrix and the cycle repeats.

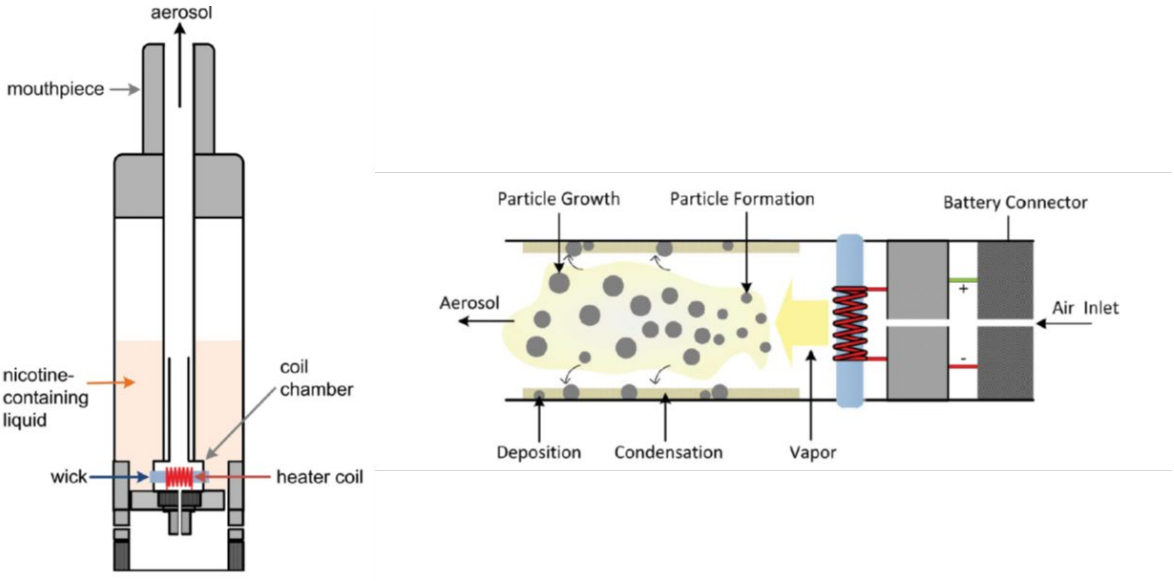

Figure 1. Aerosol generation in a coil-based ENDS. Adapted from Talih et al., 2017.[2]

B. CLASSIFICATION OF ENDS

ENDS are broadly classified by product power and the extent to which the product is accessible or modifiable by the user. ENDS not intended to be refilled are referred to as "closed". Conversely, "open" ENDS are refillable. Additionally, closed ENDS generally limit the extent to which its components (e.g., atomizer, coil, wicking, tank, battery, etc.) can be manipulated by the consumer and open ENDS may contain both user accessible and replaceable components. Closed ENDS tend to be simpler to operate, lower powered, and are marketed towards new or more casual ENDS users. Open ENDS tend to be higher powered and are marketed towards enthusiasts.

ENDS have evolved significantly in both form and function over the past decade. As a result of these rapid and extensive changes in product design, it is typical to see ENDS loosely classified by generation. These generations correspond broadly to the combination of characteristics prevalent during the different times of product development. The first marketed ENDS were typically low powered, nonadjustable, and non-refillable products that resembled combusted cigarettes ("cig-a-likes"). As a result, products with these characteristics are referred to as first generation. Second generation products vary more in appearance, allow greater manipulation by the user, can be refillable, and tend to have higher power and aerosol production than first generation products. Third generation products are characterized by the highest power and aerosol production, and typically allow the user to significantly manipulate the product hardware and operation. Smaller low-power systems (pod systems) with easily replaceable "pods", which are combination atomizer and e-liquid reservoirs, have been rapidly growing in popularity since 2015, particularly among youth and young adults.[3,4] Recently, disposable ENDS emerged, which are low-power products designed with no replaceable parts. Disposable ENDS are typically draw-activated and meant to be disposed of once the e-liquid is depleted or the battery dies. Generally, disposable ENDS designs mimic those of the pod systems, without the replaceable component.

C. ENDS COMPONENTS

Atomizers

Generally, current ENDS use a coil and wick design for the atomizer where the heating element is wound around a porous matrix to ensure efficient transfer of heat to the e-liquid. Along with product power, the number of coils and wicks in the atomizer determine the amount of e-liquid aerosolized in a given time. The number of coils in current ENDS generally range from one to twelve. Coils are made from a variety of different metals and alloys. Some of the more popular coil materials include Kanthal (an iron-aluminum-chromium alloy), Nichrome (an iron-nickel-chromium alloy), nickel, stainless steel, and titanium. Fibrous and flexible materials are typically used as the porous matrix (i.e., wick). Cotton, rayon, and silica are among the most commonly used adsorbent materials.

There are many different styles of atomizers available. Some more common styles include cartomizers, clearomizers, rebuildables, and pods. Cartomizers are typically disposable atomizers. The name, often shortened to 'carto,' is a portmanteau of cartridge and atomizer, as this is essentially a cartridge of e-liquid and an atomizer combined into one compact piece. Early atomizer designs had e-liquid cartridges that were plugged into an atomizer by the user. In a cartomizer, the coil lies inside a shell (most often

metal), surrounded by a large amount of wicking material soaked with e-liquid. Although cartomizers are sometimes user-refillable, the user is typically not meant to access the heating coil and the wicking elements.

A clearomizer, sometimes shortened to 'clearo', is a type of atomizer with a transparent e-liquid reservoir tank surrounding the heating element. The design allows users to see the level of e-liquid in the atomizer during use. Clearomizers typically use a horizontal wick and coil design. Some clearomizers house the coil on top, and some on the bottom. Many models and versions are available in different sizes. They tend to be refillable, and the atomizer portion of the product is not usually meant to be user accessible.

A "rebuildable" is an atomizer that requires the user to construct and install both the coil and the wick, as opposed to typical clearomizers, which use pre-assembled cartridges. There are two major types of rebuildables:

- RDA (rebuildable dripping atomizers): Rebuildable dripping atomizers ("drippers") contain one or more coils used with a threaded wicking material. The user drips fluid into the RDA and capillary action draws the liquid into the wicks.
- RTA (rebuildable tank atomizers): Rebuildable tank atomizers are very similar to clearomizers except the aerosolizing apparatus can be modified by the user. These give the user more flexibility in coil resistance, wicking material, and coil design.

A pod is a small atomizer designed to work with pod systems. They commonly snap into the product power source to facilitate rapid and easy replacement by the user. They are available in both pre-filled and refillable designs. In contrast to other ENDS, which typically rely on button activation, pod systems are often breath activated. Pod systems are also referred to as "pod-mods", "vape pods", "mini vapes" or "pod vapes".

In many open ENDS, components such as the atomizer, are interchangeable. This is an attractive feature for some ENDS users and components can often be exchanged with ease. For example, a 510 sized connector is far more common among different components than other thread sizes, and adapters are readily available for less common thread sizes. Consequently, it is typical for open ENDS users to experiment with or use a variety of new atomizer designs.

Power Source and Electronics

The power delivery system (PDS) of an ENDS is comprised of a battery and the electronics that control the flow of current between the battery and the atomizer. The battery refers to the assembly of one or more electrochemical cells that supply the electrical power to the coil. The controlling electronics can be complex and are often software controlled. Commonly, the PDS will include circuitry that activates the product (either by the press of a "firing button" or by sensors that detect pressure draw), and regulates voltage and wattage. More sophisticated products may have temperature control, LCD displays and may allow charging and communication via USB. The PDS may also include physical design elements such as battery ventilation holes to allow for gas to escape in the event of a battery failure.

ENDS may be powered by replaceable or integrated batteries. Non-rechargeable batteries are classified as primary batteries, and rechargeable batteries are classified as secondary. Some disposable ENDS use primary batteries, but some use secondary batteries even though disposable ENDS are not designed to allow the battery to be recharged. As with many other products, most ENDS use lithium ion-based batteries. A battery's longevity is measured as milliamp-hour (mAh). Batteries are available for purchase with different mAh ratings. The larger the mAh rating, the longer the battery will last on one charge.

D. E-LIQUID INGREDIENTS

Carrier Solvents

Propylene glycol (PG) and vegetable glycerin (VG) are typically the most abundant ingredients in e-liquids and typically account for >85% of the e-liquid mass. PG (bp = 188 °C) and VG (bp = 290 °C) are relatively high boiling point alcohols and e-liquid solvent mixtures ranging from 100% PG content to 100% VG content have been used. Currently, e-liquids most commonly use solvent ratios between 70:30 PG:VG and 30:70 PG:VG. The primary function of PG and VG is to form the aerosol that carries nicotine and flavor to the user. Whereas e-liquids with higher PG content are linked to a stronger "throat hit" and reportedly transfer more e-liquid and nicotine per puff, higher VG content is associated with larger aerosol droplets.[5] Some e-liquids may contain water, but generally in smaller quantities than PG or VG.[6]

Recently, two patents reported using 1,3-propanediol as a carrier solvent.[7,8] While both patents claim use of 1,3-propanediol lessens or prevents the formation of toxic by-products (e.g., carbonyls), only one patent reports carbonyl yields. In this patent, formaldehyde yields produced by 1,3-propanediol are reported to be below the limit of detection (LOD), while acetaldehyde and acrolein yields are lower than those produced by PG and VG.[7] A publication compared the aerosol properties when using 1,3-propanediol to aerosol properties produced using both PG and VG.[9] The results found aerosol produced from e-liquids containing 1,3-propanediol had similar aerodynamic properties to aerosol produced from e-liquids containing PG and VG, while also suggesting no thermal decomposition using a differential thermal analysis.

Nicotine

E-liquids can be nicotine-free, but most often contain nicotine concentrations ranging from 3 mg/mL to 60 mg/mL (0.3–6.0% by volume), depending on the nicotine formulation used. E-liquids are formulated using free-base nicotine or nicotine salts, and the overall nicotine concentration can be much higher for nicotine salt containing e-liquids (22–60 mg/mL)[10,11] than for free-base nicotine containing e-liquids (3–21 mg/mL).[12,13] Nicotine salts naturally occur in tobacco.[14] While various salt formulations have been investigated for use in ENDS products,[15,16] the most common currently used nicotine salt formulations are nicotine benzoate, nicotine levulinate, and nicotine lactate.[15,17] One recent publication also reports use of nicotine salicylate, nicotine malate, and nicotine tartrate in e-liquid formulations.[18]

Flavors and Dyes

Most e-liquids are flavored, and flavor is an important determinant of product appeal. Several thousand flavored e-liquids are currently available, ranging from those that mimic the taste of traditional tobacco products (e.g., tobacco and menthol) to those flavored like candy, fruit, and dessert. E-liquid flavors

typically contain the same GRAS components found in food flavorings and these compounds can be present in significant levels. A study by Behar et al. showed 12 of the most common e-liquid flavor ingredients, cinnamaldehyde, menthol, benzyl alcohol, vanillin, eugenol, p-anisaldehyde, ethyl cinnamate, maltol, ethyl maltol, triacetin, benzaldehyde, and menthone are often present in concentrations above 1 mg/mL in e-liquids and can transfer efficiently into the aerosol (mean transfer efficiency = 98% across all compounds and two different voltages).[19] Various sugars including fructose and glucose,[20] as well as sugar derivatives such as sucralose and sugar alcohols,[21,22] are used as sweeteners, and synthetic food colorings (e.g., Allura Red AC, Brilliant Blue FCF, Tartrazine and Brilliant Blue FCF) have been identified in commercial e-liquids.[23] Though many of these ingredients are considered GRAS because they are used in food, the inhalation toxicity of many of these ingredients has not yet been investigated.

E. PRODUCT CHARACTERISTICS

Engineering

ENDS vary greatly in design. As such, the design parameters necessary to evaluate product performance may also vary with product design. However, the following parameters are common to most ENDS and are critical for product evaluations, as they are expected to impact product performance across most ENDS designs: coil temperature (maximum and operating temperature), coil specifications (physical structure, configuration, material composition), particle size distribution (count and mass distribution, $PM_{0.1}$, $PM_{2.5}$), wick specifications (wicking rate, material, weight), and control board specifications (power draw and operating range, power regulation, cut off voltage/amperage, mode of control).

ENDS aerosols are highly dynamic and their physical characteristics may vary in time in response to environmental conditions. Consequently, particle size distribution and particle count measurement devices often introduce non-negligible experimental error, thereby biasing the reported results.[24-27] This is likely due to the required dilution of ENDS aerosols by all non-optical measurement techniques. Standardization of ENDS aerosol measurement methods is needed to allow relative comparison of results across products.

ENDS power correlates positively with the production of aerosol mass[28-30] and changes in the aerosol particle size distribution median diameter.[31] Changes in the particle size distribution, particle count, and aerosol mass generated by an ENDS determines deposition patterns within the user's respiratory tract,[26] and models suggest ENDS aerosols have a higher rate of deposition than non-dynamic aerosols (e.g., carbon or soot-based aerosols from combustion, aerosols with solid rather than liquid particles).[32] There is evidence the particle concentration for ENDS are higher than for combusted cigarettes and human respiratory airways can be exposed to high doses of compounds such as propylene glycol. Further, there is evidence the median size of the particles may be slightly smaller for ENDS, as compared to combusted cigarettes. ENDS aerosol particles are in the fine or ultrafine range and thus can penetrate deep into human lungs and deposit there, mostly by diffusion and sedimentation.[33] However, additional research is important to fully quantify the aerosol dynamics upon inhalation.[25]

Chemistry

E-liquid pH may help determine the nicotine state (e.g., free-base or salt), which may affect the addictive potential of the ENDS. The pH of e-liquids is typically measured by diluting the e-liquid with water (typically a 1:10 ratio) and measuring with a pH meter.[18,34,35] This method is limited, as dilution changes the solvent system, which may impact the accuracy of the measured pH.[36] A few other methods have been developed that can measure pH indirectly by determining the nicotine salt to free-base nicotine ratio directly and then calculating the pH. These indirect measurements were done by NMR[11] and colorimetric analysis.[37] However, these methods may have chemical interferences.[38] For example, when flavoring concentrations were high, nicotine was not detected by NMR when the nicotine concentration was less than 3 mg/mL.[38] In addition, while some studies assume the aerosol pH is similar to the e-liquid pH, one study investigating JUUL reported differences between the pH of the e-liquid (5.4) and the aerosol (6.1).[39]

Several studies have analyzed the chemical stability of various compounds in e-liquids. Bansal et al. performed a thermal degradation stability study on nicotine in pure nicotine liquid, an aqueous nicotine solution, and six different nicotine concentration e-liquids, each containing one of two flavors (mint and watermelon).[12] Thermal degradation after storage at 60°C for up to 10 days showed e-liquids have minimal reduction in nicotine content, while pure nicotine liquids and aqueous nicotine solutions showed almost 6.5% and 17% reduction in nicotine content, respectively. Among e-liquids studied, one mint flavored sample (3 mg/mL) showed 8.7% nicotine degradation, while other samples showed less than 5% nicotine degradation. Therefore, this study suggested the presence of other ingredients in e-liquid formulations may help stabilize the nicotine against degradation. However, this study only investigated two e-liquid flavors.[12] Further, a patent filed in 2016 stated adding polyols, such as mannitol, erythritol, xylitol, or sorbitol, along with chelating agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and nitrilotriacetic acid (NTA), increases the stability of both free-base nicotine and nicotine salt e-liquids by preventing the formation of free radicals or by reacting with free radicals.[22] However, no studies demonstrated if these chemicals affect aerosol delivery or user exposure.

In addition to nicotine stability, other publications have investigated the stability of some flavor ingredients present in e-liquids. In one paper, researchers noted acetoin in e-liquid converts to diacetyl, and the conversion rate was affected by the PG:VG ratio of the e-liquid.[40] Lastly, another publication found flavored aldehydes, such as benzaldehyde, cinnamaldehyde, citral, vanillin, and ethyl vanillin, generally begin to form acetal compounds with PG within a day of the e-liquid being manufactured and suggested stricter testing may be required to ensure reaction products are not formed in manufactured e-liquids before reaching the user.[41]

E-liquids are commonly packaged in plastic containers that contain co-polymers, plasticizers, colors, and other additives that may migrate or leach into tobacco products. Thus, extractables and leachables from the containers are important to consider when examining e-liquid stability. Extractables are any compound that can be pulled out of the packaging material under extreme conditions. Leachables are compounds likely to be transferred to a product under normal storage conditions. One study found contaminant leaching from packaging is higher in liquids and products having high water content.[42] A

review article also reported liquids facilitate the migration of contaminants from the packaging to the product through solubility, diffusion, and other active transport systems.[43] Thus, e-liquids used with both open and closed ENDS are expected to be susceptible to leaching plastic constituents. Further information on leachable and extractable compounds is in Section 2C "Toxicants Other than Nicotine."

Microbiology

E-liquid ingredients of microbiology concern generally include humectants and preservatives. Preservatives are added to prevent undesirable microbial growth and humectants function to control tobacco product moisture, which in-turn controls microbial growth; therefore, both ingredients may impact microbial stability of the tobacco product during shelf-life. E-liquids typically include humectants such as PG, VG, and water that may affect the moisture content of the product.[1] Geiss et al. reported e-liquid water content varies, 9.5–25.9%, based on the humectant composition of the e-liquids.[44] The moisture content of a given product is a measure of the total amount of water (bound and unbound) in the product. This is not a good indication of the amount of water available to support microbial growth because only unbound water is available for microbial growth. The amount of water available for microbial growth in a product is described in terms of water activity (a_w).[45,46] Although a_w has not been studied in e-liquids, research indicates the a_w limit varies with different solutes and humectants. Products with a high a_w tend to support microbial growth.[47]

PG and VG also function as preservatives. The antimicrobial activity of PG and VG has been demonstrated individually, under laboratory controlled conditions, against specific microbial strains, and at pre-determined concentrations.[48,49] However, no research studies to date have determined to what extent PG and VG together, at different concentrations, could act as antimicrobial agents or show any antimicrobial activity within e-liquids. Information on microbial contaminants in e-liquids is in Section 2C "Toxicants Other than Nicotine."

SECTION 2. INDIVIDUAL HEALTH RISKS OF ENDS

The health risks of ENDS, particularly following long-term use, are not fully understood. As with other tobacco products, the health effects of ENDS use are most likely to arise from the chronic exposure to nicotine and other toxicants present in the aerosol. This section summarizes the available literature concerning the delivery of nicotine by ENDS, the abuse liability and risk of dependence posed by these products, the toxicants that have been identified in e-liquids and aerosols, in vitro and in vivo toxicity assays of e-liquids and e-liquid aerosol, and the adverse medical effects and injuries associated with ENDS use.

A. NICOTINE EXPOSURE

Overview of Studies Evaluating Nicotine Exposure

Nicotine is the primary addictive substance in tobacco products, and the rate, degree, and total amount of nicotine delivery significantly impacts product abuse liability. Elevated nicotine concentrations and faster rates of nicotine delivery increase products' abuse liability due to the rapid absorption of nicotine into the brain.

Nicotine pharmacokinetic (PK) parameters are used to evaluate the rate and extent of nicotine exposure from a product, typically measured in blood plasma. Nicotine PK can be evaluated with prescribed or directed use of an ENDS, typically 10-15 puffs over five minutes of use. Nicotine PK characteristics include maximum nicotine concentration levels reached (C_{max}), the time it takes to reach C_{max} (T_{max}), and the area under the "concentration vs. time" curve (AUC_{0-t}; or AUC) which assesses the total nicotine exposure. Nicotine PK is an important abuse liability measure and is often used to compare tobacco products with known abuse liability, such as combusted cigarettes (high abuse liability) and nicotine replacement therapy (NRT; low abuse liability) to newer products without established abuse liability (e.g., ENDS).

Long-term studies (i.e., chronic nicotine exposure) typically measure cotinine in blood, urine, or saliva. Sometimes studies also measure total nicotine equivalents (TNE; often measured as TNE-6). Overall, nicotine exposure studies suggest that exclusive ENDS users and dual ENDS and cigarette users are exposed to high levels of nicotine and may titrate their use behaviors to achieve nicotine exposures akin to cigarette smokers.[50-56] These data suggest ENDS abuse liability may be generally similar to that of combusted cigarettes.

Product Characteristics That Influence Nicotine Yield and Delivery

Nicotine delivery (the amount of nicotine that reaches the user) is influenced by many factors including nicotine yield (the amount of nicotine heated and released into the ENDS aerosol), the physical and chemical characteristics of the aerosol, and use behavior. Nicotine yield and the characteristics of the aerosol are in turn impacted by e-liquid nicotine concentration, e-liquid composition, and product characteristics.

Product Design and Power Level

Nicotine yield in the aerosol is a function of nicotine concentration in the e-liquid and the volume of e-liquid aerosolized. As such, product characteristics and parameters that increase the quantity of e-liquid aerosolized simultaneously increases nicotine delivery. In general, the quantity of e-liquid aerosolized in a given time is determined by the flow of the e-liquid to the wick(s) and the rate and efficiency with which heat is transferred to the e-liquid. Thus, the size of each coil and wick, the number of coils, and the temperature of the heating element determine the total mass of e-liquid aerosolized. The heating element temperature is a function of the applied voltage, coil materials, coil geometry, and time. As voltage and puffing time increase, the temperature at the heating element increases.[57,58] Further, the electrical resistance of the heating element impacts the power output of the ENDS since power is inversely proportional to resistance. Accordingly, for a given coil type and e-liquid combination, aerosol mass[28-30] and nicotine yield increases with voltage,[1,59,60] power,[1,59,60] and coil temperature.[61,62]

An increase in aerosol temperature may increase nicotine yields[63,64] and the risk of thermal injuries.[65-67] In one study, one volunteer who was an experienced ENDS user was asked to sensorially evaluate inhaled aerosol temperature as power increased from 5 to 25W. The volunteer noted the aerosol temperature felt warmer as power increased and the aerosol generated at 20W was too hot.[66]

The type of ENDS (e.g., first generation, tank) influences nicotine delivery and subsequent exposure.[68-72] Since nicotine yield is a principle factor in determining nicotine delivery, products that produce lower nicotine yields tend to have poorer nicotine delivery. The first-generation ENDS ("cig-a-likes") delivered substantially less nicotine than later generation ENDS, and nicotine exposure and absorption rates were lower and slower than combusted cigarettes.[68,69,73,74] In some cases, nicotine PK parameters following use of first-generation ENDS were comparable to the use of NRT.[75-77] For example, in a cross-over study, combusted cigarette smokers with 6–7 days experience using ENDS, smoked one own-brand combusted cigarette, used three Vuse Solo ENDS (filled with 14, 29, and 36 mg/mL nicotine concentration e-liquids) *ad libitum* for 10 minutes, and used NRT nicotine gum for 30 minutes. While plasma nicotine concentrations were significantly higher with the combusted cigarette, nicotine C_{max} did not differ between either the 29 or the 36 mg/mL ENDS or the NRT gum; nicotine C_{max} associated with the 14 mg/mL ENDS was significantly lower than with the NRT gum.[76] Thus, the abuse liability of some first-generation ENDS may be lower than that of combusted cigarettes. In a sample of dual users, 24-hour *ad libitum* use showed nicotine exposure for variable-power tank ENDS users was similar to combusted cigarettes, whereas lower nicotine exposure was found for "cig-a-like" and fixed-power tank users.[78]

Newer generation ENDS deliver greater amounts of nicotine to users than first generation ENDS].[79,80] One clinical study evaluated nicotine exposures from own brand combusted cigarettes and several different types of ENDS in a cross-over study design. Combusted cigarette smokers were able to sample six different ENDS (including disposable [V2, 3.96 V, 18 mg/mL nicotine concentration], rechargeable [Green Smoke, 3.8 V, 24 mg/mL nicotine concentration], and mod-style ENDS [iTazte, 6.06 V, 24 mg/mL nicotine concentration]) for up to one week to gain familiarity with the products. Participants smoked one own brand cigarette and used ENDS for 10 puffs. Plasma nicotine C_{max} was highest following use of the combusted cigarette, but varied after use of the different ENDS; the mod-style ENDS was associated with the highest nicotine C_{max}. Nicotine AUC following use of one combusted cigarette or the mod-style ENDS was significantly higher compared to that from the other two ENDS, indicating users' nicotine exposure varies, at least in part, by ENDS design.[81] Additionally, some studies reported similar nicotine T_{max} values between newer ENDS and combusted cigarettes, suggesting rapid pulmonary absorption of nicotine from some ENDS.[17,82,83] One controlled laboratory study investigated the effects of three power settings (4.3, 6.7, 9.6 W) on nicotine delivery; in general, higher product power resulted in greater nicotine delivery.[80] In a study of experienced ENDS users, products set to higher power and lower coil resistance were associated with greater plasma nicotine concentrations.[84] Additional research will help better define the effects other ENDS product settings have on users' nicotine PK and exposure.

E-liquid Composition - PG:VG Ratio

Research suggests the PG:VG ratio in e-liquids may impact nicotine yield and delivery. A study by El-Hellani et al. indicated there may be a correlation between the PG:VG ratio in the e-liquid and the nicotine delivery to the user.[13,85] Kosmider, et al. showed at low power (4.3 W), the higher PG content e-liquid delivered more nicotine than the 50:50 PG:VG e-liquid or higher VG content e-liquid.[80] However, at higher powers (9.6 W), the nicotine delivery for all three PG:VG ratio e-liquids tested was similar. Prévôt, et al. also showed a slightly higher nicotine delivery when using a higher PG content e-liquid as compared to a higher VG content e-liquid.[86] The extent of the correlation between PG:VG ratio and

nicotine delivery is unclear as it may also depend on the engineering parameters of the device, such as power and temperature, and the amount of e-liquid that can be aerosolized.

Aerosol characteristics such as particle size and distribution are important factors that determine how nicotine yield translates to nicotine delivery. Most of the nicotine mass in the aerosol is located within the liquid droplets.[87] It is well understood from aerosol drug delivery that the deposition of these droplets within the lung plays a critical role in the delivery of constituents to the blood. In general, drugs are absorbed more rapidly and efficiently if they reach the distal portions of the lungs.[88] ENDS aerosols comprised predominantly of droplets, which reach the terminal bronchi and alveoli (typically those droplets ≤ 1.0 µm in diameter), are expected to deliver nicotine more rapidly than those comprised of larger droplets that deposit higher in the respiratory tract.

Some reports show the solvent ratio may affect the physical characteristics of aerosol, but the results are inconsistent. For example, Prévôt, et al. studied two solvent ratios, 80:20 PG:VG and 20:80 PG:VG, and found the solvent ratio did not impact the particle size distribution or mass median aerodynamic diameter (MMAD).[86] However, the same group investigated the impact of different powers on the same physical characteristics of the aerosol, using the same two solvent ratios.[29] In that study, there was a reported moderate effect of PG:VG ratio on the MMAD. Specifically, the observed MMAD was larger when the VG content was higher, but this effect was only seen at 7W. At 13W and 22W, the observed MMAD were comparable for the two different ratio e-liquids. Researchers also observed aerosol output was much higher when VG content was higher. Baassiri, et al. similarly showed the MMAD increased when the e-liquid solvent ratio went from 100% PG to 100% VG.[5] Additionally, they showed higher VG content also increases the average particle size (3.5 µm mass median diameter (MMD) from 2.3 µm MMD) and particle size distribution (maximum modal diameter shifted from 35 nm to 160 nm). In general, the particle size distribution for all PG:VG ratios studied showed similarities; most particles were found between 0.5–1 µm, but the concentration found at each size range varied by PG:VG ratio.

The effect of PG:VG ratio on the nicotine yield and aerosol characteristics appear to translate into human nicotine exposure. In a randomized, cross-over clinical study measuring nicotine PK, participants used an eGo style ENDS with 18 mg/mL e-liquid in four PG:VG ratios (2:98, 20:80, 55:45, 100:0) for two 10 puff bouts. Although plasma nicotine concentrations did not differ among differing PG:VG ratios in bout 1, plasma nicotine concentrations were significantly higher with the 100:0 e-liquids compared to 2:98 and 20:80 ratios following bout 2. During bout 1, nicotine AUC was significantly greater in the 100:0 condition compared to the 2:98 condition,[89] suggesting e-liquids with a higher PG content may be associated with greater nicotine exposure. In general, e-liquids with higher PG content tend to produce more aerosol than e-liquids with higher VG content;[5] however, this effect is most likely to be observed with an ENDS operating under lower power.[80]

E-liquid Composition - Flavors

There is conflicting information about the potential correlation between e-liquid flavors and nicotine yield. El-Hellani et al. showed a significant correlation between flavors and nicotine yield,[13] while Zhao et al. found differences in flavors did not affect nicotine yield.[62] El-Hellani et al. investigated 27 products

from 10 different commercial ENDS brands with various designs and flavors. The Zhao et al. paper investigated the effect of three flavors (tobacco, menthol, and fruit) on aerosol emissions in a controlled system where the ENDS brand, ENDS type, puffing protocol, and operational voltage were fixed. Although the sample size investigated by Zhao et al. is limited, the change in e-liquid flavors, while keeping all other parameters fixed, indicates flavors may impact overall aerosol emissions (the chemical output of ENDS) characteristics.

Additionally, Zhao et al. evaluated the effect of e-liquid flavor on physical aerosol characteristics and found some correlation between e-liquid flavor differences and particle size distribution, where the particle number concentration was highest in menthol flavor (3.3×10^6 particles/cm^3) and lowest in fruit flavor (1.4×10^6 particles/cm^3).[62] It is unclear if this effect is related to differences in compounds or concentration of compounds used in the different flavor profiles. A limited number of studies have investigated the link between e-liquid flavor and physical aerosol characteristics, so further investigation is important.

Some evidence also suggests e-liquid flavor may impact nicotine PK and exposure.[90,91] A cross-over study evaluated nicotine PK of strawberry and tobacco-flavored e-liquids (with nearly identical measured nicotine concentrations) with the KangerTech mini ProTank 3 (1.5 Ω; 3.7 V, 1000 mAh battery, 9.1 W). After a 15-puff prescribed use session, nicotine intake did not differ between flavors; however, nicotine C_{max} was 22% higher (non-significant increase) following use with the strawberry flavored e-liquid. During *ad libitum* use, nicotine intake was 45% higher (non-significant increase) and plasma nicotine exposure ($AUC_{0\text{-}90}$) was significantly greater after use with the strawberry flavored e-liquid than the tobacco-flavored e-liquid.[90] Voos et al.[92] also found different flavors can produce different nicotine exposures among daily smokers. In a 20-puff prescribed use session, puff duration and C_{max} varied depending on e-liquid flavor. **Error! Bookmark not defined.**[Error! Bookmark not defined.]

Due to the limited and conflicting nature of the evidence available, further studies are important to fully understand the extent to which flavors affect nicotine delivery and exposure.

Nicotine Concentration

In general, e-liquid nicotine concentration is positively correlated with plasma nicotine concentrations.[76,77,93-95] For example, similar dose-dependent increase in plasma nicotine concentrations was also observed with both an eGo[93] and eVic Supreme style ENDS.[94]

Nicotine Formulation (Free-base vs. Nicotine salts)

Various patents have been filed for investigating the use of one or more nicotine salt formulations for use in ENDS products.[15,16] The patent literature indicates some nicotine salts, like nicotine benzoate used in JUUL and nicotine lactate used in myblu INTENSE, may deliver nicotine more quickly and efficiently than free-base nicotine.[15,17] Figure *2* (adopted from JUUL's patent[15]) shows when tested in ENDS and combusted cigarette users, JUUL's formulation containing nicotine benzoate has comparable C_{max} and T_{max} to a Pall Mall cigarette, and several other nicotine salts outperform free-base nicotine. Some patent literature has also investigated the use of more than one organic acid in complex with nicotine, or salt co-crystals.[15,16]

Two studies investigated the nicotine aerosol yields from a JUUL. Talih et al. compared the data they gathered for the JUUL against data gathered for other ENDS in a previous study and a Marlboro Red cigarette.[39] When comparing nicotine yields per 15 puffs, Talih et al. found the yields to be a bit higher for the JUUL than the combusted cigarette, but comparable. Since it is known JUUL uses nicotine benzoate in the e-liquid, the Talih study data in combination with the PK data shown in the JUUL patent, indicate nicotine salts in e-liquids may allow for comparable nicotine delivery to combusted cigarettes.[15]

Finally, one study by Duell et al. used proton NMR to determine the fraction of nicotine in the free-base form (α_{fb}) in various e-liquids, where α_{fb} range from 0 to 1.[36] Two flavored JUUL e-liquids were analyzed and determined to have α_{fb} of 0.05 and 0.07. Duell et al. noted products with high nicotine concentrations and low α_{fb} mean the product will have a less harsh tasting aerosol with a high nicotine delivery that makes the product more appealing. The conclusions of the paper indicate this may be an important part in the youth vaping epidemic. Another study by Duell et al. reports the fractions of free-base nicotine to nicotine salt in e-liquids labelled as nicotine salt typically range from 0.1 to 0.19 while the fractions in non-salt nicotine e-liquids range from 0.43 to 0.98.[11] This study also mentions nicotine salts were theorized to reduce the harshness of nicotine in combusted cigarette smoke in the mid-1900s to explain why flue cured tobacco was less harsh compared to other tobaccos.

In keeping with the patent claims, emerging evidence suggests nicotine formulation can affect nicotine PK and exposure. Compared to e-liquids with free-base nicotine, e-liquids formulated with protonated nicotine (i.e., nicotine salts) could produce different nicotine absorption rates, overall nicotine exposure, and throat or upper airway sensations.[17,18] Similar to some free-base nicotine-containing ENDS, emerging evidence suggests e-liquids with nicotine salt formulations can reach or exceed nicotine exposure compared to combusted cigarettes.[10,96] Talih et al.[39] reported the nicotine yield from 15 puffs from a JUUL, a nicotine salt-containing product, was equivalent to the nicotine yield from 1–2 combusted cigarettes; other nicotine salt-containing products (i.e., Sourin, Phix, Bo) also had high nicotine yields.[10] Nicotine salt-containing ENDS have been found to deliver nicotine more rapidly than ENDS with free-base formulations,[17,72,97] suggesting the nicotine salt formulation may increase nicotine exposure. Compared to free-base nicotine, nicotine salts in e-liquids make inhalation easier (e.g., less irritating), particularly at high nicotine concentrations;[39,98-100] experiences may affect use behavior and puff topography.

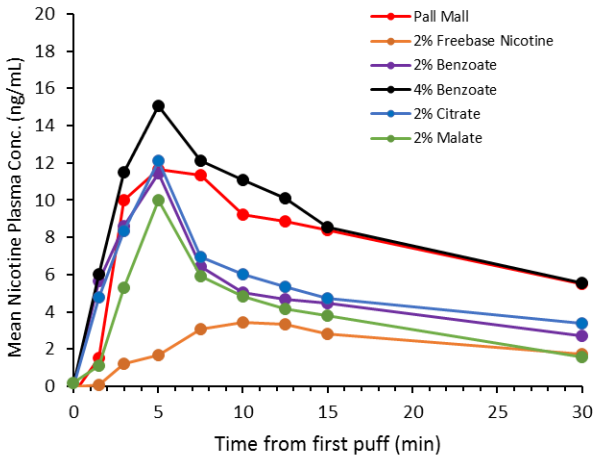

Figure 2. Comparison of mean plasma nicotine level in 24 subjects following 10 puffs (one puff each 30 s for 5 minutes) of Pall Mall cigarette and the same unspecified ENDS filled with e-liquids containing free-base nicotine and various nicotine salts. The mass of e-liquid delivered by the test ENDS was not reported. (From Bowen, et al. US Patent 9215895B2)[15]

These PK studies have several limitations. Small sample sizes limit generalizability to the population. Because some studies evaluated the plasma nicotine PK in inexperienced ENDS users who are known to have lower nicotine exposures than experienced ENDS users, the findings may not be generalizable to, or informative for, experienced ENDS users. The prescribed puffing regimens (typically 10–15 puffs) provide only one (estimated) representation of use behaviors, and may not accurately represent typical and intense puffing behaviors.[101] Furthermore, these studies often do not measure e-liquid nicotine concentration, which often varies from the labeled value, or nicotine yield, which impacts nicotine delivery and PK. Additionally, some studies assessed nicotine PK following use of several products with varying nicotine concentrations, and the specific effects of product or nicotine concentration cannot be determined.

User Behaviors that Influence Nicotine Exposure

Tobacco product use behavior (e.g., topography, frequency of use, switching or cessation) plays a critical role in nicotine exposure. Nicotine exposure from ENDS has the potential to exceed that of a combusted cigarette due to variability in user behavior (e.g., puff duration or volume, use frequency, length of use session). ENDS use behavior can vary widely among users and across products. While smoking a single combusted cigarette is limited to approximately 10 puffs over 10 minutes,[102] a single occasion of ENDS use has fewer topography limits due to a substantially larger e-liquid volume capacity (e.g., some ENDS state their tank or cartridge is equivalent to 200 puffs). ENDS users have reported "grazing" (puffing little and often throughout the day) or "chain vaping" (using constantly), with difficulty reporting quantity and frequency of their use.[103-106] Additionally, some ENDS support *mouth-to-lung* inhalation similar to smoking (e.g., first taking a puff into the mouth, and holding it in the mouth for a short period, then inhaling the aerosol bolus by taking in a deep breath of air; "the mouth-hold phase physically restricts the maximum puffing volume") and *direct-to-lung* ("the mouth-hold phase is omitted and the user takes the puff directly into their lungs in a much larger volume, single inhaling motion"; this second

approach relies on ENDS having low flow impedance characteristics).[107] Finally, ENDS design may lend itself to more frequent use (particularly among youth and young adults) because the products may be easier to hide (e.g., "stealth vaping") and can be used in more places than combusted cigarettes.

Puff Topography

Topography provides a quantitative measure of smoking or ENDS use behaviors (e.g., number of puffs, puff volume, velocity, length), and can be used to evaluate compensatory behavior (i.e., changing puff topography to achieve desired nicotine delivery), assess differences in behavior across products (ENDS or inhaled tobacco products) and populations, and inform human exposure and aerosol emissions testing for nicotine and other harmful or potentially harmful constituents (HPHCs). Differences in ENDS product characteristics and e-liquid ingredients can result in changes to puff topography and, thereby, affect exposure to nicotine and HPHCs. Topography can be measured during prescribed smoking or ENDS use, which may restrict puff timing or volumes to standardize between-subject comparisons, or *ad libitum* smoking or ENDS use, which represents more naturalistic smoking behavior. Smoking topography instruments have been adapted to measure ENDS topography (e.g., Clinical Research Support System [CReSS], Smoking Puff Analyzer [SPA]), but they may not be compatible with all ENDS. Several instruments have been made specifically for some types of ENDS (e.g., Wireless Personal Use Monitor [wPUM], E-cigarette Topography Instrument [eTOP]) and some ENDS are able to record select topography metrics (e.g., Joyetech eVic). Although self-report and video recording can be used to measure puff topography, their utility is limited based on low reliability and validity of self-report and incomplete topography metrics that can be collected from video coding (i.e., no measure of puff volume or flow rate). Topography instruments have not been found to interfere with behavior or acute effects of ENDS use.[108,109]

Several validation studies of topography instruments have been conducted. Mikheev et al.[110] report the CReSS and SPA-M have some limitations, but generally have good accuracy for puff volume and duration, with SPA-M having a more consistent response. They note with both topography instruments "visible liquid deposits were [observed]… after prolonged use… The deposits accumulated with time and could affect accuracy of the pressure transducers responses." Behar et al. (2015) validated the CReSS Pocket topography instrument for "cig-a-likes" and determined the CReSS ENDS mouthpiece did not alter topography.[111] Cunningham et al.[112] validated a patent-pending topography instrument used for industry studies (modified version of SA7) in the UK with a "cig-a-like" and a second-generation ENDS. Kosmider et al.[101] recommend using a typical and intense puffing protocol for aerosol analytical testing; this approach may also be relevant for estimating potential human exposure. Because product characteristics may impact ENDS topography, Cunningham et al.[112] caution bridging study results across products may not be appropriate: "… product characteristics influence puffing topography and, therefore, the results obtained from a given e-cigarette might not read across to other products."

ENDS puff topography may be quantitatively different from smoking puff topography and can vary substantially across users (i.e., based on individual differences). Several studies have found variation in topography patterns among ENDS users, including differences in puff duration, volume, and interpuff interval[101,113,114], patterns of short, medium, or long puff clusters,[114] and ratios of light vs. heavy use sessions (based on puffs per session and puff volume, flow rate, and duration).[113] A recent survey of 979

adult ever JUUL users found daily users report use sessions lasting 3–10 (68%) or >10 (20%) minutes, taking 2–10 (79%) or >10 (19%) puffs.[115] Moreover, ENDS topography may change with user experience, such that experienced ENDS users typically take longer, larger puffs and achieve greater nicotine exposure compared to smokers with limited or no ENDS experience[93,116,117] or limited experience with smoking (i.e., established vs. non-established).[118] Finally, ENDS users have been found to modify topography (i.e., changes to puff duration, puff volume, e-liquid volume consumed) to titrate nicotine exposure based on nicotine concentration[52,93,94,119-121] and ENDS power settings.[59,84] However, studies measuring nicotine delivery in addition to topography note nicotine titration or compensation tends to be incomplete.[79,94] Finally, two longitudinal studies found evidence of nicotine titration and compensatory behavior: ENDS users reported decreased e-liquid nicotine concentrations over time, but increased consumption volume; measured salivary cotinine was maintained.[122,123] Thus, reducing e-liquid nicotine concentrations (e.g., for the means of smoking cessation) may not result in reduced nicotine exposure due to user compensation and use of higher-powered ENDS products.

Puff topography is important to consider because it can directly impact nicotine exposure. Puff duration is known to influence nicotine yield and exposure.[116,124,125] Studies report experienced ENDS users can achieve plasma nicotine concentrations comparable to or higher than those following combusted cigarette smoking (15-20 ng/mL).[72,78,93,120,126] Experienced ENDS users have also been shown to reach similar plasma nicotine concentrations faster (i.e., T_{max}) while using an ENDS than when smoking a combusted cigarette.[96] Adjusting topography patterns and product voltage or wattage settings to achieve desired nicotine levels may predict successful switching to ENDS.[127] Moreover, inexperienced ENDS users are also able to achieve nicotine concentrations similar to combusted cigarettes.[17,93,119,128] Other factors such as sub or above ohm power settings[59,84] and flavors may impact both puff topography and nicotine exposure.[92,129,130] Effects of flavors on topography and nicotine exposure may be partially driven by pH-associated sensory effects.[130] Finally, a number of user and product behaviors may affect topography, including pre-activation button press, initial clearing puff for box-mod users, double puffing, the positioning or angle of the ENDS in the mouth, "mouth wicking", and other behaviors.[107] Moreover, "some device manufacturers incorporate puff duration limiters into their devices, with durations as short as 5 s in some cases, to limit the potential for dry wick and the consequential off tastes and elevated carbonyl emissions".[107]

Several study limitations affect the interpretation of topography results. First, small sample sizes typically limit analyses by product type, nicotine concentration, e-liquid flavor, and other product attributes that may affect topography. Second, studies typically do not characterize study e-liquids; e-liquid labeling is not always accurate. Third, the study cohort is typically homogeneous, limiting generalizability to other users or other product types. Fourth, comparing studies is challenging due to differences in study design (e.g., participant experience with the study product, use of own-brand vs. study brand product or e-liquid, state of nicotine withdrawal, method for recording topography). Finally, some ENDS may not be compatible with topography instruments and researchers would therefore need to use non-instrument-based topography methods to record behavior; video recording and coding of topography and self-report measures capture fewer metrics and are more sensitive to error or bias.

User Experience

Studies suggest ENDS topography may change with user experience, which may impact subsequent nicotine exposure. For example, experienced ENDS users typically take longer, larger puffs and achieve greater nicotine exposure compared to smokers with limited or no ENDS experience.[52,59] Studies demonstrated experienced ENDS users can attain higher C_{max} plasma nicotine concentrations than inexperienced ENDS users.[93,116] Further, some data demonstrate experienced ENDS users' plasma nicotine C_{max} can reach similar levels to those of combusted cigarette smokers,[109,126] which approximate 15–20 ng/mL, and in some cases may be higher than with combusted cigarette smokers.[93,120] In a cross-over study, experienced ENDS users used an eGo style ENDS (3.3V, 1000 mAh battery, 7.3 W cartomizer, 36 mg/mL nicotine e-liquid) for 10 directed puffs, and plasma nicotine concentrations increased by 17.9 ng/mL;[93] some participants' nicotine boost was more than twice the typical nicotine boost associated with combusted cigarettes. In addition, inexperienced ENDS users may also achieve plasma nicotine levels similar to own brand combusted cigarettes.[17,93,119,128] For example, in a cross-over study, inexperienced ENDS users' average plasma nicotine boost from 10 directed puffs on an eGo style ENDS (3.3V, 1000 mAh battery, 7.3 W cartomizer, 36 mg/mL nicotine e-liquid) was 6.8 ng/mL.[93] In another within subject study, inexperienced ENDS users' (n=18) average plasma nicotine concentrations increased from 2.2 (SD=0.7) ng/mL to 9.8 (4.9) ng/mL after 10 puffs and to 11.5 (9.3) ng/mL after 90 minutes of *ad libitum* use of JUUL.[131]

Dual users of ENDS and combusted cigarettes can achieve similar plasma nicotine concentrations to experienced ENDS users, but results are mixed. In one study, nicotine PK was measured in dual users during prescribed and *ad libitum* use of own brand ENDS and combusted cigarettes. Following prescribed use (one puff every 30 seconds, for 15 puffs among "cig-a-like" users and 10 puffs for fixed- and variable-power users), nicotine C_{max} was significantly lower and T_{max} was significantly longer, and systemic nicotine exposure was lower after a single ENDS use compared to a single combusted cigarette use.[132] Following 24-hours of *ad libitum* use, ENDS produced lower nicotine exposure than combusted cigarettes for the majority of users; however, 25% of users were exposed to more nicotine from ENDS than combusted cigarettes (predicted by more frequent ENDS use or greater dependence).[78] In another study with dual users, participants smoked a combusted cigarette or used a JUUL *ad libitum* for 5 minutes and the nicotine PK (C_{max}, T_{max}, AUC) was similar. Compared to other ENDS, JUUL had significantly shorter nicotine T_{max} and higher nicotine C_{max} and $AUC_{0 \geq 30}$ (T_{max}=4 min vs. 6.3 min; C_{max}=28.9 ng/mL vs. 10.6 ng/mL; $AUC_{0 \geq 30}$=366.4 vs. 200.5, number of puffs=12.5 versus 17.0).[96]

Product Misuse and Alternative Use

Product misuse may increase nicotine exposure and e-liquid consumption, thereby increasing abuse potential and exposure to harmful constituents. Changing e-liquid formulation, including nicotine concentration, and ENDS product settings may affect the quality and quantity of aerosol consumed. Additionally, contamination of the e-liquid or unintentional dermal and oral exposure may have negative health effects. Dripping, the process of applying e-liquid to the heating coil of a direct drip atomizer or rebuildable dripping atomizer, and cloud chasing, the process of trying to produce the largest or densest aerosol cloud, are two behaviors with little research on prevalence and resulting exposure. Stealth use, the practice of using ENDS discreetly, especially among youth and young adults, increases abuse liability

by increasing the potential for higher rates of use and thereby nicotine exposure; studies on prevalence and behaviors of stealth use are limited. Other types of misuse, such as modifying e-liquids, refilling a closed ENDS, and modifying software or hardware, require further documentation and evaluation.

Dripping

ENDS users report dripping, the process of applying e-liquid to the heating coil of a direct drip atomizer or rebuildable dripping atomizer, provides greater aerosol yields, stronger throat hit, and a better flavor.[133,134] The higher temperatures involved with this behavior may increase toxicant yield. Moreover, the process of dripping is generally repeated every few puffs to avoid "dry puffs," which produce an aversive taste and may expose users to more toxicants. However, adding more e-liquid to the heating coil can be challenging for users, as too much e-liquid can flood the coils and prevent aerosol production. Aside from one study of high school students in Connecticut that found a quarter of those who have ever used ENDS report dripping,[134] the prevalence of this practice is relatively unknown. The authors concluded additional research in this area is needed to further understand the health impact of dripping products (e.g., rebuildable dripping tank atomizers [RDTAs]) vs. other ENDS), and the prevalence of dripping among ENDS users.

User Modifications

A semi-structured interview of ENDS users who have experience modifying ENDS described some types of modification and their reasons.[135] Participants reported replacing coils with the intent to increase cloud density, adjust nicotine delivery, or alter sensory experiences (e.g., "throat hit"). Participants also reported changing wicks with the intent to reduce exposure to harmful chemicals, adjust the speed of wicking, and alter the taste of the e-liquid. They also replaced batteries for the intended purpose of addressing safety concerns (e.g., rumors of old batteries exploding), greater cloud production, and wattage adjustment (commonly reduced wattage). About half of participants endorsed changing the chipsets (computer chips) in their products to allow for better control over preheating, wattage, and temperature. Finally, e-liquids were modified (refilling closed pods, changing e-liquid flavor and nicotine content, and mixing e-liquids to create novel flavor-nicotine combinations) with the intent to save money, to address health reasons ("organic products"), to optimize flavor and nicotine strength, or to add other substances (e.g., CBD, THC). The authors note users have reported a decline in the prevalence of user modifications due to increased product quality; however, some users (referred to as hobbyists) may continue "more extreme" modification practices (e.g., building their own coils for aesthetic reasons, called "coil art").

Stealth Use

In addition to modifying topography for nicotine titration, some users may modify ENDS topography (e.g., "stealth vaping") in places or social situations where ENDS use is inappropriate or socially discouraged (e.g., school, work); such "stealth vaping" practices seek to reduce a visible aerosol cloud, such as by "deep inhale" (diluting the aerosol with air or letting it dissipate in the mouth or lungs before exhaling), "second inhale" (subsequent inhalation of air to dilute aerosol), or swallowing the aerosol during exhalation.[136,137] Users may also exhale into a backpack, under clothing, or into a napkin or paper towel; or use hoodies and backpacks manufactured to conceal ENDS use.[137] In a survey of experienced adult ENDS users, 64.3% reported ever "stealth vaping," where they modify topography to reduce a

visible aerosol cloud.[136] An online search of "stealth" products and "e-juices" identified ENDS described as "stealthy," "sleek," "mini body," "ultra portable," "low profile," "ultimate discretion," "private," and "discreet" that resemble USB sticks, asthma inhalers, pens or highlighters, car key fobs, mobile phones, and small electronic devices (e.g., remote control, MP3 player, iPod).[137] Some e-liquid characteristics designed to produce low aerosol plumes, such as low odor, high concentrations of nicotine by weight (e.g., 5% nicotine salt), and PG:VG ratio, may reduce detection. It is unknown how "stealth vaping" may affect exposure to nicotine and other toxicants.

Biomarkers of Nicotine Exposure

While cotinine accounts for most urinary metabolites of nicotine, additional metabolites make up the total nicotine equivalents (TNE; often measured as TNE-6).[138] The half-life of nicotine is about 1–2 hours and its metabolite cotinine has a half-life of about 16–19 hours.[139] Long-term studies (i.e., chronic nicotine exposure) typically measure cotinine in blood, urine, or saliva, but some long-term studies also measure total nicotine equivalents (TNE; often measured as TNE-6). Biomarkers of nicotine exposure can be evaluated under lab or in the real-world conditions. This section focuses on the biomarkers of nicotine exposure in three subpopulations: exclusive ENDS users, combusted cigarette smokers, and dual users of ENDS and combusted cigarettes.

ENDS can expose users to high amounts of nicotine.[56,123,140-144] ENDS have a large range of potential nicotine exposures due to variability in product characteristics (e.g., e-liquid nicotine concentration and formulation, pH, power) and user behavior (e.g., puff duration, use frequency, length of use session).[114,145] For example, in a clinical study where experienced ENDS users used own brand ENDS (varying products and e-liquid nicotine concentrations) *ad libitum* for 90 minutes, plasma nicotine C_{max} and AUC_{0-90} values were widely variable (C_{max} varied from 1.6 ng/mL to 29.7 ng/mL; AUC_{0-90}: 65–1669 ng/min*mL).[114] When exclusive ENDS users use newer generation ENDS (i.e., not "cig-a-likes"), nicotine exposure is generally similar to exposures associated with combusted cigarette smoking. For example, an analysis of Wave 1 PATH biomarker data found adult, exclusive ENDS users had significantly lower urinary TNE levels than combusted cigarette smokers,[146] whereas a cross-sectional analysis among American Indian smokers found urinary TNE levels were similar among exclusive ENDS users and combusted cigarette smokers.[50] Mixed results, likely due to differing ENDS types, were observed among dual users: a study, evaluating Wave 1 PATH data, demonstrated dual users had higher TNE levels than combusted cigarette smokers,[52] whereas another observed urinary TNE levels were similar among dual users and exclusive combusted cigarette smokers.[50]

Longitudinal studies that evaluate switching behaviors from combusted cigarette smoking to ENDS use (including dual use) also have mixed findings regarding overall nicotine exposure. A five-day forced switching study to exclusive ENDS (rechargeable blu ENDS) use, dual use of ENDS and own brand combusted cigarettes, and smoking cessation found ENDS users had significantly lower plasma TNE levels than baseline (own brand combusted cigarette smoking), but dual use did not affect overall nicotine exposure.[53] In another study, combusted cigarette smokers were encouraged to use the M201 ENDS (pen-style) for two weeks and refrain from smoking; urinary TNE were not different between baseline combusted cigarette smoking and two weeks of ENDS use,[141] but this study did not evaluate the extent of dual use or its impact on nicotine exposure. However, the literature suggests dual ENDS and

combusted cigarette users have nicotine exposures that are similar to or higher than those of combusted cigarette smokers.[50-52] Study interpretations are limited because switching studies recruit combusted cigarette smokers who are inexperienced with ENDS (which has been shown to affect use behaviors and nicotine exposure).[93] Furthermore, some study protocols that assess product switching may not allow enough time for combusted cigarette smokers to become familiarized with ENDS to adequately represent longer-term nicotine exposure associated with ENDS use. Study interpretations are further limited by the ENDS product characteristics participants used to replace combusted cigarette smoking, as some products may be outdated and not effective at delivering nicotine.

A few studies have measured nicotine exposure in youth or young adult ENDS users and have documented significant nicotine exposure in this vulnerable population. Although findings should be replicated, one study conducted in 2017-2018 found youth, daily exclusive ENDS users had higher urinary cotinine levels than nondaily users (315.4 vs. 1.69 ng/mL); pod users had higher urinary cotinine levels than non-pod users.[97] However, higher urinary cotinine concentrations (769–1202 ng/mL) were documented in a study among older youth, aged 16-20, who used ENDS for at least 10 days in the past month.[147] A study in youth found daily exclusive ENDS users had salivary cotinine concentrations of 93.0 ng/mL and non-daily ENDS users had salivary cotinine concentrations of 19.9 ng/mL; exclusive (daily and nondaily) JUUL users had lower salivary cotinine levels (36.1 ng/mL).[148] One small study evaluated nicotine exposure associated with ENDS pod use among youth and found exclusive nicotine salt pod users had higher urinary cotinine concentrations than previously reported values for youth combusted cigarettes smokers.[10] Furthermore, in a study that examined changes in youth ENDS use behaviors over a 12-month period, salivary cotinine concentrations increased with time,[149] indicating continued ENDS use and greater nicotine exposure with time, although smoking prevalence also increased over time.

Overall, nicotine exposure studies suggest exclusive ENDS users and dual ENDS and combusted cigarette users are exposed to high levels of nicotine and may titrate their use behaviors to achieve nicotine exposures akin to combusted cigarette smokers.[50-56] These data suggest ENDS abuse liability may be generally similar to that of combusted cigarettes.

Additionally, two studies evaluated secondary nicotine exposure. Ballbè et al.[150] analyzed home airborne nicotine and urinary and salivary cotinine levels for non-smokers who lived with combusted cigarette smokers, ENDS users, or people in neither group. They found geometric mean salivary cotinine levels were highest for those who lived with smokers (0.38 ng/mL), followed by those who lived with ENDS users (0.19 ng/mL), and then those who lived with neither group (0.07 ng/mL). A study by Johnson et al.[151] analyzed urinary biomarker concentrations after 34 subjects (19–30 years old who did not use or live with anyone using tobacco products) were asked to attend 4 separate ENDS events (with between 150–1500 attendees) for approximately 6 hours. Findings included statistically significant increases in concentrations of nicotine (urinary and salivary cotinine and urinary trans-3'-hydroxycotinine) and quantified internal dose biomarkers of secondhand exposure to ENDS.

Conclusions for Section 2.A. Nicotine Exposure Data

ENDS can deliver a large range of nicotine and some products can expose users to similar PK profiles as combusted cigarettes. Nicotine delivery and exposure is based on several factors, including user

experience, use behavior, and product characteristics. As a result, nicotine exposure from ENDS use can vary, in rate and extent, to a greater degree than combusted cigarette smoking. Experienced ENDS users have higher plasma nicotine concentrations than inexperienced ENDS users[93,116] and can achieve plasma nicotine concentrations comparable to or higher than those following combusted cigarette smoking (15–20 ng/mL).[72,78,93,120,126] Inexperienced ENDS users can achieve nicotine concentrations similar to combusted cigarettes[17,93,119,128] and sub or above ohm power settings[59,84] and flavors[90,92,129] may impact nicotine exposure. E-liquids with nicotine salts are easier (e.g., less irritating) to inhale at high nicotine concentrations[39,98-100] reach or exceed nicotine exposures and absorption rates associated with combusted cigarettes[10,96] and other ENDS with free-base nicotine formulations.[17,72,97]

B. ABUSE LIABILITY

In addition to nicotine pharmacokinetics, a product's likelihood of continued use and abuse liability can be estimated using measures of reinforcement such as: behavioral economic measures of product purchasing or valuation, subjective effects of a product's use experience, and measures of withdrawal, craving, or dependence upon discontinuation of product use. Self-reported subjective effects (e.g., drug "liking", "satisfaction", willingness to take the drug again) are widely used measures of reinforcing efficacy and abuse liability for drugs. Drug "liking" has been shown to be the most sensitive and reliable subjective effects measure of abuse liability.[152] This measure is typically recorded on a bipolar VAS scale and used during drug self-administration studies. A drug's impact on sensory effects (e.g., sight, smell, taste, and mouth feel), mood, and physical symptoms (e.g., dizziness) may also affect abuse liability and can also be measured on a VAS scale. Generally, drugs with greater positive subjective ratings have greater abuse liability.

Behavioral Measures

Purchasing Tasks

Behavioral economics methods provide a framework for addressing drug reinforcement. Behavioral economic tasks measure amount of consumption or self-administration of an addictive substance across different prices. In the context of tobacco products, researchers have examined consumers' hypothetical tobacco purchase choices, which have been shown to be concordant with real purchase estimates[153] and tobacco product consumption.[154,155] Studies can be designed to compare between products that contain tobacco and nicotine (e.g., ENDS vs. combusted cigarettes; between different types of ENDS; ENDS vs. NRT). The task can be completely hypothetical, where participants complete the task without previous exposure to the study product; alternatively, participants can have prior experience with the product, sample the product during the study, and take one of their choices home for use. Behavioral economics tasks for tobacco products can include a Cigarette or ENDS Purchase Task (CPT, eCPT, Experimental Tobacco Marketplace) and Delay Discounting. These tasks measure an addictive substance's elasticity or sensitivity of changes in consumption relative to changes in price across different products; if an addictive substance is less sensitive to price increases, it is considered to have greater abuse liability.[156]

Behavioral economics studies of ENDS generally support substitutability between ENDS and combusted cigarettes, such that increasing cost (e.g., financial, effort or behavioral) of combusted cigarettes increases the likelihood of switching to ENDS.[157-160] When selecting potential substitutes for combusted

cigarettes in behavioral economics studies, ENDS tend to have a higher acceptability than other tobacco products and NRT.[157-159] However, ENDS purchasing was found to be more elastic than combusted cigarettes purchasing, such that participants were more willing to forego ENDS use at increased prices but would need a bigger incentive (e.g., greater cost increase) to switch from or reduce smoking combusted cigarettes.[157,161] These findings suggest ENDS have a lower abuse liability than combusted cigarettes. Several factors related to ENDS users and products can affect behavioral economics outcome measures, including: 1) past experience with and current use of tobacco products (e.g., combusted cigarettes, ENDS);[159,160,162,163] 2) state of nicotine abstinence or withdrawal vs. satiation during the study;[164] 3) participant gender;[158] and 4) variability in ENDS product characteristics and e-liquid composition, including e-liquid flavors and nicotine concentrations,[159,161] although PG:VG ratios were not found to play a role for smokers with minimal ENDS experience.[3]

These studies have several study limitations. Behavioral economics studies have been conducted primarily among dual users of ENDS and combusted cigarettes or exclusive smokers with limited or no ENDS experience, limiting generalizability of outcomes. Small sample sizes limit the ability to conduct subgroup analyses (e.g., by gender, product experience). Some studies have had challenges with study question comprehension (i.e., determined by nonsystematic responding), unfamiliarity with some tobacco product options, and non-compliance (i.e., when taking the selected products home for use). Generalizability to the marketplace is also limited in study designs with multiple tobacco product options.

Choice Procedure

Choice procedure tasks measure relative reinforcement of an addictive substance and other reinforcers or rewards (e.g., other addictive substances, money). The Multiple-Choice Procedure (MCP) has been used to evaluate preference for addictive substances relative to money in human subjects; this task is well-suited to examine relative abuse liability across different doses and delivery platforms (e.g., ENDS vs. combusted cigarette).[156,165]

In smokers with minimal ENDS use experience, two studies found ENDS had a significantly lower abuse liability than own-brand combusted cigarettes[128,161] and significantly higher abuse liability than a nicotine inhaler.[128] Barnes et al.[161] also found the flavored ENDS had a higher abuse liability than the unflavored ENDS.

In a study of male, experienced ENDS users, own-brand ENDS had a significantly higher MCP crossover point ($1.35) compared to a study ENDS (eGo) filled with own-brand e-liquid at 0 mg/mL ($0.83) and the highest available nicotine concentration ($0.88) and a nicotine inhaler ($0.72).[166]

In a study among youth and young adults (aged 16–20) who were current ENDS users, nicotine level and menthol concentration did not affect MCP values;[147] the authors note their metric of puff number (vs. money) may not be an appropriate unit or dose of measurement in this task.

Data on the effects of PG:VG ratio on abuse liability is limited; two studies show no effects of differential reinforcement (used different ratios and different populations).[3,167] Using an unflavored e-liquid participants (current ENDS users) reported disliking, Harvanko et al.[167] found no difference in reinforcing

effects (i.e., MCP) across PG:VG ratios. Smith et al.[168] recruited smokers with minimal ENDS experience and found minimal effect of PG:VG ratios on product choice; the most popular preference, when available, was to abstain from using the product.

Drug Discrimination

Drug discrimination measures evaluate whether a product, in this case nicotine, has discriminative stimulus (and thereby pharmacologic) effects, indicating differences or similarities in pharmacological mechanisms of action.[156]

In a discrimination pilot study of PG:VG ratios, Schneller et al.[169] found established ENDS users were unable to consistently distinguish among the tested PG:VG ratios. This study has several limitations, including small sample size and issues with product taste (occasional "burnt" taste).

Subjective Effects

Nicotine

Subjective effects, including product liking and satisfaction (i.e., hedonic ratings) related to nicotine, inform tobacco product abuse liability. These measures may assess relative product liking or satisfaction (i.e., in comparison to other tobacco products or NRT) or whether a user feels the tobacco product delivers enough or too much nicotine.

Many studies have investigated the relative hedonic subjective effects of ENDS and find combusted cigarettes are rated higher than ENDS,[76,77,162,170,171] and ENDS are typically rated higher than NRT.[76,77,170] In an abuse liability study of Vuse ENDS with three different nicotine concentrations (14, 29, and 36 mg/mL), participants used own brand combusted cigarettes, the three Vuse study products, and NRT nicotine gum. Mean maximum "liking" and "intent to use again" scores were highest for the own brand combusted cigarette, followed by all three Vuse ENDS, and NRT gum,[76] indicating the abuse liability of these ENDS may lie between combusted cigarettes and NRT gum. Among different ENDS products, "liking" scores may only differ between nicotine-free and nicotine-containing e-liquids;[172,173] ratings between ENDS with different e-liquid nicotine concentrations did not differ.[94,174,175] Although ENDS users had different nicotine exposures associated with *ad libitum* use of 6 and 24 mg/mL e-liquids in a tank-style product, "satisfaction" was not different between the products.[94] A study measuring satisfaction among smokers following use of JUUL, combusted cigarettes, and IQOS found combusted cigarettes scored higher than both JUUL and IQOS.

Subjective measures of effect, including "enough nicotine" and "dizzy," are also rated lower for ENDS compared to combusted cigarettes among adult combusted cigarette smokers[17] and youth tobacco users.[176] Perkins et al.[172] reported moderate nicotine concentration-dependent effects for subjective items related to nicotine intake, including "how much nicotine" and "head rush/buzzed", when participants used ENDS with 0, 12, 24, and 36 mg/mL nicotine. In a cross-sectional sample of youth, Kong et al.[177] found pharmacological effects ("buzz") among the top reasons for liking JUUL among users, and frequency of JUUL use was associated with several pharmacological effects ("buzz", ability to concentrate, nicotine level).

There were several limitations to these studies. First, the extent of ENDS experience and the inclusion of own brand combusted cigarette comparators may influence or affect subjective liking of ENDS. These studies are also limited due to their small sample sizes, open-label design, and limited study populations, which may limit generalizability to other populations. Finally, flavors may mitigate the effects of nicotine's bitter taste.[178,179]

Flavor

E-liquid flavorings can modulate the sensory (e.g., sweetness, cooling, irritation), preference, liking, rewarding, and reinforcing effects of e-liquids, thereby facilitating ENDS use and increasing abuse liability. Flavors can affect topography,[129,130] nicotine exposure,[90] and switching behavior.[180] Additional research on the effect of flavors on reinforcement, exposure, and behavior is important to further understand their potential impact on abuse liability.

Flavors have been found to impact subjective liking and preference scores, and therefore also impact abuse liability. A study comparing reward and reinforcement of flavored and unflavored e-liquids found flavoring enhances the subjective rewarding and reinforcing value of ENDS with nicotine, and thus their abuse liability in young adult smokers.[181] One neuroimaging study found sweet-tasting e-liquid potentiates the reinforcing effects of nicotine in ENDS, resulting in heighted brain cue-reactivity response; however, similar effects were not observed for subjective outcomes (e.g., liking ratings).[182]

The effects of flavors may differ by populations based on age group and smoking history. For example, one survey study found youth ENDS users prefer alcohol, fruit, and "other" flavored e-liquids, whereas adults disproportionately preferred non-sweet flavors (i.e., tobacco, menthol, mint, coffee, spice).[183] Young adult smokers report liking fruit and menthol flavors more than tobacco flavor, and these flavors enhance sweetness and smoothness and reduce the bitterness of nicotine.[184,185] Experienced smokers may prefer the tastes or sensations associated with nicotine delivery (e.g., bitter or sour taste, irritation or throat hit), whereas newer ENDS users may prefer flavors that mask or reduce aversive tastes (e.g., bitterness or sourness) and irritation or throat hit from nicotine content, by increasing perceived sweetness or coolness.[173,178,184] A study measuring effects of aroma (oral, misted administration of nicotine-free e-liquids) in non-established ever-smokers showed fruity aromas enhanced VAS ratings of sweetness, confectionary aromas enhanced ratings of pleasantness and decreased ratings of bitterness, and menthol and burnt aromas had no effect on taste-related outcome measures.[186]

Menthol may have unique effects due to its effects on taste, coolness, and nicotinic receptors. DeVito et al.[179] found menthol but not fruit flavor e-liquids reduced aversiveness of nicotine; fruit but not menthol flavor increased appeal of nicotine-free e-liquid. A study of e-liquid menthol concentration among young (aged 16–20) ENDS users found menthol improves the taste and perceived coolness of ENDS, even at low concentrations.[147] These studies may be limited because a combusted cigarette smokers' preference for menthol vs. non-menthol combusted cigarettes may have influenced subjective effects for menthol vs. non-menthol ENDS. For example, in a study of predominately menthol smokers who sampled three e-liquid flavors (i.e., menthol, burley-tobacco, slim-tobacco), menthol-flavored ENDS scored higher in liking compared to the two types of tobacco-flavored products.[175]

Studies of flavors, ingredients, and subjective effects have several limitations. Study design and study product (e.g., ENDS product characteristics, e-liquid composition) differ among studies, and the e-liquid or ENDS used in the study may differ from participants' own preferred brand. Own brand e-liquid flavors may be preferred over study brand flavors.[90] Comparison products in these studies may not be representative of the marketplace, and the study population may not generalize to other populations (e.g., age, sex, race, tobacco product use history, ENDS use experience); small sample sizes also limit the ability to conduct subgroup analyses (e.g., by demographics, product experience). Nicotine content and absorption may affect sensory perceptions, and studies may not perform e-liquid characterization (labeling may be inaccurate). Finally, short exposure periods may affect product perceptions.

E-liquid Composition

E-liquid ingredients (e.g., PG:VG ratio) can modulate the sensory (e.g., sweetness, cooling, irritation), preference, liking, rewarding, and reinforcing effects of e-liquids, thereby facilitating ENDS use and increasing abuse liability. The PG:VG ratio has been documented to affect topography[89] and nicotine exposure.[80,89] Research on other e-liquid ingredients that may affect reinforcement, exposure, and behavior is important to further understand their impact on abuse liability.

Studies of PG:VG using different ratios (and different populations) report mixed results for sensory and subjective effects. Harvanko et al.[167] used an unflavored e-liquid participants (current ENDS users) reported disliking, and found differences in aerosol cloud visibility (i.e., higher VG content) and sensory effects like throat hit (i.e., more sensation with PG:VG mixture than PG or VG alone), but no differences in liking ("like the effects," "want to use the electronic cigarette again," "enjoy the electronic cigarette").[167] In a study of current ENDS users, Spindle et al.[89] found lower product satisfaction and higher throat hit for the 100% PG liquid. Smith et al.[168] recruited smokers with minimal ENDS experience, and found minimal effect of PG:VG ratios on subjective effects, also reporting stronger throat hit for the highest PG concentration liquid (70% PG) and no difference in aerosol cloud production perception. Studies that systematically vary PG:VG ratio and measure effects on abuse liability and behavior are limited.

Dependence, Craving, Withdrawal

Measures of craving, withdrawal, and dependence reflect a user's psychological and physiological dependence or addiction and are associated with current and future tobacco product use, including cessation outcomes. A substance's ability to suppress craving or withdrawal suggests abuse liability in dependent individuals.[156]

Several questionnaires validated for smoking or nicotine have been modified for ENDS to measure craving, withdrawal, and dependence associated with ENDS (e.g., Fagerström Test for Cigarette Dependence [e-FTCD], Nicotine Dependence Syndrome Scale [e-NDSS], Wisconsin Inventory of Smoking Dependence Motives [e-WISDM], Hooked on Nicotine Checklist [HONC], ENDS Addiction Severity Index (EASI)). Additionally, several new measures have been developed and validated: Penn State Electronic Cigarette Dependence Index (PS-ECDI),[187,188] Patient-Reported Outcomes Measurement Information System (PROMIS) Nicotine Dependence for ENDS dependence (PROMIS-E) for youth and adults, which has been renamed the E-cigarette Dependence Scale (EDS),[189-191] and Questionnaire of Vaping Craving

(QVC).[192] A psychometric evaluation of the PS-ECDI, e-FTCD, and e-WISDM support these measures' validity; all three were strongly correlated with one another and significantly correlated with self-reported addiction to ENDS and number of days used per week.[193]

Surveys of adults also show evidence for ENDS dependence in adult exclusive ENDS users and dual users of ENDS and combusted cigarettes. Moreover, withdrawal symptoms occur when stopping ENDS use for both former[194] and never smokers,[195] supporting that ENDS can establish and maintain dependence. Combusted cigarette dependence scores tend to be higher in exclusive combusted cigarette smokers than dual users of combusted cigarettes and ENDS[143] and exclusive ENDS users;[196,197] dual users report being more dependent on combusted cigarettes than ENDS[198] (but not among all studies[197]). Dual users who reduce their combusted cigarette consumption likely use ENDS to supplement their nicotine intake (e.g., nicotine metabolites were similar between groups of exclusive smokers and dual users).[51,143] ENDS dependence may correlate with more frequent ENDS use[190,198,199] and use of higher nicotine content e-liquids.[190,199] Among long-term ENDS users, ENDS-related dependence (PS-ECDI) was stable over an average of 3.7 years.[200] Population level studies of dependence[197] are unable to account for variability in nicotine delivery across different ENDS and may not be generalizable.

Several studies have evaluated the extent to which ENDS reduce craving and alleviate withdrawal symptoms, and found both combusted cigarettes and ENDS significantly reduce craving and withdrawal symptoms.[53,81,93,94,128,131,164,171] A PATH analysis evaluated the prevalence of withdrawal symptoms among ENDS users and combusted cigarette smokers and found ENDS users reported significantly fewer withdrawal symptoms than combusted cigarette smokers upon quitting or reducing use.[201] When dual ENDS and combusted cigarette users used their own brand ENDS or combusted cigarettes after 16 hours of abstinence, withdrawal symptoms decreased equally; however, combusted cigarettes suppressed the urge to smoke to a greater extent than ENDS.[164] Similarly, in a cross-over study where combusted cigarette smokers smoked an own brand combusted cigarette and used six different ENDS (with limited familiarity) for 10 puffs upon overnight abstinence, all products significantly reduced withdrawal symptoms without differences among products.[81] Studies also observed nicotine concentration-related effects on craving and withdrawal symptoms, such that higher e-liquid nicotine concentrations alleviate more symptoms.[93,128]

Several studies support that youth ENDS users experience nicotine dependence symptoms. Data from a youth sample from Connecticut high schools (n = 520, data from 2017) showed dependence symptoms were associated with characteristics previously shown to confer risk for frequent ENDS use and tobacco combusted cigarette dependence in youth (i.e., being in a higher grade, initiating ENDS use at an earlier age, using ENDS more frequently, using nicotine e-liquid and higher nicotine concentrations, smoking combusted cigarettes).[189] Using data from the Texas Adolescent Tobacco and Marketing Surveillance System survey (April 2016 to June 2016), Case et al.[202] found dual ENDS and combusted tobacco product users (n = 41) reported more dependence symptoms, were less interested in quitting, and were less likely to make a past-year quit attempt than exclusive ENDS users (n = 91). Two studies measured dependence among youth and young adults who were pod-based and non-pod-based ENDS: McKelvey et al.[203] compared ever users of pod-based ENDS, other ENDS, and combusted cigarettes among youth and young adults (survey conducted in California, 2018) and found no difference in mean dependence

scores between pod-based and other ENDS users who endorsed any dependence symptoms. Pod-based ENDS were used on more days in the past month and week than the other ENDS and combusted cigarettes, and use of other ENDS and combusted cigarettes was more common for pod-based ENDS users.[203] In a secondary analysis of youth and young adult ENDS users (survey conducted in Stony Brook Children's outpatient offices, 2017-2018), Boykan et al.[97] found pod-based ENDS users endorsed more nicotine dependence questions (five questions were selected from several established scales; these are not validated for use together), and more endorsement of dependence questions were correlated with higher urinary cotinine levels. Pod-based ENDS users were also more likely to report daily use compared to non-pod ENDS users, and pod users were younger than non-pod users in this sample.[97] Vogel et al.[204] surveyed high school youth at baseline and six months later (n = 444, 2016-2017) and found youth endorsed symptoms on the HONC scale, with presence of one more symptoms associated with higher use frequency, intensity, greater use of other tobacco products, and smoking more combusted cigarettes daily; dependence symptoms at baseline were associated with greater odds of using ENDS at 6 months and more frequent use (i.e., more days, sessions, and puffs per session). Finally, in a study that examined changes in youth (aged 13–18) ENDS use behaviors over a 12 month period, nicotine dependence (PSECDI) and salivary cotinine concentrations increased with time,[149] indicating continued ENDS use and greater nicotine exposure with time.

These studies on ENDS dependence and withdrawal and craving symptoms have several limitations. Unvalidated measures of ENDS dependence (including those modified from combusted cigarette-based questionnaires) may not be strong measures of dependence in a new context. Furthermore, because ENDS experience affects use behavior and exposure,[93,113] product familiarity may impact withdrawal and craving scores, particularly when compared to symptoms associated with combusted cigarettes. Furthermore, small sample sizes limit analyses by demographics and tobacco product history. Generalizability of findings may be limited by convenience samples and recruitment of user subgroups (e.g., exclusive ENDS users, dual ENDS, and combusted cigarette users). Finally, there may be potential response or recall bias and accuracy issues due to the reliance on self-report data, especially among youth;[205] biochemical confirmation of ENDS and combusted cigarette use is not typically done for survey studies.

Conclusions for Section 2.B. Abuse Liability

ENDS are reinforcing and may be suitable substitutes for combusted cigarettes. Product characteristics, including e-liquid flavors and nicotine content and delivery, affect reinforcement. Behavioral economics studies generally support substitutability between ENDS and combusted cigarettes, such that increasing cost (e.g., financial, effort, or behavioral) of combusted cigarettes increases the likelihood of switching to ENDS. ENDS product characteristics, including flavors and nicotine concentration affect reinforcement and subjective liking, and thereby abuse liability. Evidence of ENDS dependence has been found for both adults and youth ENDS users.

C. EXPOSURE TO TOXICANTS OTHER THAN NICOTINE

HPHCs and Other Potential Toxicants Identified in E-liquids and ENDS Aerosols

Much of the published literature on emissions from ENDS has focused on quantifying potentially harmful compounds associated with the use of combusted tobacco products (e.g., volatile organic compounds

(VOCs), formaldehyde, acetaldehyde, acrolein, tobacco specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs)). Although these studies address certain aspects of ENDS emissions, they do not provide a complete picture of ENDS aerosol chemistry. However, more recent studies are broadening the scope the previous work and beginning to identify toxicants specific to ENDS use. The quantity and identity of potential toxicants emitted from ENDS are dependent on highly variable product characteristics, including product type, components, power, and how the device is operated.[1] The toxicant profile of the aerosol depends on several factors, including the ingredients or toxicants in the e-liquid and the transfer efficiency of those ingredients or toxicants to the aerosol.

Carbonyls and Related Compounds

Investigation of HPHCs and other potential toxicants in ENDS aerosols has primarily focused on carbonyls and other thermal degradation products. The NASEM report identifies numerous known and potentially toxic carbonyl compounds detected and quantified in e-liquid aerosol, including formaldehyde, acetaldehyde, acrolein, acetone, propanal, hydroxy acetone, acrylamide, butanal, glyoxal, and methyl glyoxal.[206] Propylene oxide and glycidol are two carcinogenic epoxide intermediates formed during the decomposition of PG and VG to acetaldehyde and acrolein.[207] These two compounds are respiratory irritants, similar to acetic acid and formic acid.[208] Most reports indicate ENDS produce lower quantities of carbonyls and other oxidized organic compounds than combusted cigarettes, however carbonyl output is strongly influenced by the specific characteristics of the ENDS.

Carbonyls can react with hydroxyl-containing compounds such as PG and VG to produce acetals and hemiacetals. Acetals exist in equilibrium with their parent carbonyls and can serve as a pool of carbonyl generating entities. Several papers have reported the presence of hemiacetals and acetals both in e-liquid and in the aerosol.[209,210] Formaldehyde acetals and hemiacetals generated by the reaction of formaldehyde with PG and VG have been detected by NMR in collected ENDS aerosol.[211,212] Erythropel et al. found acetals also form between flavoring compounds, like ethyl vanillin, and PG in the e-liquid and that up to 40% of flavor aldehydes react with PG to form acetals in the e-liquid, with carryover rates into the aerosol ranging from 50–80%.[41]

Carbonyls and other oxidized species in the aerosol arise primarily from the temperature-driven decomposition of PG, VG, and various flavor components. The thermal degradation of PG and VG has been well studied and nearly all low molecular weight carbonyls identified in e-liquid aerosols can arise from reactions involving PG and VG.

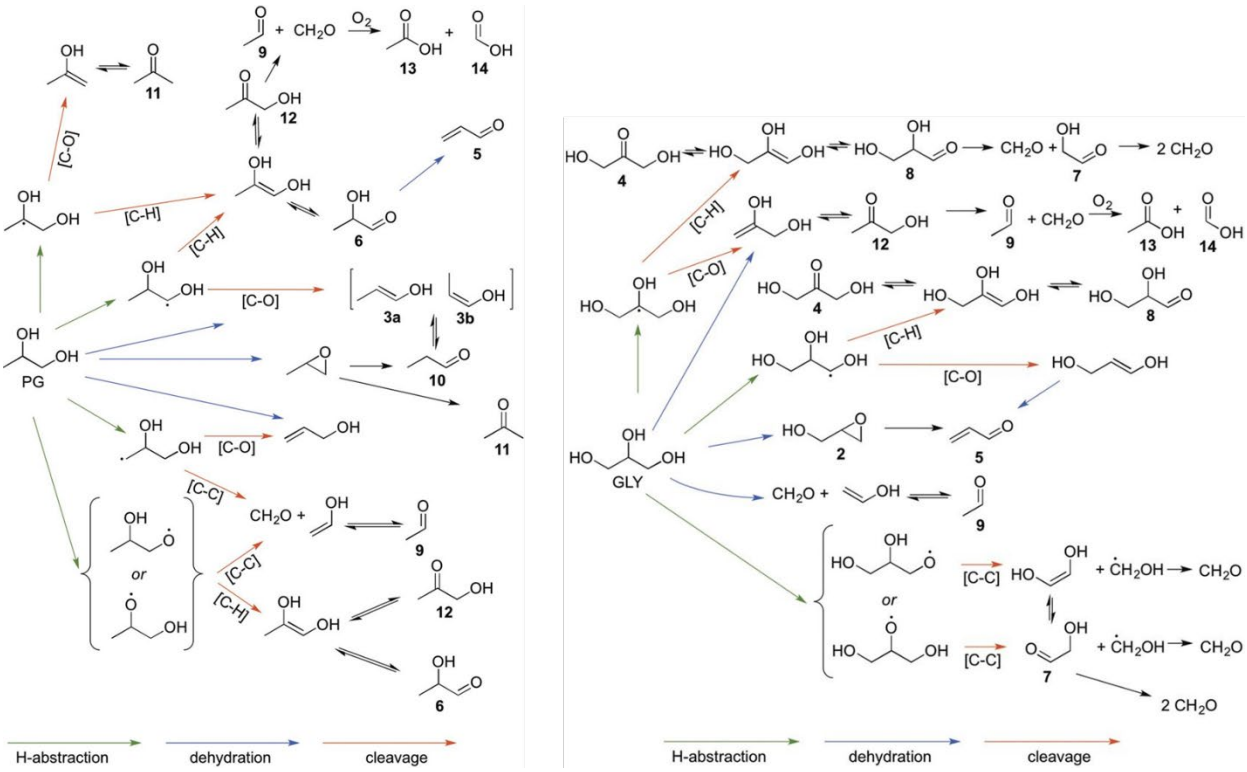

Figure 3. Degradation pathways of PG and VG. (From Jensen et al. 2017)[208]Error! Bookmark not defined.

Both PG and VG can produce the same carbonyls (e.g., formaldehyde), but according to Geiss et al., PG oxidation is involved primarily with the production of acetaldehyde,[66] while Gillman et al. confirmed VG decomposition is responsible for the production of acrolein.[213]

Flavors have also been reported to increase the carbonyl emissions of ENDS. Both triacetin and sucralose have been investigated, and in both cases, increased quantities in the e-liquid resulted in a higher measured quantity of carbonyls in the aerosol.[21,214] Vreeke et al. investigated the triacetin degradation mechanism using carbon-13 labelled triacetin.[214]**Error! Bookmark not defined.** They found when triacetin degrades, it releases acetic acid, which then catalyzes the degradation of both PG and VG. Therefore, the higher the concentration of triacetin, the more acetic acid released, and the more carbonyls formed. In a different study, Duell, et al. found degradation of sucralose results in the release of hydrochloric acid, which can also catalyze the decomposition of PG and VG.[21]

Studies have also reported flavor mixtures increase carbonyl yields. Khlystov and Samburova,[215] Klager et al.,[216] and Qu et al.[217] have all reported increasing quantities of flavoring in the e-liquid generally correlate with higher levels of carbonyls in the aerosol. Gillman et al. report multiple flavored e-liquid formulations, including apple, tropical, tobacco, and coffee flavors, resulted in increases in acetaldehyde compared to non-flavored e-liquid formulations.[218] The effect of these flavors on formaldehyde depended on the flavor formulation. Apple increased formaldehyde yields, tobacco lowered formaldehyde yields, and coffee and tropical flavor did not significantly affect formaldehyde yields.[218]

The flavors did not significantly affect acrolein yields.[218] Thus, the choice of flavor may selectively influence the carbonyl yields produced by ENDS. However, these studies do not evaluate the specific constituents of the flavors to determine which specific compounds may increase carbonyl production. However, esters are common flavor components. It is a plausible hypothesis the hydrolysis of esters during aerosolization of the e-liquid may produce enough acid to catalyze the degradation of PG and VG.

There is general agreement among most studies in the NASEM report that coil temperature has a critical influence on carbonyl production. It can be difficult to accurately measure coil temperature during use. As a result, most studies measure carbonyl production as a function of product power or voltage. Within a given product, increasing voltage or power typically translates into increased coil temperature. Some studies report an increase in carbonyl production with increasing voltage and power, even if only one product was studied.[13,213,219] However, it is difficult to extrapolate these results to different products. Talih et al. suggested using product power divided by the coil surface area as an indirect measure of coil temperature and was able to correlate carbonyl production among products using this approach.[220]

Studies in the NASEM report indicate carbonyl production begins to occur at coil temperatures somewhere above 200°C and rapidly increase as the temperature rises. Additionally, the position of the coil and the wicking rate can increase the possibility of "dry puff" conditions, which can increase aldehyde emissions.[213,221] Acrolein production is reported to occur when the coil temperature reaches 270°C,[222] and formaldehyde production increases dramatically above 350°C.[223] However, two recent studies investigated the nicotine and carbonyl emissions from a JUUL, a known low power product, and found measurable quantities of formaldehyde.[39,224]

Using a controlled pyrolysis reactor, Saliba et al. examined which carbonyls were produced at specific temperatures in the presence of different coil materials.[225] In the reactor, PG vapor was passed across different material coils at different temperatures. The group found when using a Kanthal wire, methylglyoxal and acetaldehyde start forming almost immediately, but the peak quantity is present at 256 °C. In contrast, formaldehyde has two local maxima, and is present in high concentrations at both 256 °C and 560°C, implying there is more than one mechanism by which formaldehyde is present in aerosols. Additionally, the study found the "aging" of a coil is important as well. While new nichrome wires showed the lowest production of all investigated aldehydes under test conditions, the used wires turned black after testing. The experiment was rerun with the blackened, "aged" coils. The aged nichrome produced higher overall aldehyde yields and higher formaldehyde yields at 256°C than any other coil material tested. The limitation of this study is that it did not use an actual ENDS or e-liquid.

TSNAs and PAHs

Pharmaceutical grade nicotine may be used in e-liquid manufacturing. Pharmaceutical grade nicotine has strict levels of allowed impurities, including TSNAs, which may contribute to why lower levels of TSNAs are reported in e-liquids.[226] A few studies reported low levels of N-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), minor tobacco alkaloids, and PAHs in e-liquids.[227,228] One study evaluated 105 refill e-liquid brands purchased from 11 ENDS companies in the Korean market and detected TSNAs in the concentration ranges of 0.34–60.08 µg/L.[228] The same study also reported concentration ranges of NNN (0.22–9.84 µg/L), NNK (0.11–11.11 µg/L) and N-

nitrosoanabasine (NAB; 0.09–62.19 µg/L). Another study evaluated three e-liquids and showed only NAB was found at trace levels in two of the commercial e-liquids (1.2 and 2.3 ng/g), while the third e-liquid contained both 1.5 ng/g NAB and 7.7 ng/g NNN.[229] TSNAs and certain PAHs are reported to transfer from the e-liquid to the aerosol, but measured aerosol levels of these compounds are typically much lower than those in combusted cigarette smoke, and may be below the LOD. [6,227]**Error! Bookmark not defined.** More recent studies investigated TSNA aerosol yields from ENDS, but they do not provide a large or broad enough sample size to make substantive conclusions.

TSNAs are produced from naturally occurring tobacco alkaloids during the curing process. PAHs are primarily produced during combustion of tobacco, but can be introduced during tobacco processing if the processing involves exposure to smoke or combustion products. TSNAs and PAHs are not expected to form during aerosolization of e-liquids, but, as noted earlier, can transfer to the aerosol if they are present in the e-liquid.[6,227] The nicotine extract may be the most likely source of PAHs and TSNAs detected in the e-liquid or aerosol.

VOCs

PG and VG are readily volatilized and are typically the most abundant organic compounds in the aerosol. The relative composition of PG and VG in the aerosol is approximately that of the e-liquid. Studies evaluated in the NASEM report identified several VOCs in e-liquid samples including benzene, toluene, xylenes, styrene, and phenolic compounds.[230,231] Volatile and semi-volatile compounds commonly used in flavorings such as ethyl acetate, ethanol, methanol, pyridine, acetylpyrazine, and 2,3,5-trimethylpyrazine have also been identified in e-liquid aerosols. Furans such as furural and 5-hydroxymethylfurfural have been identified in the aerosol of e-liquids sweetened with sugars.[232] Except for flavoring components, volatile organics were generally quantified at levels in the nanogram (ng) to low microgram per milliliter (µg/mL) range in the e-liquids tested, and are measured in the aerosol at levels lower than those typically seen in combusted cigarette smoke.

With regard to possible combustion products as a potential source for aerosol VOCs, a recent report by El-Hellani et al. found more powerful sub-ohm products can produce carbon monoxide (CO) and small hydrocarbon gasses (e.g., methane, ethylene, and acetylene) in the aerosol.[233] Wagner et al. investigated the presence of combustion-related HPHCs in e-liquids and the associated aerosols produced by low power devices (i.e., “cig-a-likes”).[6] They did not detect any of the nine compounds investigated (3 aromatic amines, 5 VOCs, and B[a]P) in the commercially available e-liquids or the associated aerosols.

VOCs in ENDS aerosol are a combination of volatile ingredients and the thermal degradation products of e-liquid constituents. As with carbonyl production, coil temperature plays a significant role in VOC generation. Currently, potential thermal degradation products for most e-liquid components are not fully identified. Coil material and the degree of coil contamination appear to affect VOC production, but there is insufficient data to understand the extent to which these attributes contribute.

Metals

E-liquids and e-liquid aerosol have been reported to contain metals including nickel (Ni), chromium (Cr), cadmium (Cd), Lead (Pb), copper (Cu), aluminum (Al), tin (Sn), and manganese (Mn). Olmedo et al. showed the metal content of e-liquid is higher after the e-liquid is in contact with the atomizer and tank, suggesting the source of metals in the e-liquid is the metallic components of the product.[234] Olmdedo et al. also showed metals may transfer into the aerosol if present in the e-liquids. The concentration of a metal in the aerosol may even exceed that in the e-liquid itself.[234,235] The concentration of metals in ENDS aerosols varies within and between brands.[1]

The most likely source of metals from the device is the atomizer assembly,[236] including the heating coil,[29] the batteries, and solder joints.[235] Metals can leach passively into the e-liquid during storage or be released actively from the coil during heating. The specific e-liquid and metal components that promote the dissolution of metals has not been studied in detail. Most solubilized metals have low volatility, especially in the ionized form. The appearance of metal in the aerosol is likely the result of boiling at the surface of the coil causing bulk e-liquid containing the dissolved metal to be thrown into the aerosol. Product designs that promote the boiling of e-liquid may therefore facilitate the transfer of any dissolved metals to the aerosol. One study investigating two low-powered pre-filled ENDS and two high-powered refillable tank ENDS found as the power output increases, the metal concentration increases in the aerosol.[237] The higher power ENDS had higher metal yields in the aerosols than the two lower power ENDS. In addition, Zhao et al. [237] found levels of arsenic, tungsten, and uranium in the e-liquid and the aerosol were comparable, suggesting an efficient transfer rate. However, the levels in both matrices were close to the limit of detection, making the transfer efficiency data difficult to interpret. Contrary to Olmedo et al. and Zhao et al., Palazzolo et al. reported the transfer efficiency of metals from e-liquids to aerosol are low.[238] The quantity of metal in the aerosol may be generally dependent on the quantity leached into the e-liquid, but the exact relationship is unclear.

Flavors

The flavor constituents used in e-liquids are typically approved for use in food. However, there is limited information regarding the inhalation toxicity of some flavor components. The NASEM report states many known respiratory toxicants or irritants such as diacetyl, acetyl propionyl, cinnamaldehyde, benzaldehyde, and eugenol are commonly identified in e-liquids and aerosol.[1] The inhalation toxicity of many other structurally similar flavor components have yet to be fully evaluated. As discussed previously, flavors may increase the levels of carbonyls and other thermal decomposition products in the aerosol, although it is unclear whether this is a direct or indirect effect. One study by Moldoveanu et al. investigated the presence of harmful flavor compounds like diacetyl and acetyl propionyl in e-liquids and associated aerosols and found no detectable levels.[239]

Microbial Contaminants

A study published in 2015 evaluated 14 brands of refill e-liquids for the presence of microorganisms (total aerobic microbial count [TAMC], and total yeast and mold count [TYMC], *Staphylococcus aureus* and *Pseudomonas aeruginosa*). Results showed TAMC and TYMC in all samples were ≤ 1 colony forming unit (cfu)/mL and they all tested negative for *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[240] A

study published in 2019 showed cartridges ("cig-a-likes") and e-liquids (refillable e-liquid bottles) are contaminated with microbial constituents such as bacterial endotoxins and fungal cell wall constituents.[241] Endotoxins, part of the outer membrane of gram-negative bacteria, and [1→3]-β-D-glucan (glucan), a fungal cell wall constituent, may result in increased risk to public health because they have been associated with development of respiratory symptoms, reduced lung function, inflammation, or asthma.[241] The study assessed a total of 75 products (37 "cig-a-likes" and 38 e-liquid bottles) with the highest nicotine content, including four flavors (tobacco, menthol, fruit and other), from the 10 top-selling brands in the United States. Results showed endotoxin concentrations were over the LOD (0.1–1.6 endotoxin units [EU]/mL) in 17 of 75 products tested (23%), and glucan concentrations were greater than LOD (0.0125–0.2 ng/mL) in 61 of 75 products (81%). The study also reported the glucan concentration in cartridges was 3.2 times higher compared to the e-liquid samples and glucan concentrations in tobacco- and menthol-flavored products were 10.4 and 3.5 times higher than concentrations found in fruit-flavored products. E-liquid cartridges contain wicks made of cotton or other fibers,[242] and endotoxins and glucans are biological contaminants of cotton fibers.[243] Thus, contamination of cartridge wicks may be a source of endotoxin and glucan contamination and might contribute to higher concentrations of glucans in cartridges than e-liquids. Lee and Christiani analyzed 54 JUUL pods, including eight flavors (Virginia tobacco, classic tobacco, menthol, cucumber, fruit, mango, mint, and crème), to determine the presence of endotoxins and glucan. Results demonstrated that endotoxin levels of all tested samples were below LOD. Nevertheless, glucan levels were over the LOD in 46% of the samples. The mean concentrations of glucan in all tested JUUL products were 0.14 ng/mL (range 0.03–86.30 ng/mL). This study also concluded the glucan levels in tobacco and menthol-flavored products were substantially higher (307 and 1,353 times higher, respectively) than other flavored JUUL pods tested in this study.[244]

Other Potential Toxicants

Several studies have investigated the presence of different free radical species in ENDS aerosols, and found them to be present, but not enough research has been done to evaluate how the quantities in ENDS aerosols may affect the user.[224,245,246] Another report found sucralose in e-liquids degrades into potentially toxic compounds, such as (±)-3-Chloro-1,2-propanediol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP), which transfer into the aerosol, even at relatively low temperatures.[247]

An additional source of potential toxicants from ENDS is the materials leached from the container closure system (the cartridge, capsule, or refill container) into the e-liquid. Oh and Shin reported the presence of two plasticizers, diethyl phthalate (DEP) and diethylhexyl phthalate (DEHP), in replacement e-liquids.[248] A follow-up paper by Wei et al. modified Oh and Shin's methodology to investigate six other phthalate plasticizers and organophosphate flame retardants in e-liquids.[249] Wei et al. found dimethyl phthalate (DMP), an irritant, and DEP in 80% and 35% of the e-liquids tested, respectively. However, DEHP was not observed in any e-liquid. A third study examined multiple methodologies to measure plasticizers in e-liquids, including GC-MS, GC-MS-MS, LC-UV, LC-MS, and LC-MS-MS.[250] Of these methods, GC-MS-MS had the best sensitivity with the lowest limit of quantification (LOQ). However, the number of plasticizers measured were limited, as heavier plasticizers such as diphenyl, diisononyl, and diisodecylphthalates are not volatile. LC-MS and LC-MS-MS were the best methods for plasticizers not

measurable by GC-MS-MS. DEP, DEHP, dibutyl phthalate (an irritant), and dimethyl terephthalate (an irritant) were reported in 10–64% of the 39 e-liquids examined. The heavier phthalates were below the LOD for all 39 e-liquids.[250] One limitation is none of the studies examined how efficiently these chemicals transfer into aerosols. The findings from these three studies demonstrate the importance of examining leachable and extractable data for e-liquids to determine which chemicals can leach into e-liquids and their risk potential with time.

Computational Exposure and Toxicity Modeling

Several computational modeling studies evaluated the intake and particle deposition of inhaled ENDS aerosol compounds, including PG, VG, and nicotine; nicotine physiologically based pharmacokinetic (PBPK) modeling; an exposure assessment for pulegone, a cancer risk assessment for arsenic; and modeling of toxicity based on in vitro and clinical studies. Similarly, a study by Frati et al. (2020), used machine learning to identify homogenous clusters within larger data sets, drawing conclusions from two separate acute clinical studies.[251]

Jabba and Jordt (2019) estimated risks associated with pulegone content in mint/menthol-flavored ENDS and smokeless tobacco compared to combusted menthol cigarettes.[252] Pulegone exposure is associated with species- and sex-specific carcinogenesis in rodents and is classified by IARC as a possible human carcinogen (2B). Daily pulegone exposure from ENDS compared with combusted menthol cigarette was higher across all investigated user groups. Estimated pulegone intake from smokeless tobacco was higher in light and heavy users when compared with combusted menthol cigarette. The modeled effect using mathematical calculations identified potential adverse health risks associated with pulegone intake from tobacco products. However, this calculation differs from human data on the amount of pulegone absorbed and metabolized by ENDS users and needs further validation.

Kovar et al. (2020) developed a multi-pathway PBPK/pharmacodynamic (PD) model that describes nicotine PK in humans via four different routes of exposure, including intravenous, inhalation (combusted cigarettes and ENDS), oral (nicotine gums), and transdermal (nicotine patches), using the commercial software PK-Sim and Mobi (for dermal exposure).[253] The PBPK model used nicotine and cotinine PK profiles reported in 34 clinical studies for calibrating and evaluating model performance. The PBPK model also included the ability to predict brain tissue nicotine concentration and PK differences via inhalation vs. transdermal routes, as well as comparing normal vs. poor CYP2A6 metabolizers. Interindividual variability was estimated using Monte Carlo simulations of a virtual population of 100 individuals. The PBPD arm of the model describes internal nicotine dosimetry in relationship to heart rate and circadian rhythm in humans and that the cardiac effect was better linked to plasma nicotine concentrations than the heart or brain concentrations. Overall, the use of a commercial software that included assumptions and methods that are not readily available to the public limit the usage of this model. Without the access to the software built-in database and a clear illustration of the assumptions used and the supporting scientific data, it is impossible to fully evaluate the model and therefore its application is limited.

A cancer risk assessment study by Liu et al. (2020) focused on levels of arsenic species in 17 e-liquids (0–5% nicotine) in Canada and China based on market share.[254] Aerosols generated from eight e-liquids

were condensed for analysis. Six known arsenic species (including inorganic arsenate, arsenite and monomethylarsonic acid) and three new arsenic species not yet identified were detected. The authors estimate that ENDS users could inhale approximately 4 mg/m^3 inorganic arsenic species which is approximately half the permissible OSHA exposure limit (10 mg/m^3). The authors used this information to estimate lifetime cancer risk and noted a potential increased risk to develop lung cancer (1.5×10^{-4}), which is 150 times higher than the US EPA's goal of one per million risk.

Marescotti et al. (2020) evaluated a layered framework comprising of real-time cellular analysis, phenotypic high-content screening studies, and gene expression analysis, to evaluate the potential impact of e-liquids and their corresponding aerosols.[255] Primary NHBE cells from one donor were treated with e-liquid (40:40 PG:VG, 0.6% nicotine, and up to 5.7% for the flavoring compound) for 30 minutes to 24 hours. Computationally derived scores were used to quantify toxicity, phenotypic effect, and impact on the transcriptome, post exposure. Based on this modeling, citronellol was identified as the most cytotoxic flavor compound. The authors recommended screening flavoring substances individually and in mixtures for a more accurate assessment of their toxic effects.

Pourhashem et al. 2020, developed a computational model for investigating inhaled ENDS aerosol transport and deposition in the human upper respiratory tract.[256] Air flow properties, including velocity, temperature, and concentration of the aerosol constituents for the liquid and vapor phases were predicted. Results demonstrated low liquid deposition for the aerosol constituents, including glycerol, nicotine and PG compared to aerosol deposition. Simulations also indicated low vapor concentration for glycerol resulted in the lowest total deposition of this constituent compared to nicotine and PG. Although this is a preliminary in silico study, the authors' computational approach may eventually become a more practical method to estimate depositions of aerosol constituents in the upper respiratory tract, when compared to clinical studies.

Computational modeling, such as particle deposition modeling integrated with PBPK and in silico predictive toxicology has become a valuable tool for the estimation of internal dosimetry of ENDS aerosols in the respiratory tract and target tissues/organs, and evaluation of potential toxicity of compounds based on structure, metabolism, and activity.

Biomarkers of Exposure

Tobacco use, including ENDS use, exposes users to nicotine and various HPHCs in combusted cigarette smoke and ENDS aerosols. These substances and their metabolites can be measured in human biofluids (e.g., blood, urine, saliva, exhaled breath) as biomarkers of exposure (BOE). Because data regarding the long-term health effects of ENDS are currently limited, data from clinical studies that measure changes in systemic BOE can serve as intermediate outcomes to the possible health effects of ENDS use. Biomarker studies may also provide information regarding actual use behavior (e.g., exclusive use, switching or reduction in smoking, dual or poly tobacco use, misuse) and consumption patterns (e.g., use frequency of use, amount consumed, topography) across various user populations. Populations of interest for ENDS use include current exclusive combusted cigarette smokers, exclusive ENDS users, individuals who smoke combusted cigarettes and use ENDS concurrently (i.e., dual users of combusted

cigarettes and ENDS), poly-tobacco users, and individuals who do not use any tobacco products (i.e., non-users).

Combusted cigarette smokers are exposed to numerous HPHCs found in combusted cigarette smoke including nicotine and tobacco alkaloids, CO, TSNAs, PAHs, VOCs, carcinogenic aromatic amines, and metals.[257] Several of these HPHCs have also been detected in ENDS aerosols, though at much lower levels than those measured in combusted cigarette smoke,[258,259] suggesting ENDS may expose users to fewer HPHCs and thereby reduce BOEs for smokers switching to ENDS. However, due to the wide variety of ENDS and e-liquids, some ENDS may also expose users to HPHCs that are semi-specific to ENDS use, such as those compounds formed when propylene glycol or flavorants are heated and metal-containing ENDS parts undergo corrosion.[260] Therefore, in addition to measuring reductions in BOE associated with combusted cigarette smoking, it is important to identify and measure BOE that may be specific to ENDS use.

Studies that measure changes in systemic levels of BOE when combusted cigarette smokers switch to ENDS may allow for inferences to be drawn regarding whether switching to ENDS impacts the health of combusted cigarette smokers and dual users. For example, if combusted cigarette smokers who switch to ENDS experience significant reductions in BOE, then switching to ENDS may provide a health benefit for combusted cigarette smokers through reduced exposure to HPHCs. However, if combusted cigarette smokers have no change in BOE after switching to ENDS, then ENDS use may provide no reductions in exposure to HPHCs. Combusted cigarette smokers who switch to dual use (i.e., concurrent use of combusted cigarettes and ENDS) represent the majority of adult ENDS users;[146] however, the impact of switching to dual use on users' exposure to HPHCs and corresponding BOE has not been thoroughly investigated. Furthermore, dual use is not well defined in the literature. Broadly, dual users may include smokers who use ENDS and reduce their cigarettes per day (CPD), or, smokers who use ENDS but do not reduce their CPD. For dual users who reduce their CPD, it is unclear if reductions in CPD reduce BOE or provide health benefit. For example, even light and social smokers continue to have similar risk for cardiovascular disease as daily smokers[261-263] and they continue to have increased risk of cancers and all-cause mortality.[264] Dual users, who maintain their CPD, may be exposed to HPHCs associated with combusted cigarette smoking and HPHCs detected in ENDS aerosols, possibly resulting in higher levels of BOEs in dual users compared to exclusive combusted cigarette smokers or exclusive ENDS users.[146] Altogether, switching studies may provide understanding of how BOE levels change when combusted cigarette smokers switch to exclusive ENDS use or dual use. Additional studies are important to assess changes in BOE when combusted cigarette smokers switch to exclusive ENDS use or dual use.

Based on the results of studies published to date, the biomarkers of exposure for the following constituents and toxicants are important for understanding user exposure to ENDS: TSNAs, VOCs, heavy metals, and propylene glycol.

TSNAs

TSNAs are in tobacco smoke[265,266] and TSNAs and their metabolites are used to measure tobacco use and exposure[267-269] Many TSNAs are carcinogenic and contribute tumorigenesis in the lung, esophagus, liver, pancreas, and oral cavity of animals and humans.[270,271] The most well-studied TSNAs include NNK, a

potent lung carcinogen, and NNN, an esophageal carcinogen.[271,272] NNAL (4-(methylnitrosamino)-1–(3-pyridyl)-1-butanol), the primary metabolite of NNK, is a particularly useful BOE because it has a relatively long half-life of 10–45 days in biological fluids, is tobacco-specific, and is itself a lung carcinogen, thereby making it an indicator of tobacco exposure and cancer risk.[273] The glucuronides of NNAL and NNN, including NNN-glucuronide, NNAL-N-glucuronide, NNAL-O-glucuronide, have been measured in the urine of combusted cigarette smokers and smokeless tobacco users.[269,274] While other TSNAs including NAB and N'-nitrosoanatabine (NAT) have been quantified in human urine unchanged,[274-276] and their byproducts have been measured in humans (i.e., NNN-glucuronide, NAT-glucuronide, NAB-glucuronide), these are not as well characterized as NNAL and their biological half-lives have not been established.[274-276]

Studies comparing biomarkers of TSNA exposure in exclusive combusted cigarette smokers, exclusive ENDS users, dual users of ENDS and combusted cigarettes, and non-users show exclusive ENDS users have higher levels of TSNAs relative to non-users[146] and lower levels of TSNAs relative to exclusive combusted cigarette smokers and dual users of ENDS and combusted cigarettes.[71,146,277-279] For example, a cross-sectional analysis of data from Wave 1 of the PATH study (2013-2014) found exclusive daily ENDS users had higher levels of four TSNA biomarkers (NNAL, NAB, NNN, NAT) relative to non-users; however, exclusive ENDS users had lower levels of TSNA biomarkers relative to daily combusted cigarette smokers and dual users.[146] Notably, dual users of ENDS and combusted cigarettes had the highest levels of urinary NNAL, NAT, and NAB.[146] These results may be explained by the comparable cigarette consumption across dual users (15.1 CPD) and exclusive combusted cigarette smokers (15.4 CPD). Another cross sectional-analysis found exclusive ENDS users and users of nicotine replacement therapy (NRT) had significantly lower levels of urinary NNAL compared to exclusive combusted cigarette smokers, dual users of combusted cigarettes and ENDS, and dual users of combusted cigarettes and NRT;[279] no differences in urinary NNAL were observed across exclusive combusted cigarette smokers and dual users of combusted cigarettes and ENDS.[279] Another study discovered similar levels of NNAL for dual users (ENDS and combusted cigarettes) and exclusive combusted cigarette users in hair samples taken from 76 pregnant women.[280]

Exclusive ENDS users are exposed to lower levels of TSNAs relative to combusted cigarette smokers and dual users of ENDS and combusted cigarettes;[71,146,277,278] however, exclusive ENDS users' exposure to TSNAs is higher than that of non-users.[146,278] When combusted cigarette smokers switch to exclusive ENDS use, they experience significant reductions in TSNA biomarkers;[51,140-142,281] however, when combusted cigarette smokers switch to dual use, their exposure to TSNAs remains the same[51] or they experience low to modest reductions in TSNA biomarkers.[140] More research is important to understand whether dual users (of ENDS and combusted cigarettes) experience increases,[141] decreases,[140] or comparable levels of TSNA biomarkers[51,279] compared to exclusive combusted cigarette smokers. Further, because the number of cigarettes per day a dual user smokes likely influences exposure to TSNAs, additional studies are important to further understand the relationship between combusted cigarette consumption and TSNA exposure in dual users.

Goniewicz et al.[10] calculated urinary cotinine concentrations for 22 adolescents who had used JUUL or similar pod systems in the previous seven days. They found these individuals had median cotinine levels

higher than levels previously reported for adolescent established combusted cigarette smokers. They also reported these young people had very low levels of NNAL.

VOCs

VOCs are in tobacco smoke as well as in ENDS aerosol; however, there are multiple other sources of VOCs including food, environmental, and work-related exposure, which make measurement of VOC metabolites in urine nonspecific to tobacco exposure. The health effects of VOC inhalation particularly associated with smoking and using ENDS have been studied; however, due to various sources and numerous toxicities of VOCs, a large amount of the literature describes their pharmacology and health effects in the other populations not related to tobacco use. Several VOCs cause cardiovascular and lung damage, and some are known or suspected respiratory carcinogens;[271,282] therefore, reductions in users' exposure to VOCs may represent a reduction in risk of harm and of disease development.

Acrolein and other carbonyl compounds (formaldehyde, acetaldehyde, crotonaldehyde) form during heating of VG or glycerol-derived fats (e.g., triglycerides), making VOCs biomarkers of particular interest for ENDS that contain PG and VG in their e-liquids.[260,283]

Biomarkers of acrolein (AC) exposure likely reflect a combination of inhaled AC from tobacco smoke or aerosol and endogenous inflammatory responses and lipid peroxidation. The levels of AC metabolite, 3-HPMA[284] was correlated with increased risk of cardiovascular disease, and numerous studies have linked AC exposure to dyslipidemia, platelet activation, and thrombosis, which are well-known risk factors for cardiac and cerebral complications.[285-287] Acrylonitrile (AN) metabolizes in humans to 2-cyanoethylmercapturic acid (CYMA, also abbreviated as CNEMA or CEMA). AN is acutely toxic to humans at relatively low levels; however, prolonged exposures were not associated with the increased cancer risk.[288] Acrylamide (AA) produces AA mercapturic acid (AAMA) and AA epoxide glycidamide (GAMA). A recent review identified the link between AA exposures and increased risks of ovarian, endometrial and breast cancer,[289] and it is neurotoxic effects studied in animals[290] and humans with possible effect of the peripheral nervous system with prolonged exposure even at low doses.[291] When combusted cigarette smokers switched from smoking to ENDS use, the crotonaldehyde metabolite 3-hydroxy-1-methylpropyl mercapturic acid (HMPMA, sometimes abbreviated as HPMMA) decreased, although it did not decrease in dual users.[140] Crotonaldehyde is recognized as a carcinogen, and it shares some toxicity with AC.[146,292] In an observational study, a propylene oxide metabolite (2-HPMA) was found in higher levels in urine of ENDS users than in non-users but lower than in dual users,[278] it was lower in ENDS users than in smokers and similar to the abstention arm.[293] At high temperatures, which might be encountered in some ENDS, propylene oxide could be formed from propylene glycol[277] and it is classified as a possible carcinogenic by IARC.[271] Ethylene oxide, acrylonitrile, and vinyl chloride metabolize to 2-Hydroxyethylmercapturic acid (HEMA) that was detected in urine of ENDS users in concentrations similar to NRT users, and lower than in dual combusted cigarette and ENDS users.[146] Exposures to ethylene oxide is associated with lymphohematopoietic and breast cancers.[140,278,279] Several other VOCs (benzene, ethylene, 1,3-butadiene) metabolize to HEMA, and have been in urine of ENDS users in small concentrations similar to the abstention arm.[293]

Rubinstein et al.[278] compared urinary and salivary biomarker levels in adolescents studying 67 exclusive ENDS users, 16 dual combusted cigarette and ENDS users, and 20 never-users of combusted cigarettes and ENDS. They found exclusive ENDS users had lower benzene, ethylene oxide, acrylonitrile, acrolein, and acrylamide levels compared to dual users and higher acrylonitrile, acrolein, propylene oxide, acrylamide, and crotonaldehyde levels than non-users. St. Helen et al.[293] conducted a two-arm counterbalanced, cross-over study in 36 healthy dual users of ENDS and combusted cigarettes and measured urine metabolites of acrolein, acrylamide, acrylonitrile, 1,3-butadiene (MHBMA-1+2), 1,3-butadiene (MHBMA-3), benzene, crotonaldehyde, ethylene, methylating agents, propylene oxide. ENDS users in the study were found to have lower levels of measured VOCs and metabolites compared to users of combusted cigarettes in the study. However, ENDS users in the study with the highest levels of the benzene metabolite S-PMA, were found in low-powered ENDS users and users of fruit and tobacco flavors.

Urinary concentrations of 3-HPMA, CYMA, AAMA, GAMA, 2-HPMA, HMPMA, HEMA and SPMA were significantly lower in the urine of ENDS users compared to combusted cigarette smokers.[140,146,277,279,293] In a few published studies comparing ENDS users, combusted cigarette smokers, and dual users of ENDS and combusted cigarettes, the urinary levels of BOE for VOCs resembled those found in combusted cigarette smokers or were found to have low to modest reductions.[286] Although systemic exposures of BOE of VOCs in ENDS users are often lower than in combusted cigarette smokers, VOCs in ENDS users are detectable and present at levels higher than in non-smokers, and the potential harms of these exposures are not yet clear. Because the urinary concentrations of BOE of ethylene oxide, vinyl chloride, 1,3-butadiene, propylene, propylene oxide and benzene were found at trace levels in ENDS users,[140,293] additional studies are important to further understand these BOE for ENDS use.

Heavy Metals

Because of the various other sources of exposure to metals (e.g., food, water, pharmaceutical and dental products), metals are non-specific to tobacco use. Nevertheless, human exposure to metals from tobacco products is important as some metals have deleterious effects on the body and can cause acute and chronic toxicity in humans.[294] Heavy metals such as arsenic, cadmium, chromium, lead, and mercury are toxic, are known to induce damage to multiple organs even at low concentrations, and are classified as IARC known or probable human carcinogens.[271,294-296]

Importantly, one study showed a correlation between the presence of nickel and chromium in ENDS aerosol and human exposure to these metals.[297] Metals including cadmium, lead, strontium,[146,298,299] chromium, nickel,[297] selenium, and zinc[300] have been detected in the biofluids of ENDS users. ENDS users are exposed to higher levels of metals such as cadmium and lead compared to non-users.[146,298] Cadmium exposure in exclusive ENDS users is lower than[146] or comparable to that of combusted cigarette smokers and dual users.[298,299] Although more research is needed, one study shows exclusive ENDS users are exposed to higher levels of chromium and nickel compared with dual users of ENDS and combusted cigarettes.[297] A more recent study found male ENDS users had elevated blood cadmium levels compared to non-smokers, which the authors speculated is the result of leaching from the atomizer chamber.[301] Lead exposure is similar across exclusive ENDS users, combusted cigarette smokers, and dual users.[298,299] Daily ENDS users are exposed to significantly higher levels of strontium compared to 'someday' ENDS

users and daily combusted cigarette smokers.[146] However, data combining 'daily' and 'someday' users show no differences in strontium levels across non-users, ENDS users, and combusted cigarette smokers; compared with these groups, dual users show the highest levels of strontium exposure.[146] Additional studies are needed to determine whether combusted cigarette smokers and dual users experience significant reductions in metal exposure when switching to ENDS. Further, many ENDS users are former combusted cigarette smokers and because some metals have long biological half-lives (e.g., cadmium, lead), additional studies are important to discern whether exposure to some metals is due to ENDS use or prior combusted cigarette smoking.

Propylene Glycol

PG is a primary constituent of e-liquids and serves as a carrier for nicotine and flavorants.[260] PG and VG comprise as much as 95% of e-liquids;[302] consequently, high concentrations of PG and VG are present in ENDS aerosols.[44,303] Despite the presence of VG in ENDS liquids and aerosols, the studies conducted to date, suggest VG is unlikely to be viable as a BOE for ENDS use as urinary since plasma VG levels do not increase in ENDS users following use.[304]

Pharmacokinetic assessment of PG in rats and dogs show absorption of PG following pulmonary inhalation occurs rapidly, and inhalation of PG produces high systemic concentrations of PG.[305] In humans, PG is metabolized to D- and L-lactic acid and further into D- and L-lactate; L-lactate occurs endogenously whereas D-lactate is suggestive of exposure to PG.[282,306,307] In humans, PG is metabolized to D- and L-lactic acid and further into D- and L-lactate; L-lactate occurs endogenously whereas D-lactate is suggestive of exposure to PG[282,306,307] and may warrant further study as a potential biomarker of PG exposure.[260] PG has a short biological half-life of approximately 4 hours with approximately 12–45% of PG excreted in urine unchanged.[308]

A few studies have characterized PG exposure in ENDS users' urine[304,309,310] and blood plasma;[304] however, the extent to which plasma D-lactate is increased following PG exposure from ENDS use has not been investigated. A small observational study of 42 daily ENDS users (using 1st, 2nd, and 3rd generation ENDS) and 50 controls (who did not use ENDS or other tobacco products) showed significantly higher levels of urinary PG in ENDS users (25.6 mcg/mL) following use of their own brand ENDS and e-liquid, compared to controls (9.8 mcg/mL).[309,310] Further, when ENDS users abstained from ENDS for 12 hours, urinary levels of PG decreased significantly to levels comparable to controls (9.7 mcg/mL). In a controlled in-patient industry study conducted in Germany, 20 ENDS users used a tank-based ENDS (Eleaf iStick attached to an Aspire Nautilus mini tank; e-liquid 12 ng/mL nicotine, 50:50 PG:VG) and 5 combusted cigarette smokers smoked a combusted cigarette spiked with a stable isotope-labeled tracker to isolate and characterize PG exposure from ENDS and combusted cigarettes.[304] Results showed, following ENDS use, urinary and blood plasma PG levels increased from baseline and, generally, PG increases in plasma and urine followed a similar pattern to nicotine (values not provided). However, for combusted cigarette smokers, there were no increases in urinary and plasma PG from baseline.[304]

Additionally, one small study (n = 40) evaluated potential biomarkers of dual ENDS and combusted cigarette use and found nicotelline and NNAL may provide a measure of combusted tobacco use in dual use studies, since levels were found to be low in exclusive ENDS users.[311] Nicotelline can provide

information on recent combusted tobacco product use (e.g., hours to days) and NNAL may help assess non-ENDS tobacco product use occurring over a longer timeframe (e.g., weeks). While these biomarkers may be beneficial, this study was completed on a small sample size in one geographical area, limiting the generalizability of the results.

Conclusions for Section 2.C. Exposure to Toxicants Other than Nicotine

Several studies showed the presence of various HPHCs and other potential toxicants in ENDS e-liquids or aerosol. Various studies showed the presence of harmful and potentially toxic carbonyls (e.g., formaldehyde, acrolein, acetaldehyde) and metals (e.g., lead, nickel, chromium) in the e-liquids and aerosol. One study even showed the presence of carbon monoxide in the aerosol when using high powered ENDS with a sub-ohm coil. Additionally, several potentially toxic flavor aldehyde compounds have been identified in various e-liquids or aerosol (e.g., diacetyl, cinnamaldehyde). New studies indicate the possibility of hemiacetal and acetal formation between aldehydes and humectants, including both flavor aldehydes and HPHC aldehydes (e.g., formaldehyde). Most biomarker studies show ENDS users who completely switch to ENDS from combusted cigarettes have significantly lower biomarker levels for metabolites of potentially harmful compounds, except nicotine. However, for dual users, the pattern is less clear.

D. STUDIES INVESTIGATING THE PHYSIOLOGICAL EFFECTS OF ENDS USE

Respiratory

In Vitro Studies

The NASEM report discussed several in vitro studies that tested ENDS aerosol, aerosol extract, and e-liquid and aerosol constituents, with or without nicotine, to evaluate the respiratory effects of ENDS.[1] Most of these in vitro studies compare effect levels for ENDS with those of combusted cigarettes. The NASEM report discussed ENDS in vitro studies and effects conducted across eight commonly used respiratory cell lines, such as the bronchial epithelial cell line (BEAS-2B, NCI-H292), primary human bronchial epithelial cells (HBEC), normal human bronchial epithelial (NHBE, NHBE48) cells, and bronchial epithelial cells from cancer cell lines (A549, NCI-292).

Scheffler et al. exposed primary NHBE cells obtained from healthy tissue from a 75-year-old patient with non-small cell lung cancer (NHBE48), an immortalized cell line created by transfecting NHBE48 cells with cyclin-dependent kinase 4 and human telomerase reverse transcriptase genes (CL-1548), and adenocarcinoma human alveolar basal epithelial cells (A549) using air-liquid interface (ALI) to ENDS aerosol (0% and 2.4% (24 mg/mL) nicotine concentrations), mainstream combusted cigarette smoke (10 K3R4F cigarettes each puffed six times), or clean air in a CULTEX RFS compact module.[312] For both ENDS aerosols and combusted cigarette smoke, the levels of oxidative stress were highest in primary NHBE48 cells, followed by the immortalized CL-1548 cells, and finally the A549 cells. Also, in agreement with the cell viability data, accumulation of oxidative stress with either ENDS aerosol was only a fraction of that seen with combusted cigarette smoke. Overall, the study uses a new immortalized NHBE cell line for in vitro toxicity testing of ENDS and provides evidence ENDS aerosols are cytotoxic and produce oxidative stress, albeit at lower levels than those produced by combusted cigarette smoke.

Another study by Taylor et al. investigated whether treatment with aqueous extracts from aerosols of two ENDS ("cig-a-like", cartomizer style; and a closed modular product) or combusted cigarettes (reference 3R4F) elicits cellular stress responses in a human bronchial epithelial cell line (NCI-H292).[313] The authors analyzed cellular ratios of reduced glutathione (GSH) to the oxidized form (GSSG), generation of ROS, and transcriptional activation of gene antioxidant response element (ARE) as an indirect indicator of Nrf2 activation and nuclear translocation. Caspase 3/7 activity was also measured as a marker of initiation of apoptotic responses to oxidative stress. A concentration-dependent induction of cytotoxicity was observed following exposure to combusted cigarette smoke aqueous extract. By contrast, no cytotoxicity was detected with either type of ENDS aerosol extracts. Similarly, when various dilutions of aqueous ENDS extracts were applied to cells (including undiluted extracts), there was activation of caspase 3/7 of up to 40% compared to controls, but no changes in apoptosis. Although oxidative stress and ROS generation significantly increased with combusted cigarette smoke, none of the endpoints of were affected by the aqueous ENDS extracts.

Overall, the NASEM report concluded for the respiratory effects of ENDS aerosol exposure from in vitro studies, 1) "there is substantial evidence that e-cigarette aerosols can induce acute endothelial cell dysfunction, although the long-term consequences and outcomes on these parameters with long-term exposure to e-cigarette aerosol are uncertain;" and 2) "there is substantial evidence that components of e-cigarette aerosols can promote formation of reactive oxygen species/oxidative stress. Although this supports the biological plausibility of tissue injury and disease from long-term exposure to e-cigarette aerosols, generation of reactive oxygen species and oxidative stress induction are generally lower from e-cigarettes than from combusted cigarette smoke." It was noted the in vitro and ex vivo studies would be more informative and representative of the human condition if aerosols rather than liquid ENDS solutions are used and if primary, instead of immortalized, cell lines are used.

Since the NASEM report was published, additional in vitro studies support the report's conclusions. These in vitro studies provided further detail on the respiratory effects of ENDS, including oxidative stress and ROS formation, cell dysfunction, cytotoxicity, and inflammation. Notably, several recent in vitro studies investigated commonly known ENDS, such as JUUL and Blu, while other studies have focused on flavors toxicity, including menthol, cinnamaldehyde, eugenol, and ethyl maltol.

Omaiye et al. reported cytotoxicity when human bronchial epithelial cells (BEAS-2B) were treated with 0.02, 0.06, 0.2, 0.6, 2, and 6 total puff equivalents (TPE) (1 TPE is equivalent to 1 puff/mL of culture medium) of aerosol condensate from JUUL with eight commercially available JUUL flavors for 24 hours. Cell viability and cytotoxicity were determined using the dimethylthiazol-diphenyltetrazolium bromide (MTT) colorimetric assay, neutral red uptake (NRU), and lactate dehydrogenase (LDH) assays.[98] Aerosol condensate and e-liquids for all flavors were cytotoxic using the MTT and NRU assay, but none were cytotoxic using LDH assay. Overall, aerosols were more cytotoxic than e-liquids, and liquid to aerosol phase transfer was estimated to be 39–62%. In addition, using correlation analysis, the main predictors of cytotoxicity were estimated to be the concentrations of nicotine and ethyl maltol in JUUL pods.[98] Hua et al. treated mouse neuronal stem cells (mNSC) and human bronchial epithelial cells (BEAS-2B) with 20 popular flavored e-liquids (as assessed from an internet survey and local and online sales information) and 10 flavor compounds at concentrations of 0.001–1% for 48 hours, and determined cytotoxicity using

the MTT assay.[314] The relative potency, based on cytotoxicity, for 10 compounds commonly found in these flavors was as follows: ethyl maltol > furaneol > maltol > ethyl vanillin > vanillin > benzyl alcohol > ethyl butanoate > triacetin > acetoin > ethyl acetate; 80% of these compounds were determined to be cytotoxic at 1% concentration (ethyl acetate and acetoin were not cytotoxic).

A study by Muthumalage et al. evaluated ROS generation and epithelial dysfunction by exposing BEAS-2B and 16-HBE pulmonary epithelial cells, and U937 monocytes to aerosols from 7 JUUL and 2 other pod flavors using acellular and cellular assays.[315] Aerosol from all JUUL pod flavors except Mango generated acellular (H_2O_2 µM equivalents) ROS. With respect to cellular ROS, two JUUL flavors (Cool Cucumber, Classic Menthol) and the Just Mango-Strawberry Coconut flavor showed high mitochondrial superoxide production. Aerosol exposure of all flavors increased inflammatory mediators and growth factors. Exposure to JUUL pod flavors, resulted in epithelial barrier dysfunction in 16 HBE cells. In addition, JUUL flavors induced DNA damage in U937 monocytes. However, this was an acute exposure experimental design and the study did not identify constituents in flavored e-liquids or aerosols that may be contributing to the observed effects. Sohal et al. also demonstrated treatment of BEAS-2B cells and primary human airway smooth muscle cells with ENDS aerosol condensate significantly increased the inflammatory marker interleukin (IL)-8; and extracellular matrix proteins (collagen 1A1 and fibronectin), which facilitate epithelial mesenchymal transition (EMT); and increased mitochondrial activity (glycolysis—measured by extracellular acidification rate, and mitochondrial uncoupling—measured by proton leak).[316] These results suggest ENDS exposure may elicit inflammation, airway scarring and remodeling via ECM in the lung. In a study by Ween et al., primary HBE cells were treated with 100% ENDS aerosol extract (18 mg/mL nicotine, 3 apple flavors), 10% combusted cigarette smoke extract (1R5F reference cigarette) or air, for 24 hours.[317] ENDS aerosol extracts of all three apple flavors (but not nicotine, PG, or VG alone) resulted in necrosis (measured by Syntox Green) and apoptosis (measured by Annexin V), and decreased efferocytosis (i.e., removal of apoptotic cells by macrophages) assessed by attenuated expression of the apoptotic cell recognition receptors, CD36 and CD44. Secretion of inflammatory cytokines (TNF-α, IL-6, IP-10, MIP-lα and MIP-1β) was decreased for all flavor variants. The study provides evidence that not only flavors, but also base e-liquid constituents (i.e., PG, VG) may influence the expression of apoptotic cell recognition receptors. In addition, the study shows that nicotine alone also reduced efferocytosis and decreased the expression of cytokines.

In an ex vivo study by Song et al. (2019), lung inflammation (cell counts and cytokines), global gene expression, and DNA methylation were examined using bronchioalveolar lavage (BAL) and brushings from 73 subjects (42 never-smokers, 15 ENDS users, and 16 combusted cigarette smokers).[318] There were significant differences among never-smokers, ENDS users, and smokers for inflammatory cell counts and cytokines. ENDS users had statistically significantly higher IL-1β, IFNγ and IL-6 levels in BAL fluid compared to never-smokers, and higher IL-1β than smokers. For differential gene expression and DNA methylation, ENDS users were more like never-smokers. There were 181 transcripts that were modulated in the ENDS group; the top ten included MUC5B, MUC5AC, ZNF445, REEP1, ABHK4, LINC00589, and TMPRSS3. In addition, there were 14 CpGs related to ENDS use (lower levels: RHBDL2, TTC16, ZNF815, and 3 intergenic CpGs); (higher; AMZ1, KRT12, NOX5/MIR548H4 colocalized, NRF1, and

4 intergenic CpGs). Modulated genes corresponded to smoking-related pathways, including those for xenobiotic metabolism, aryl hydrocarbon receptor signaling, and oxidative stress.

In an ex vivo study by Ghosh et al. (2019), 14 ENDS users (4 females, mean age 26 years), 14 combusted tobacco smokers (6 females, mean age 29 years), and 14 never-smokers (10 females, mean age 25 years) underwent bronchoscopy, and biomarkers of nicotine exposure and inflammation and protease levels were measured.[319] Users of ENDS and combusted cigarette smokers had significantly higher BAL neutrophil elastase, matrix metalloprotease (MMP)-2 and -9 levels, compared to never-smokers. Protease inhibitor (A1AT, SLPI, TIMP1 and TIMP2) levels in BAL fluid did not differ between ENDS users, combusted cigarette smokers and never-smokers. In vitro, immune cells from BAL were treated with 3% diluted PG:VG (55:45), 3% diluted PG:VG with 18 mg/mL nicotine or 3 mM nicotine. Peripheral blood neutrophils and BAL macrophages treated with nicotine (PG:VG + nicotine and 3 mM nicotine) induced protease release and increased cytosolic Ca^{2+} levels. In addition, levels of nicotine markers (nicotine, cotinine and 3'-hydroxycotinine) in sputum and BAL fluid of ENDS users were significantly higher than never-smokers (who did not use ENDS), suggesting exposure to nicotine from ENDS aerosols may be sufficient to cause protease release from macrophages and potentially increase overall lung proteolysis. It is plausible higher levels of nicotine may be present in ENDS users' airways during actual inhalation.

Behar et al. exposed human pulmonary fibroblasts (hPF) and lung epithelial (A549, CCL-185) cells in vitro to aerosols with flavors and showed increased cytotoxicity (using the MTT assay), with greater toxicity at higher voltages (5V versus 3V).[19] Berkelhamer et al. (2019) evaluated the potential toxicity of flavored solutions on immature lungs. Pulmonary artery smooth muscle cell cultures, collected from fetal, neonatal and adult ewes were treated with nicotine-free flavored solutions, pure PG or VG.[320] Based on the data obtained from viability studies and vasoreactivity analysis, the authors concluded the immature lung may be more susceptible than adult lung tissue to flavored ENDS solution-induced toxicity. Higham et al. showed incubation of primary bronchial epithelia cells (BECs) (from COPD patients and healthy non-smokers) with ENDS aerosol extract resulted in statistically significantly increased levels of LDH—a marker of cellular damage levels.[321]

Another study by Scott et al. reported alveolar macrophages from 8 never smokers treated with 0–2% ENDS aerosol condensates for 24 hours showed statistically significant, dose-dependent, decreases in cell viability, and increased apoptosis and necrosis (possibly mediated by the PI3K signaling pathway) for both 0 and 36 mg/mL nicotine aerosol condensates (with more pronounced effects among the 36 mg/mL nicotine compared to the 0 mg/mL nicotine condensate).[322]

The more recent literature provided further evidence that exposure of respiratory cell monolayer or 3D cultures to ENDS leads to a host of new toxic effects: increased ROS,[315] increased DNA damage,[315] decreased cell viability,[19,98,314,320,321] increased necrosis and apoptosis,[317,322] decreased removal of apoptotic cells by macrophages; enhanced bacterial replication and virulence;[323] enhanced susceptibility to respiratory infections due to increased bacterial airway adhesion to respiratory airway cells[324] and decreased phagocytic activity of alveolar macrophages;[322,323,325] increases in protease levels[316,319] and inflammatory responses;[318,325,326] enhanced epithelial-to-mesenchymal transition (change of cell structure, and enhanced cell migration), which may be relevant to cancer;[327,328] airway epithelial cell cilia

dysfunction;[329-331] chloride ion channel dysfunction,[332,333] changes in protein expression[334], gene expression and epigenetic markers.[318,335]

In Vivo Studies

The NASEM report discussed several in vivo studies related to the effects of ENDS on respiratory outcomes. It was noted animal studies in combination with in vitro studies have provided insights into the potential health effects associated with ENDS use.

Werley et al. conducted a 90-day inhalation study in Sprague-Dawley rats, followed by a 42-day recovery period with nose-only exposures to low-, mid-, and high-dose levels of aerosols composed of vehicle (VG and PG mixture); vehicle and 2.0% nicotine; and flavor mixture.[336] Exposures to 1 mg/L aerosol for 16, 48, and 160 minutes delivered daily targeted aerosol total particulate matter (TPM) doses of 3.2, 9.6, and 32.0 mg/kg/day, respectively; while it appears aerosols were administered, TPM was used as a dosing metric. Treatment-related effects following 90 days of exposure included dose-related decreases in thymus and spleen weights, and increased BALF lactate dehydrogenase, total protein, alveolar macrophages, neutrophils, and lung weights; and changes in body weight, food consumption, and respiratory rate. This in vivo study in rats provides some insight for identifying a threshold effect level based on bodyweight decreases at the mid-dose level for each formulation, equivalent to a daily TPM exposure dose of approximately 9.6 mg/kg/day. Histopathology changes appear to be isolated to the nasal mucosa. Limitations of this study include the capacity to extrapolate these findings to human exposures and how the findings from the ENDS and unknown flavor mixtures used in the study may compare or be generalized to ENDS. Further, lung weights and body weights are crude measures of effect.

Another study by Laube et al. exposed whole-body 10-week-old male C57BL/6 mice to ENDS aerosol containing PG alone or PG in combination with 2.4% nicotine for 20 minutes per day for either 1 or 3 weeks.[337] Young adult male mice exposed to PG aerosol had statistically significantly higher mucociliary clearance (MCC) than mice exposed to PG and nicotine aerosol. This study suggests daily exposure for 3 weeks to PG and nicotine slowed MCC compared with exposure to PG alone. Similarly, Sussan et al. (2015) showed impaired bacterial lung clearance in 8-week-old male C57BL/6 mice exposed whole-body to ENDS aerosol (NJOY menthol bold, 1.8% nicotine) for 3 hours per day for 2 weeks.[338] Together, these studies provide evidence exposure to ENDS aerosols during adolescence and early adulthood can result in significant impairments in lung function, even in the absence of lung inflammation.

Toxicity, oxidative stress, and inflammatory response in mice and human airway epithelial cells were examined by Lerner et al., where whole body exposure to ENDS aerosol in C57BL/6J mice increased pro-inflammatory cytokines (IL-6, MCP-1, IL-1α and IL-13) in bronchoalveolar lavage fluid, while diminishing glutathione levels in the lungs. In the same study, ENDS aerosol exposure to human airway epithelial cells (H292) in an ALI system resulted in increased secretion of inflammatory cytokines IL-6 and IL-8.[339] Collectively, exposure to ENDS aerosols or e-liquids produces increased oxidative and inflammatory responses in lung cells and tissues that might lead to health consequences.

The NASEM report concluded "there is limited evidence of adverse effects of e-cigarette exposure on the respiratory system from animal and in vitro studies." The report also summarized the limitations for the in vivo studies of respiratory effects of ENDS, stating 1) "animal studies that have examined the effects of e-cigarettes on respiratory outcomes have used different e-cigarette products, pumps, solutions, and exposures, limiting the ability to compare results among studies," 2) there were "confounding factors such as aerosol temperature and particle size that have not been taken into account," 3) "not all studies evaluating the effects of nicotine aerosols on lung inflammation, MCC, and lung immune responses have included biomarkers of systemic nicotine absorption, which would help to standardize exposures in animal studies," and 4) "the utility of studies using whole-body exposures in animal models when examining health effects of e-cigarette aerosols is limited because this type of exposure may overestimate or underestimate an exposure in the human condition."

Since the NASEM report was published, additional published in vivo studies extend the report's findings, providing further evidence ENDS aerosol exposure leads to respiratory toxicity. Several identified in vivo studies found other respiratory effects of ENDS exposure. Additionally, two studies found changes in expression of circadian rhythm genes and proteins expressed in lung tissue after exposure to ENDS aerosol, although the studies have limited relevance to respiratory toxicity and disease.[340,341] Key respiratory toxicology-related in vivo studies of ENDS published after the NASEM report are discussed below.

In a study by Reinikovaite et al. male Sprague Dawley rats exposed, whole-body, for 5 weeks to Blu ENDS aerosol (Classic tobacco flavor, 12 mg/mL nicotine) showed statistically significant emphysematous lung damage (increased alveolar airspace area and loss of capillary vasculature) when compared to controls (room air). In the same study, similar changes in airspace area and vasculature were seen in a group injected subcutaneously with nicotine, which suggests a role for nicotine in short-term emphysema-related pathologies.[342]

In a study by Khosravi et al., anesthetized guinea pigs were exposed to a single puff of aerosol from a KangerTech product (Subtank Mini 0.5 ohm coil, 5 V, 50 W) with six different brands of e-liquids (Old Kentucky, Blu, eVo, NJOY King, JC, and V2 Cig) and nicotine concentrations of 0, 12, and 18 mg/mL.[343] Delivery of a single puff of ENDS aerosol (diluted 1:1 with air; 12 mg/mL nicotine) into the lung triggered an immediate and transient bronchoconstriction for greater than 2 minutes. However, the increase in airway resistance was almost completely abolished by a pretreatment with either intravenous injection of atropine, or inhalation of aerosolized lidocaine, suggesting the bronchoconstriction was elicited by cholinergic reflex mechanism, and probable stimulation of airway sensory nerves. Electrophysiological recording confirmed a pronounced stimulatory effect on vagal bronchopulmonary C-fibers. In contrast, these effects were not seen with a pre-treatment with nicotinic acetylcholine receptor antagonists, or with ENDS aerosols without nicotine, indicating a critical role of nicotine. A limitation of this study is no experiments were conducted to assess the effects of multiple puffs, which better mimics human behavior, and resulting bronchoconstriction mechanisms such as neuroinflammation.[343]

Ha et al. evaluated the whole-body exposure to combusted cigarette tobacco smoke (4 CPD), ENDS aerosol (3 second puffs, followed by 20 seconds of room air for 82 per day, 5 days/week for 16 weeks)

with or without nicotine or air on cytokine expression in murine larynx.[344] This study found an induction of IL-4 with combusted cigarette smoke and ENDS aerosol containing nicotine but not ENDS aerosol without nicotine. Levels of TGFβ2, TGFβ3 and IL-10 were not statistically significantly different from air controls. No significant changes were found for 24 other cytokines/chemokines evaluated in the test conditions. A major limitation of the study was the small sample size with only 4 animals per group.[344]

An industry study by Phillips et al. reported a 90-day OECD study of 6-week-old male and female Sprague Dawley rats nose-only exposed to ENDS aerosols (containing 0 or 23 µg/L nicotine and three different PG:VG concentrations) or vehicle for 6 hours per day, 5 days per week for 13 weeks. The study reported measurements of multiple endpoints, beyond just respiratory outcomes, and concluded there were no toxicologically relevant adverse effects from exposure to aerosol containing PG and VG alone or from PG and VG with nicotine.[345] However, the study did not adequately report their data, so the results are difficult to independently evaluate. The ENDS aerosols were also diluted to target concentrations using filtered conditioned air, but it is unclear whether these diluted concentrations are representative of the aerosol exposure to either PG:VG or to nicotine that would occur with ENDS use. In addition, with respect to the statistical analysis, the resulting effects from ENDS aerosol and combusted cigarette smoke are often not compared to the relevant control (e.g., comparing to sham but not to vehicle control).

Bahmed et al. (2019) evaluated the effects of ENDS aerosol exposure on human alveolar type II (ATII) cells and in a mouse model. ATII cells from lung tissue of healthy donors were exposed to ENDS aerosol (0 or 24 mg/mL nicotine) and cell analysis was performed 24 hours postexposure.[346] For the in vivo study, wild-type C57BL/6 mice and DJ-1 KO mice were exposed whole body to ENDS aerosol (24 mg/mL nicotine). Authors have shown previously (Messier et al., *Cell Death Dis* 4: e573, 2013) that DJ-1 induces NRF-2 mediated antioxidant defense in ATII cells in combusted cigarette smokers**.** ENDS aerosol exposure to human ATII cells resulted in significant increases in IL-8 levels, DNA damage and apoptosis (assessed by increased p-53-binding protein expression). DJ-1 deletion sensitized these cells to mitochondrial dysfunction as detected by high mitochondrial superoxide production, decreased mitochondrial membrane potential, and calcium elevation. Dysregulation of oxidative phosphorylation complexes in ENDS exposed DJ-1 KO mice was also noted. DJ-1 KO were more susceptible to ATII cell apoptosis and lung injury upon exposure to ENDS aerosol compared with WT mice. The authors conclude that that DJ-1 deficiency sensitizes ATII cells to ENDS induced damage leading to lung injury.

In a study by Madison et al. (2019), female C57BL/6J mice were exposed to ENDS aerosol (60:40 PG:VG only or PG:VG with 33 mg/mL nicotine), combusted cigarettes or room air for 4 months.[347] In contrast to combusted cigarette smoke exposure, mice receiving ENDS aerosol for 4 months failed to develop pulmonary inflammation or emphysema. However, ENDS exposure altered lung lipid homeostasis in alveolar macrophages and epithelial cells. Alveolar macrophages isolated from the BAL of ENDS exposed mice (with or without nicotine), showed enhanced lipid accumulation and increased number of lysosomes. In addition, ATII cells from ENDS-exposed animals exhibited morphological changes in lamellar bodies with increase in the number of poorly organized, irregular organelles. Lipidomic and structural analyses indicated aberrant phospholipids in alveolar macrophages (phosphatidylcholine-, phosphatidylserine-, and phosphatidylethanolamine-based lipids, with an enrichment of disaturated

phospholipids and cholesterol esters) and increased surfactant-associated phospholipids (DPPC, MPPC and PPoPC). Attenuated innate immunity resulted in enhanced lung inflammation and tissue damage in ENDS-exposed mice infected with influenza. Notably, these changes were independent of nicotine content of ENDS. This was a detailed the in vivo study, which established the effect of ENDS solvents, independent of nicotine, on lung lipoprotein biology and alveolar macrophages. ENDS exposure disrupted both the lipid and protein components of pulmonary surfactant, increased phospholipid pools in the airway and reduced surfactant proteins SP-A and SP-D. Further studies are needed to evaluate how exposures to ENDS restructure membrane properties of lung macrophages and affects immune response.

In a study by Wang et al. (2019), C57BL/6J mice were exposed to ENDS aerosols (PG alone or PG with 25 mg/mL nicotine) for 2 hours per day for 3 consecutive days [348]. Female mice exposed to PG and PG with nicotine aerosols showed significant increases in inflammatory cell influx (increased neutrophils and $CD8a^+$ T-lymphocytes) in BAL fluid compared to controls, while male mice showed no differences. Overall, pro-inflammatory mediators (TNFα, IL-3, IL-4, IL-9, IL-12p70, IL-13, IL-17α, IFNγ, KC, GM-CSF, eotaxin, MIP-1α, MIP-1β, and RANTES) were significantly increased in the BAL fluid of mice exposed to PG alone and PG with nicotine compared to controls. For IL-3, IL-4, IL-9, IL-12p70, IFNγ, GM-CSF, Eotaxin, and MIP-1β, exposures to PG with nicotine aerosol were significantly higher than PG alone. PG alone significantly augmented the lung levels of various homeostasis/repair mediators (PPARγ, ADRP, ACTA2, CTNNB1, LEF1, β-catenin, E-cadherin, and MMP-2). This was associated with increase in protein abundance and altered gene expression of lipogenic markers (PPARγ, ADRP) and myogenic markers (fibronectin, α-smooth muscle actin and β-catenin). In summary, this study evaluated the effects of PG with and without nicotine on lung inflammation in a sex-dependent manner to demonstrate that acute exposure to PG without nicotine can induce oxidative stress in the lung.

In a study by Glynos et al. (2018), male C57BL/6 mice were exposed for four sessions/day for 3 days or 4 weeks to ENDS aerosol (PG:VG or PG:VG + 18 mg/mL nicotine or PG:VG + 18 mg/mL nicotine+ Nobacco American Tobacco flavor), combusted cigarette smoke or air.[349] Overall, exposure to ENDS aerosols, especially PG:VG + nicotine + flavor significantly increased BAL fluid cellularity (macrophage and protein concentrations), lung protein carbonyls, and oxidative stress markers comparably or more than combusted cigarette smoke. In short, ENDS aerosol triggered inflammatory responses and adversely affected respiratory mechanics. Flavor in ENDS aerosol exacerbated these effects. Strengths of the study include, the use of air and combusted cigarette smoke as negative and positive controls with PG:VG, PG:VG + nicotine, and PG:VG + nicotine + flavor, which helped attribute changes to vehicle, nicotine, or flavors. Inclusion of a sub-chronic exposure protocol in addition to the 3-day acute exposure allowed comparison and better characterization of pulmonary changes. In conclusion, the data indicate all ingredients of ENDS in the study including PG and VG induce lung inflammation and cause changes in respiratory mechanics at the dose used. However, the authors only observed high lung injury score in mice exposed to combusted cigarette smoke.

In this study by Cirillo et al. (2019), male Sprague Dawley rats were exposed to ENDS aerosol (3.5V, 1.5 Ω and 0.25 Ω coils, PG:VG 50:50 and 10% red fruits flavor), for 3 hours/day for 28 days.[350] Aldehydes (i.e., formaldehyde, acetaldehyde, and acrolein) in aerosol showed 3- to 7-fold increases as resistance

decreased from 1.5 to 0.25 Ω. Exposure to ENDS aerosol at the lower resistance coil setting showed perturbation of the antioxidant enzyme activity (increased superoxide dismutase, glutathione; decreased catalase), increased phase-II enzymes (UDPGT, glutathione S-transferase), increased ROS levels, elevated xanthine oxidase and P450-linked monooxygenases activity (increased CYP1A1, CYP2E1; decreased CYP2B1/2), and increased lipid hydrogen peroxides in the lung tissues, compared to the higher resistance group. Significantly reduced CCL3 gene expression was noted with exposure to the 0.25 Ω, compared to the 1.5 Ω coil. Disorganization of alveolar and bronchial epithelium (including detachment and loss of cilia), large areas of airflow collapse and evidence of apoptosis and necrosis were more remarkable in animals exposed to aerosol from the 0.25 Ω coils. In summary, the data suggest ENDS aerosol from low resistance coils are potentially more harmful than ENDS aerosol from higher resistance coils with exposures to higher aldehyde levels, and increased oxidative stress, inflammatory responses, and lung damage.

A study by Chapman et al. (2019) administered, intranasally, house dust mite (HDM) or sterile PBS to Balb/c mice over three. The mice were also exposed to ENDS aerosol (50:50 PG:VG, Black Licorice, Kola, Banana Pudding and Cinnacide flavors) or room air.[351] Mice challenged with HDM and exposed to nicotine-free Cinnacide flavored ENDS aerosol showed reduced airway inflammation (decreased total leukocyte cell count and eosinophils in BAL fluid) and increased peripheral airway hyperresponsiveness (measured using methacholine challenge) compared to mice challenged with HDM and exposed to room air. Compared to room air, exposure to nicotine-free Black Licorice aerosol showed a trend towards increased airway inflammation (increased total leukocytes and increased macrophages), while exposure to nicotine-free Banana Pudding aerosol increased soluble lung collagen in HDM challenged mice. In contrast, all tested ENDS containing nicotine suppressed airway inflammation but did not alter airway hyperresponsiveness or airway remodeling. In summary, flavored ENDS without nicotine in this study were found to alter allergic airways disease, but the effect is dependent upon the specific flavor (e.g., Black Licorice exaggerated airway inflammation whereas Cinnacide caused suppression).

Chung et al. (2019) evaluated the mucociliary dysfunction from ENDS aerosol in in vitro and animal models.[352] For the in vitro study, primary HBEC (donated from never-smokers without documented airway disease) were exposed to ENDS aerosol (50:50 PG:VG with 0 mg/mL or 36 mg/mL nicotine) or nebulized vapor (50:50 PG:VG with 36 mg/mL) using ALI. For the in vivo study female sheep (ewes) were exposed to ENDS aerosol (50:50 PG:VG with 36 mg/mL nicotine). In vitro, both ENDS aerosol and nebulized nicotine significantly decreased airway surface liquid (ASL) volume, and increased mucus viscosity of HBECs. Acute nicotine exposure increased intracellular calcium levels through TRPA1. Nebulized ENDS liquid containing nicotine reduced tracheal mucus velocity and elevated plasma cotinine levels, whileTRPA1 inhibitor A967079 reversed these effects. The proposed working model for mucociliary dysfunction--nicotine activates TRPA1 leading to Ca^{2+} influx, that over time results in loss of ASL hydration and increased viscosity--is illustrated in the in vitro and in vivo studies. Further, this study hints at the plausible mechanism of increased risk for chronic bronchitis in ENDS users. However, the limited number of animals and absence of proper controls when examining the role of TRPA1 in accumulation of mucus solids in sheep mucosa were limitations of this study. In conclusion, the in vitro

data for this study is complemented by in vivo study, and the results consistently indicate that nicotine containing ENDS aerosols impair mucociliary clearance.

Corriden et al. (2019) conducted ex vivo and in vivo studies to evaluate the impact of ENDS aerosol exposure on key neutrophil functions, including chemotaxis, neutrophil extracellular trap (NET) formation, and generation of ROS.[353] In the ex vivo model, the primary human neutrophils obtained from human donors were exposed to ENDS aerosol extract (50:50 PG:VG, 0 mg/mL or 24 mg/mL nicotine). In the in vivo model, female C57BL/6 mice were exposed nose-only for 1 hour/day to ENDS aerosol for 5 days/week, for 4 weeks, before infection. Ex vivo exposure to ENDS aerosol extract (0, 25%, 50, 75%, 100%) without nicotine showed dose-dependent decreases in chemotaxis toward the chemoattractant bacterial cell-well component f-Met-Leu-Phe. Treatment with ENDS aerosol extracts also altered neutrophil morphology (e.g., F-actin distribution and membrane fluidity), decreased ROS and diminished NETosis. Neutrophils treated with ENDS aerosol extract (with and without nicotine containing PG, and activated by phorbol 12-myristate 13-acetate) exhibited suppressive effects on NET formation, however, noncanonical NETosis was unaffected. In addition, exposure to ENDS aerosol extract lowered the rate of phagocytosis of *E. coli* and *S. aureus* bacterial bioparticles. The mouse model evaluated the effect of ENDS use on extravasation and chemotaxis of neutrophils in an infected space, and bacterial burden, following intraperitoneal challenge with *P. aeruginosa*. ENDS aerosol inhalation in mice led to significantly reduced total leukocytes recruited to the site of *P. aeruginosa* infection, decreased neutrophil migration recovered from peritoneal spaces, and led to a higher burden of *P. aeruginosa* compared to air controls. This was a well-designed study using both in vitro and in vivo study models to elucidate the impact of ENDS aerosols (with and without nicotine) on neutrophils. In addition, instead of pneumonia models which primarily assess the defense capabilities of macrophages, the study used a gram-negative sepsis model in which mice underwent intraperitoneal bacterial challenge for recruitment of neutrophils. One limitation was that only female mice were used for the study, therefore sex-based differences were not assessed. Future studies using samples directly from ENDS users can elucidate potential adverse effects on immune system associated with use of these products.

Human Studies

The NASEM report did not find studies examining the long-term effects of ENDS use and the development of chronic respiratory symptoms due to the newness of the products.[1] Studies have shown ENDS with nicotine can have short-term effects on lung defense mechanisms such as mucociliary clearance, urge to cough, and cough sensitivity. The report found moderate evidence of increased cough and wheeze among adolescent ENDS users and an association between ENDS use and an increase in asthma exacerbations.[1] It also found limited evidence from animal and in vitro studies of adverse effects of ENDS exposure on the respiratory system.

King et al. published results on symptoms reported by ever ENDS users from a nationally representative sample of US adults. They found the most commonly reported symptoms were cough (40.0%), dry or irritated mouth or throat (31.0%), and dizziness or lightheadedness (27.1%).[354] Using wave 2 PATH data, Li et al. report compared with non-users, risks of wheezing and related respiratory symptoms were significantly increased in current ENDS users (aOR=1.67, 95% CI: 1.23 to 2.15). Current ENDS users had significantly lower risk in wheezing and related respiratory symptoms compared with current smokers

(aOR=0.68, 95% CI: 0.53 to 0.87). No significant differences were found between dual users and current smokers in risk of wheezing and related respiratory symptoms (aOR=1.06, 95% CI: 0.91 to 1.24).[355]

Conclusions

The NASEM report concludes for the respiratory in vitro studies of ENDS, "there is substantial evidence that e-cigarette aerosols and components can induce cell dysfunction and promote formation of ROS/oxidative stress." For the in vivo studies, the NASEM report concludes studies have shown effects of ENDS on respiratory outcomes, however, the differences in study methodology limit the ability to compare results across studies. Despite these limitations, the in vitro and in vivo studies provide evidence of adverse effects of ENDS exposure on the respiratory system.

The published in vitro and in vivo studies seek to address key toxicological questions as to whether ENDS use, and the chemicals present in e-liquids (including flavors) and ENDS aerosols are associated with adverse respiratory health effects. Another important question is whether the respiratory effects reported across studies and with different parameters (e.g., cell lines, animal species, puff profiles, e-liquids, and nicotine concentrations) are generalizable to various ENDS products and whether these outcomes can be compared to other tobacco products.

The in vitro studies herein reported ENDS aerosol exposure led to oxidative stress and ROS formation, inflammation, cell dysfunction, cytotoxicity, and increased susceptibility to bacterial infections. Several studies found evidence of previously undocumented toxic effects of ENDS in vitro: 1) increases in lung protease levels; 2) changes in gene expression and epigenetic markers, and increased DNA damage; and 3) increased necrosis and apoptosis, decreased removal of apoptotic cells by macrophages.

In vivo studies also showed physiological effects, including increased protein concentration, oxidative stress markers[349,350] and antioxidant enzyme activity,[350] lipid accumulation,[347] increased inflammatory responses,[344,347-349] increased DNA damage,[346] enhanced susceptibility to the influenza virus, decreased immune responses with bacterial infections,[353] changes in respiratory mechanisms such as mucus velocity,[352] and histopathological changes, acute bronchoconstriction,[343] allergen-mediated hyperresponsiveness,[351] and airway remodeling.[342] However, a few in vivo studies reported no significant changes in bacterial vigilance and cytokine induction in mice infected with *Strep. Pneumoniae*,[356] and concluded there were no significant toxicological effects in rats with subchronic exposures to ENDS.[345]

Cardiovascular

In Vitro and In Vivo Studies

The NASEM report discussed several in vitro studies examining cellular toxicity, endothelial dysfunction and inflammatory responses in human umbilical vein endothelial cells (HUVEC), human coronary artery endothelial cells (HCAEC) and rat primary lung endothelial cells (RLEC) exposed to ENDS aerosol and combusted cigarette smoke extracts.[1]

In the NASEM report,[1] human umbilical vein endothelial cells (HUVECs) exposed to ENDS aerosol extracts in vitro showed increased ROS, reduced cell proliferation, decreased cell density, and increased apoptotic and necrotic cell death, independent of nicotine.[357-359] Exposure to combusted cigarette

smoke extracts produced more pronounced endothelial cell toxicity than ENDS aerosol extracts. In addition, increased endothelial cell migration in HUVEC, and increased expression of CYP1A1, CYP1B1, IL8, neuronal pentraxin-1 (NTPX1) and antioxidant stress response Nrf2-dependent genes (GCLM, OSGIN1, PAR4 and HMOX1) in HCAEC were specific to combusted cigarette smoke extract exposed cells only.[360] The NASEM report also found endothelial barrier disruption (determined by transcellular electrical resistance) in RLEC exposed to ENDS aerosol extracts, with nicotine as an important contributor to these effects.[361] In another study, C57BL/6 mice with brain ischemic injury were exposed to Blu ENDS aerosol for two weeks and showed increased oxidative stress (measured using CellROX)—with the antidiabetic drug metformin partially attenuating these effects, and antioxidant responses (i.e. induction of NAD(P)H:quinone oxidoreductase-1 protein).[362] Mouse brain microvascular endothelial cells (mBMEC) cultures, isolated from these mice and incubated with 5% soluble ENDS aerosol extract for 24 hours showed similar time- and concentration-dependent activation of the Nrf2 antioxidant pathway, but no increases in oxidative stress compared to controls.[362]

Since the NASEM report was published, several studies supported the NASEM report's conclusions, specifically, in vitro studies found decreased nitric oxide production in human aortic endothelial cells treated with ENDS aerosols; and an in vivo study found increased aortic stiffness and decreased cardiac function in female C56BL/6 mice exposed to ENDS aerosols and combusted cigarette smoke.

In a study by Kaisar et al. 2018, mice bEnd.3 brain cells and mBMEC treated with 5% diluted ENDS aerosol extracts in vitro showed greater mitochondrial dysfunction (increased mitochondrial membrane depolarization) compared to combusted cigarette smoke extract and positive control in mBMEC cells only. RNA and protein expression of Slc40a1 (transmembrane iron exporter) and Abcb6 (porphyrin importer) increased with combusted cigarette smoke extract, but not significantly with ENDS aerosol extract in both cell lines.[363] In another in vitro study, human aortic endothelial cells treated with ENDS flavor aerosol concentrate from vanillin aerosolized at 200 °C, and with eugenol aerosolized at 200 °C and 700 °C, showed decreased A23187-induced nitric oxide production.[364]

Lee et al. (2019) used human induced pluripotent stem cells-derived endothelial cells (iPSC-EC) obtained from three healthy donors and a high-throughput screening approach to assess endothelial integrity following exposure to six different e-liquids with varying nicotine concentrations and to serum from ENDS users (50%:50%, 80%:20% PG:VG, and 100% VG; 0, 6, and 18 mg/mL of nicotine).[365] Cytotoxicity of e-liquids varied considerably, with the cinnamon-flavored product being most potent, resulting in reduced cell viability, apoptosis, increased ROS levels, and LDL uptake, impaired tube formation and migration. This was associated with macrophage polarization into a pro-inflammatory state, with production of IL-1β and IL-6, leading to increased ROS. Exposure of iPSC-derived endothelial cells to serum of ENDS users, increased ROS, and inflammatory cytokines. An important finding is that the effect of ENDS use on mean plasma nicotine and cotinine levels were similar to combusted cigarette smoking. In conclusion, data from this acute exposure study suggested that flavored e-liquids or ENDS can impair endothelial dysfunction and may subsequently predispose to CVD. However, these in vitro studies should be validated in in vivo models, where development of cardiac pathologies may be better established using ENDS aerosol.

Wölkart et al. (2019) evaluated the effects of flavoring compounds (acetylpyridine, cinnamaldehyde, diacetyl, dimethylpyrazine, eucalyptol, eugenol, isoamyl acetate, menthol, and vanillin) on Ca^{2+}-induced cGMP accumulation, NO synthase activation, NO scavenging and blood vessel function in porcine aortic endothelial cells and Sprague-Dawley rats aortic rings.[366] Porcine aortic endothelial cells were treated with 1 mM of flavor compound for 10–60 minutes, depending on the assay, and rat aortic rings were tested with 100 µM eugenol and cinnamaldehyde and 300 µM for all other flavor compounds. Of the nine flavors compounds evaluated, only cinnamaldehyde inhibited Ca^{2+}-induced 3',5'-cyclic GMP (cGMP) accumulation and NO synthase activation (≥ 0.3 mM). Cinnamaldehyde and diacetyl inhibited NO-activated soluble guanylate cyclase with IC_{50} values of 0.56 and 0.29 mM, respectively, and caused moderate NO scavenging at 1 mM (not mediated by superoxide anions). Other compounds did not scavenge at this dose. None of the flavorings interfered with acetylcholine-induced vascular relaxation but caused relaxation of pre-contracted aortas (most potent being eugenol and cinnamaldehyde). The authors concluded that this suggests the absence of endothelial dysfunction by these flavors. This study provides some insight into NO signaling and endothelial function by ENDS flavors.

In a study by Nystoriak et al. (2019), human induced pluripotent stem cell (iPSC)-derived cardiomyocytes were treated with unheated or heated cinnamaldehyde aerosol extract (1, 10, 100 µM) for up to 48 hours to determine the effects of cinnamaldehyde on contractility, rhythmicity, electrical signaling properties, and cellular viability.[367] Cinnamaldehyde was heated to 200 or 700 °C in a drop-tube furnace, then added dropwise into a heated area, where it rapidly aerosolized, collected within a glass impinger and eluted in an ethanol solution (55% in PBS). In unheated cinnamaldehyde treated cells, cytotoxicity was seen at the high dose only (100 µM) at 24 and 48 hour timepoints. Cinnamaldehyde statistically significantly impaired contractile activity of iPSC-derived cardiac myocytes, and caused depolarization of resting membrane potential. However, heating cinnamaldehyde (at 200 or 700 °C) attenuated these effects. In summary, cinnamaldehyde impacts the function of human iPSC-CMs in vitro, yet these effects are largely attenuated after the compound is heated. Elevated temperatures, such as those achieved in ENDS e-liquids, may alter the bioactive properties of cinnamaldehyde and attenuate any direct cardioactive effects of cinnamaldehyde. In conclusion, the study provides interesting data suggesting that route of administration, constituents of e-liquids and device temperature may be important variables for cinnamaldehyde-mediated effects.

In a study Noel et al. (2019), human umbilical vein endothelial cells (HUVEC)/Tert2 were treated with flavor compounds (acetonitrile, *trans*-anethole, *p*-anisaldehyde quantified as 3-vinylbenzaldehyde, benzaldehyde, benzaldehyde PG acetal benzyl alcohol, *trans*-cinnamaldehyde, cinnamaldehyde PG acetal, estragole, eugenol, limonene, limonene oxide, linalool, menthol) commonly found in e-liquids or 1% diluted commercial e-liquids.[368] Cell lysis (lactate dehydrogenase [LDH] assay) was measured after 24 hours of treatment and metabolic activity was determined 48 hours after treatment, with varying doses of select flavor compounds (in 50:50 PG:VG solution) and select commercial e-liquids. Cinnamaldehyde increased cell lysis at multiple concentrations (5g/kg and 10 g/kg) compared to controls (PG:VG alone), and the commercial e-liquid SV70 (comprised mostly of anisaldehyde) showed high cell lysis activity. Flavoring chemicals such as cinnamaldehyde, cinnamaldehyde PG acetal and limonene, and commercial e-liquids: SV70 (anisaldehyde), VC36 (anisaldehyde), MQ90 (limonene) and PO69 (limonene oxide)

showed reductions in metabolic activity, compared to controls (untreated cells). However, eugenol and estragole did not reduce metabolic activity. Cinnamaldehyde was found to be the most deleterious for HUVEC/Tert2 cells. In this study, compounds in 34 concentrates and 21 liquids were quantified; the cytotoxicity studies for these compounds used a range of concentrations which may not represent the final concentration in the ENDS aerosol reaching the lung and the vasculature.

In a study by Olfert et al., female C56BL/6 mice were exposed, whole-body, to ENDS aerosol (38–39 puffs/hour for 4 hours/day, 5 days a week for 8 months; 18 mg/mL nicotine), 3R4F reference cigarette smoke (24 combusted cigarettes puffed over the 4 hours of exposure per day) or air (control).[369] Aortic stiffness (AS) was increased 2.5-fold in ENDS aerosol and 2.8-fold in 3R4F-exposed mice, compared with air-exposed control mice. Compared to controls, the maximal aortic relaxation to methacholine was 24% lower in mice exposed to ENDS aerosol and 33% lower in mice exposed to 3R4F cigarette smoke. Reduced fractional shortening and ejection fraction was also observed after 8 months in 3R4F exposed mice, but not in ENDS aerosol exposed mice. Histological and respiratory function data support emphysema-associated changes in 3R4F-exposed, but not ENDS aerosol-exposed mice. In summary, chronic exposure to ENDS aerosol accelerates AS, statistically significantly impairs aortic endothelial function, and may lead to impaired cardiac function, suggesting chronic use of ENDS, even at relatively low exposure levels, induces cardiovascular dysfunction.[369]

Crotty Alexander et al., C57BL/6 and CD-1 female mice exposed, nose-only, to non-flavored 50:50 PG:VG ENDS aerosol for 3 puffs per minute for 60 minutes/day, 5 days/week for 3–6 months showed increased renal fibrosis (increased collagen), increased cardiac fibrosis, increased liver fibrosis, decreased heart rate, and increased systolic blood pressure in both mouse strains. The authors also found increased circulating inflammatory markers in both mouse strains, leukemia inhibitory factor (LIF), LIX, EGF and angiopoietin 1 (Ang-1), and decreased circulating MMP-3, which is associated with degradation of collagen, fibronectin, elastin and laminin and clearance of fibrosis.[370]

Kuntic et al. (2019) conducted a murine study examining vascular oxidative damage in response to ENDS aerosol exposures.[371] For the animal study, 124 male C57BL/6 mice (age 12± 3 weeks) and 27 male *Nox2* null C57BL/6 mice (age 13± 3 weeks) were exposed to ENDS aerosol (Joyetech eVIC-VTC Mini, 0.5 ohm atomizer, 24 W; 3 second puffs, 55 mL puff volume, every 30 seconds for a total of 40 puffs/session) for 6 sessions/day over 1, 3, or 5 days. Acute ENDS aerosol exposure produced marked impairment of endothelial function in chronic smokers, as assessed by flow-mediated dilation (FMD). In mice, exposure to ENDS aerosol without nicotine was more detrimental to endothelial function, markers of oxidative stress, inflammation, and lipid peroxidation than ENDS with nicotine. These effects were absent in mice lacking phagocytic NADPH oxidase (NOX-2) or upon treatment with macitentan (endothelin receptor blocker) or bepridil (FOXO3 activator). The ENDS aerosol constituent, acrolein, recapitulated many of the NOX-2-dependent effects suggesting that ENDS aerosol exposure increases vascular, cerebral, and pulmonary oxidative stress via a NOX-2. In summary, the study found ENDS aerosol produced increases in serum oxidative stress marker (8-isoprostanes), NOX-2 activation, and reduced endothelial function. Additionally, ENDS aerosol appeared to mediate many of its adverse vascular consequences through NOX-2 activation.

In an acute inhalation study by Rao et al. (2020), adult rats were exposed to two types of ENDS, combusted cigarettes or air to evaluate vascular endothelial function, assessed as arterial FMD.[372] Ten week old male and female Sprague-Dawley rats via nose cone to ENDS aerosol from JUUL (Virginia Tobacco, 5% nicotine (59 mg/mL nicotine; 30:70 PG:VG)) or Nautilus tank (unflavored, 12 mg/mL nicotine 67:33 PG:VG); Marlboro Red cigarettes (positive control); and clean air (control) for 10 second cycles with 2 second inhalation over 5 minutes. In contrast to air exposed group, FMD was significantly impaired in animals exposed to aerosol from JUUL and Nautilus, and Marlboro Red cigarette smoke. The extent of FMD impairment did not significantly differ between the groups. Serum nicotine and cotinine levels were highest in the JUUL group, while the Nautilus group and the combusted cigarette group had comparable levels. There is substantial evidence regarding the disruptive proinflammatory effects of nicotine and ENDS on endothelial function and recent studies in humans have indicated nicotine-free ENDS can also impact vascular function adversely. Although this study found no difference in effect by type of nicotine, further studies are important to determine whether there is any difference in the effect of free nicotine and nicotine salts on the endothelium. There is substantial evidence regarding the disruptive proinflammatory effects of nicotine and ENDS on endothelial function. This study adds to a growing literature supporting the adverse impact of exposure to ENDS aerosol on vascular function.

In a study by Szostak et al. (2020), female ApoE-/- mice were exposed whole body to ENDS aerosol (PG:VG, PG:VG + 4% nicotine or PG:VG + 4% nicotine + flavoring), combusted cigarette smoke (3R4F reference cigarette) or control (filtered air) for 3 hours/day, 5 days/week for up to 6 months.[373] ENDS aerosol and combusted cigarette smoke concentrations were normalized to 35 µg/L nicotine. Formaldehyde, acetaldehyde, propionaldehyde, crotonaldehyde, acrolein, NNN and NNK levels were less in ENDS aerosol than in combusted cigarette smoke at a normalized nicotine level. Nicotine exposure measures were similar across groups (as expected due to normalization). At four months, the only statistically significant increase was in urinary oxidative stress and inflammation markers (MDA, 2,3-di-PGF2α, PGF2a, t-PGE-M and LTE4), while at three and six months the combusted cigarette smoke exposed groups only had increased whole blood counts (hematocrit, hemoglobin, erythrocytes, and reticulocytes). Cholesterol levels were elevated in combusted cigarette smoke exposed animals, but not ENDS aerosol groups when compared to controls. The authors stated there was a reduction in plaque area in the thoracic aorta in ENDS exposed groups compared to combusted cigarette smoke group, closer to controls, with the absolute value of this change reducing by six months. This trend is consistent in micro-CT data looking at similar parameters (although representative images show more disperse plaque throughout the thoracic aorta in the ENDS groups compared to sham). Changes in heart performance (ejection fraction, cardiac output, fractional shortening), as measured by echocardiography, were observed in animals exposed to combusted cigarette smoke but not ENDS. Mice exposed to the PG:VG + nicotine and PG:VG + nicotine + flavor groups had an increased isovolumic relaxation and increased myocardial performance index (negative effect), similar to the combusted cigarette smoke exposed mice. The nicotine containing ENDS aerosol exposures also increased abdominal aortic stiffness at four and six months, and carotid artery stiffness at six months, but to a lesser extent than the combusted cigarette smoke group. Significant gene dysregulation was seen in the left ventricle and thoracic aorta at three and six months in combusted cigarette smoke exposed groups, but no genes were statistically significantly dysregulated in the ENDS aerosol groups relative to sham (air

control). Overall, the study demonstrates that at normalized nicotine levels (potentially reducing exposures from the ENDS aerosols) there is a reduction in several cardiovascular performance parameters (e.g., myocardial performance index, aortic stiffening) in nicotine containing ENDS aerosol groups compared to sham exposed animals but this reduction is not as significant as that seen in the combusted cigarette smoke exposed animals. The cardiac performance data suggest that there is a long-term effect of e-liquids containing nicotine on cardiovascular performance, but this is not conveyed by gene expression. The most likely explanation is that the affected cells are diluted out as this is bulk RNA-seq. If the imaging was performed in close relation to exposure, the effects may be a holdover of the nicotine levels in the animals.

In a study by Chen et al. (2019), exercise performance and health-related profiles were examined in female mice exposed to ENDS aerosols for 14 days.[374] Eight week old female ICR mice were randomly assigned to five groups (1) vehicle; (2) air; (3) EC-0X [0 mg/day e-liquid without nicotine]; (4) EC-1X [4 mL VG+ 0.5 mg/mL nicotine] (5) EC-10X [4 mL VG + 5 mg/mL nicotine.[374] Mice were exposed to ENDS aerosol for 30 minutes/day for 14 days. At the highest dose, ENDS aerosol exposure statistically significantly reduced grip strength, decreased swimming time of the mice, and resulted in decreases in liver and muscle glycogen storage. No histopathological abnormalities in the tissues or organs of the mice were noted.

In a study by Espinoza-Derout et al. (2019), 8 week old male C57BL/6J ApoE$^{-/-}$ mice fed a western diet were exposed to ENDS aerosol (blu CIG PLUS, Gold Leaf tobacco flavor (0% nicotine) or Classic tobacco flavor (2.4% nicotine); 4 seconds/puff, 25 second interval, 8 puffs/session, 1 session every 30 minutes for 12 hours/day) for 12 weeks.[375] Exposure to ENDS aerosol with 2.4% nicotine showed statistically significantly decreased left ventricular fractional shortening and left ventricular ejection fraction, and increased atherosclerotic lesions, compared to saline. Using RNA-seq by quantitative PCR, ventricular transcriptomic analysis showed changes in genes associated with metabolism (e.g., upregulated Npas2), circadian rhythm (e.g., downregulated Per2 and Per3), inflammation (e.g., Col5a3), and apoptosis (upregulated TNFRSM12A and Hrk) in mice exposed to ENDS aerosol with nicotine in comparison with saline. Transmission electron microscopy (TEM) of left ventricles of mice exposed to ENDS aerosol with 2.4% nicotine showed ultrastructural abnormalities indicative of cardiomyopathy (including shrunken nuclei, nuclei with convoluted nuclear membrane, myofibrillar derangement, thinning and destruction). In addition, exposure to aerosol with nicotine significantly increased oxidative stress (measured by cardiac tissue malondialdehyde) and mitochondrial DNA damage in cardiomyocytes was observed.

Human Studies

The NASEM report reviewed 13 clinical intervention studies published between 2010 and 2017 that evaluated acute cardiovascular effects of ENDS use, such as short-term changes in blood pressure levels, heart rate, arterial stiffness and endothelial function, cardiac geometry and function, and oxidative stress as well as three studies that examined cardiovascular outcomes over a longer timeframe. Five clinical studies published between 2015 and 2017 found higher heart rate levels after ENDS use, whereas five earlier clinical studies published between 2010 and 2014 did not. This result may be due to greater nicotine exposure produced by more recent ENDS such as tank systems. The three longer-term studies did not find an association between ENDS use and increased heart rate. Short-term studies

generally found diastolic blood pressure, although not necessarily systolic blood pressure, generally increased after ENDS use. A small number of studies evaluated oxidative stress biomarkers, endothelial function, and arterial elasticity after ENDS use and found some evidence of short-term effects. Since the NASEM report there have been additional clinical and cross-sectional studies related to ENDS use and cardiovascular disease, but data on longitudinal cardiovascular outcomes are still limited.

In other clinical intervention studies, smokers using ENDS with nicotine significantly increased systolic blood pressure, peripheral pulse pressure, and heart rate up to 45 minutes after use, while for ENDS without nicotine, diastolic blood pressure was statistically significantly decreased for up to 30 minutes after use, compared to baseline.[376] Several crossover studies with ENDS use and combusted cigarette smoking showed increased skin tissue hypoxia for 60 minutes after ENDS use, compared to controls;[377] and in smokers, use of ENDS without nicotine resulted in slightly increased superficial blood flow, while use of ENDS with nicotine showed significantly decreased deep blood flow.[378]

A meta-analysis conducted by Skotsimara et al. analyzed 26 research articles from January 2000 to November 2017, related to ENDS use and cardiovascular effects.[379] The meta-analysis showed evidence ENDS use was statistically significantly correlated with increased heart rate, increased systolic blood pressure, and increased diastolic blood pressure. It also indicated switching from combusted cigarettes to ENDS was associated with no change in heart rate, decreased systolic blood pressure and decreased diastolic blood pressure. One limitation of this meta-analysis was the underlying studies included a variety of ENDS, e-liquids, and differences in product use, therefore the findings may not be applicable to a specific product characteristic or ingredient.[379]

In a crossover single-blind clinical study by Nocella et al., in which 20 current smokers and 20 non-smokers smoked a combusted cigarette then, after a one-week washout, took 9 puffs of an ENDS with similar nicotine concentrations, blood taken 5 minutes after ENDS use showed increased platelet aggregation, increased soluble CD40L and increased soluble P-selectin, compared to baseline. The authors concluded ENDS use may have implications for the process of thrombosis and pathophysiology of cardiovascular disease regardless of combusted cigarette smoking status.[380] A clinical study using ^{18}F-flurorodeoxyglucose positron emission tomography/computer tomography (FDG-PET/CT) imaging to track modified glucose in tissues of exclusive ENDS users and smokers showed an increased metabolic activity in the spleen and aortic wall, compared to controls. The authors suggest smoking and ENDS use may activate the "Splenocardiac Axis" (a proinflammatory pathway associated with atherosclerosis),[381] which may lead to increased risk for cardiovascular events.[382]

Data pooled from the 2016–2017 Behavioral Risk Factor Surveillance System (BRFSS) indicate there was no significant association between ENDS use and cardiovascular disease among never combusted cigarette smokers. Compared to current combusted cigarette smokers who never used ENDS, dual use of ENDS and combusted cigarettes was associated with 36% higher odds of cardiovascular disease.[383]

Conclusions

The NASEM report and subsequently published in vitro, in vivo, and clinical studies seek to address whether exposure to ENDS aerosols are associated with cardiovascular toxicity and adverse

cardiovascular health effects. The NASEM report concludes for the in vitro studies of ENDS, "there is substantial evidence that e-cigarette aerosols and components can induce endothelial cell dysfunction and promote formation of reactive oxygen species/oxidative stress." For in vivo studies, the NASEM report concludes there is "limited evidence that ENDS aerosols can cause a short-term increase in systolic blood pressure, changes in biomarkers of oxidative stress, increased endothelial dysfunction and arterial stiffness, and autonomic control."

After the NASEM report was published, several recently published in vitro and in vivo studies have provided additional evidence of the impact of ENDS aerosol exposure on endothelial cell dysfunction, oxidative stress, aortic stiffness, blood pressure, and heart rate. Studies have also found evidence of new outcomes, including cardiac myopathy, vascular endothelial dysfunction, impaired microvascular function and reduced arterial flow-mediated dilation, which suggest that ENDS exposure may impact intermediate endpoints on the pathway to clinical cardiovascular disease. Although these in vitro and in vivo studies may support the biological plausibility of hypothesized cardiovascular disease pathways, further studies are important to determine if ENDS exposure is associated with clinical cardiovascular disease outcomes (coronary heart disease, stroke, and peripheral artery disease) and subclinical atherosclerosis (carotid intima-media thickness and coronary artery calcification).

Cancer and Genotoxicity

In Vitro and In Vivo Studies

The NASEM report discussed several in vitro studies of possible mutagenicity with exposures to ENDS aerosol and combusted tobacco smoke, comparing outcomes of Ames assay in Salmonella Typhimurium strains TA98 and TA100, micronucleus formation in Chinese hamster ovary (CHO) cells, and DNA strand breaks (H2Ax immunofluorescence) in BEAS-2B cells. Most of the in vitro studies do not directly report mutagenicity or DNA damage from exposures to ENDS aerosols. A study by Breheny et al. used a cell transformation assay (CTA) in Bhas 42 mouse fibroblast cells to detect potential for initiation and promotion exposed 3 to 120 µg/mL concentrations of ENDS aerosol condensate (18 mg/mL nicotine) and total particulate matter (TPM) from the 3R4F reference cigarette. ENDS aerosol condensate-exposed cells showed no promotion or cytotoxicity, whereas TPM-exposed cells showed parallel cell growth compared to positive controls.[384] Several studies found no statistically significant induction in the number of revertants for Salmonella strains TA98 and TA100,[385] and no significant increase in micronucleus formation in Chinese hamster ovary (CHO) cells exposed to e-liquids or to tobacco smoke extract.[385] In a study by Thorne et al., human bronchial epithelial cells (BEAS-2Bs) exposed to ENDS aerosol from two types of ENDS showed no DNA double strand breaks (measured using γ-H2Ax immunofluorescence) and no statistically significant cytotoxicity. Combusted cigarette smoke from 3R4F reference cigarettes showed genotoxicity at a dose of 3.1 µg/cm^2 and cytotoxicity at 26.9 µg/cm^2.[386] In an in vitro study by Welz et al., a 3D mucosal tissue model (with fresh tissue samples of healthy human oropharyngeal mucosa) treated with tobacco flavored and base e-liquid (80% PG, 10% VG, 10% water) for 24 hours or repetitively over 5 days showed no DNA damage using the neutral comet assay (Alkaline elution DNA damage assay). In contrast, treatment with apple and cherry flavored e-liquids induced statistically significant DNA damage in both 24 hour and 5-day treatments.[387] In a study by Yu et al., immortalized human keratinocytes (HaCaT) and cells from the human non-small cell carcinoma (HNSCC)

cell lines UMSCC10B and HN30 treated with four ENDS aerosol extracts (from e-liquids with and without nicotine) showed DNA double strand breaks (using γ-H2Ax immunofluorescence) for all four ENDS extracts, although extracts with nicotine showed more pronounced responses.[388]

In an in vivo study by Canistro et al., Sprague Dawley rats exposed to ENDS aerosol (18 mg/mL nicotine) for 4 weeks showed single- and double-stranded DNA breaks (using the alkaline comet assay) and increased percentage of micronucleated reticulocytes in peripheral blood, a four-fold increase in 8-hydroxy-2'-deoxyguanine (8-OHdG) in lung tissue, and decreased levels of the antioxidant enzymes catalase, NQO1, superoxide dismutase, and glutathione S-transferase.[389]

An ex vivo study by Franco et al. studied oral cells from ENDS users, combusted tobacco cigarette smokers, and non-smokers of combusted tobacco cigarettes or ENDS, collected by scraping the oral mucosa. Compared with non-smokers, mean levels of micronucleated cells/1000 cells were 133% higher in ENDS users and 633% higher in combusted cigarette smokers, while total micronuclei/1000 cells were 160% higher in ENDS users compared to non-smokers.[390]

Since the NASEM report was published, additional in vitro and in vivo studies support the NASEM report conclusions. A study by Tommasi et al. showed no statistically significant increases in mutation frequencies in the *cII* and *supF* reporter genes in mouse embryonic fibroblasts and SV-40 transformed human fibroblasts (GM4427) treated with ENDS aerosol extracts from Blu ENDS (16 mg/mL nicotine), NJOY (18 mg/mL nicotine), and V2 (18 mg/mL nicotine), compared to control.[391] A study by Fetterman et al. reported treatment of pulmonary endothelial cells in vitro from smokers and non-smokers with 1–100 mM of common flavor compounds (vanillin, menthol, diacetyl, eucalyptol, dimethylpyrazine, isoamyl acetate, cinnamaldehyde, eugenol, acetylpyridine) for 90 minutes statistically significantly increased DNA strand breaks (TUNEL positive) at the higher concentrations, compared to ethanol vehicle controls.[391]

Al-Saleh et al. (2020) studied human lymphoblastoid TK6 cells and Chinese hamster ovary (CHO) cells, which were treated with 68 different e-liquids (at 1% concentration) for 24 hours.[392] The majority of e-liquids were shown to induce DNA damage, chromosome breakage (increased median percent micronuclei compared to untreated cells), and cytotoxic effects (decreased cell viability in TK6 and CHO cells compared to untreated cells). DNA damage, measured using comet assay tail movement, was reported for TK6 cells activated with S9 fraction (S9+) and treated with 11 out of 23 e-liquids. However, TK6 cells that were not treated with S9 fraction (S9-) showed no significant DNA damage. TK6 cells seem to be more susceptible to DNA damage than CHO cells, as TK6 cells treated with e-liquid produced a more than 3-fold higher tail migration than CHO cells for six e-liquids. CHO cells showed no comparative increases in tail migrations. It is difficult to interpret the relevance of study findings because the e-liquid was added directly to the cells, which does not represent real-life exposures.

In an in vivo study by Lee et al., male FVBN mice whole-body exposed to ENDS aerosol (50:50 PG:VG, 10 mg/mL nicotine) for 3 hours/day, 5 days/week for 12 weeks showed nitrosamine metabolite-DNA adducts (O^6-methyl-deoxuguanosine (O^6-medG)) and aldehyde-derived DNA adducts (cyclic 1,N_2-propano-dG (PdG)) in lung, bladder, and heart tissues. The O^6-medG adduct levels in the lung were three

to eight-fold higher than in the bladder and heart and PdG adducts were 25 to 60-fold higher than O^6-medG adducts in lung, bladder, and heart tissues. DNA repair (nucleotide excision repair and base excision repair) activity and proteins were also reduced in the lungs of ENDS aerosol exposed mice compared to air controls.[393] In human lung and bladder epithelial cell lines, nicotine and NNK were reported to induce DNA damage, inhibit DNA repair, and enhance mutations and cell transformation in response to UV and hydrogen peroxide (H_2O_2) exposures.[393]

A study by Tang et al. (2019), 6-8 week old male FVB/N mice were exposed whole-body to ENDS aerosol (eAerosols generator, 1.9 A, 4.0 V; vehicle, 50:50 PG:VG or 50:50 PG:VG with 36 mg/mL nicotine) or filtered air for 4 hours/day, 5 days/week for 54 weeks.[394] Nine of the 40 mice (22.5%) exposed to ENDS aerosol for 54 weeks developed lung adenocarcinomas and 23 of the 40 mice (57.5%) developed bladder urothelial hyperplasia. None of the mice in the vehicle group (PG:VG) and only 1 of the 18 mice (5.6%) in the filtered air group developed lung adenocarcinoma. One of 16 (6.3%) mice in the vehicle group and none of the 17 in the filtered air group developed urothelial hyperplasia. However, there was no difference in incidence of urothelial hyperplasia comparing mice with lung tumors to mice without lung tumors (6 of 9 (67%) versus 18 of 31 (58%), p=0.64). This was one of the first studies showing that ENDS aerosol can induce lung carcinogenesis in an animal model. In the current study, the authors do not address the precise mechanism through which ENDS induced lung tumorigenesis. However, in their previous work, using low nicotine concentrations (10 mg/mL, 3 hours/days, 5 days/weeks for 12 weeks), the authors demonstrated that ENDS exposure induced DNA damage and compromised repair activity in murine lung, heart, and bladder tissue. Moreover, nicotine and its metabolite, enhance mutational susceptibility and tumorigenic transformation of cultured human bronchial epithelial and urothelial cells. Taken together, these findings implicate ENDS aerosol as a lung and potential bladder carcinogen in an animal model. Further research (e.g., mechanistic, clinical, and epidemiological studies) is important to determine whether these carcinogenic effects extend to ENDS exposures in humans.

A study by Nguyen et al. (2018) reported offspring of female Balb/C mice whole-body exposed to ENDS aerosol showed altered expression of many epigenetic genes, such as DNA methyltransferases, histone-lysine demethylases, histone acetyltransferases, and aurora kinases in both the 0 and 18 mg/mL nicotine groups. These data suggest effects of ENDS exposure in mothers, with or without nicotine, can affect epigenetics of offspring.[395]

Human Studies

The NASEM report reviewed available studies related to cancer and ENDS use and found them very limited in number and relevance and generally lacking in methodological rigor. The report found no available epidemiological studies on the potential association between ENDS use and cancer or intermediate cancer endpoints in humans that would allow for conclusions. No additional human study data pertaining to cancer has been published since the NASEM report.

Conclusions

The NASEM report concludes 1) “there is no available evidence whether or not e-cigarette use is associated with intermediate cancer endpoints in humans. This holds true for e-cigarette use compared with use of combusted cigarettes and e-cigarette use compared with no use of tobacco products;” 2)

"there is limited evidence from in vivo animal studies using intermediate biomarkers of cancer to support the hypothesis that long-term e-cigarette use could increase the risk of cancer; there is no available evidence from adequate long-term animal bioassays of e-cigarette aerosol exposures to inform cancer risk;" 3) "there is limited evidence that e-cigarette aerosol can be mutagenic or cause DNA damage in humans, animal models, and human cells in culture;" and 4) "there is substantial evidence that some chemicals present in e-cigarette aerosols (e.g., formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis." This supports the biological plausibility long-term exposure to ENDS aerosols could increase risk of cancer and adverse reproductive outcomes. Notable in vitro studies published since the NASEM report, with incubation of TK6 cells and CHO cells with commercially available e-liquids for 24 hours, showed DNA damage, chromosome breakage and cytotoxic effects. Another in vitro study showed increased DNA damage, micronuclei and hypodiploid nuclein in treatment of CHO cells with e-liquids. A recent in vivo study showed evidence of increased incidence of lung adenocarcinomas and bladder urothelial hyperplasia in mice exposed to ENDS aerosol for 54 weeks. Taken together, these studies present new information toward assessing DNA damage and cancer incidence with ENDS exposure.

Developmental and Reproductive

In Vitro and In Vivo Studies

The NASEM report discussed an in vitro study examining cardiac directed differentiation with exposure to ENDS aerosol and combusted cigarette smoke extracts, and an ex vivo study examining lung tissue growth after exposure to nicotine.[1] The NASEM report reviewed six in vivo studies in rodents and non-human primates and reported effects on lung development with exposures to nicotine, and tobacco products with nicotine, and two in vivo studies of cardiac development and teratogenicity in non-mammalian animal models. In addition, the NASEM report discussed epidemiology studies of mothers exposed to combusted cigarette smoke during pregnancy and birth outcomes and adverse health outcomes in their children.

The scarcity of studies examining the impact of ENDS on fetal and postnatal development and reproductive health during pregnancy limits predicting health effects of ENDS aerosol exposure on the fetus and pregnant mother. Consequently, the NASEM committee also considered research on the effects of combusted cigarettes and NRT on developmental and reproductive outcomes, which may or may not reflect the actual impacts of ENDS aerosol exposure on fetal and reproductive health, but which the committee could draw on in their assessment of the health risk of ENDS to these outcomes. For example, although there are currently no studies in humans evaluating the effects of nicotine-containing or nicotine-free ENDS on fetal and childhood development and reproductive health, because ENDS often contain nicotine, data examining the effects of nicotine-only exposure on the fetus and young child may also inform the health effects of nicotine exposure via ENDS use.

In the NASEM report,[1] an in vitro study in human embryonic stem cells showed exposure to ENDS aerosol extract inhibited cardiac-directed differentiation, and the effects in ENDS extract exposed cells were less pronounced than combusted cigarette smoke extract exposed cells.[396] Two in vivo studies in mice showed whole-body exposures prenatally or early postnatally to nicotine-containing ENDS aerosol impaired alveolar growth, decreased lung cell proliferation, and delayed alterations in risk taking

behaviors as adults.[397,398] Studies in zebrafish demonstrated exposure to ENDS aerosol extract impaired cardiac development.[396]

The NASEM report also discussed a number of developmental and reproductive studies on nicotine-only exposure. One ex vivo study in prenatal lung tissue reported exposure to nicotine stimulated lung branching through α7 nicotinic acetylcholine receptors, possibly contributing to dysanaptic lung growth.[399] Three additional studies conducted subcutaneous nicotine exposure in rodents and non-human primates during prenatal or early post-natal exposure and showed developmental aberrations. In one of the studies, nicotine exposure in pregnant rhesus monkeys led to offspring with reduced total body weight, alveolar hypoplasia, and upregulation of α7 nicotinic acetylcholine receptors in airway cartilage and vessels of fetal lungs.[400] In addition, prenatal nicotine exposure in mice resulted in decreased forced expiratory flows and decreased airway diameters[401] and both prenatal and postnatal nicotine exposure in mice led to transient changes in lung development, including increased linear intercepts of lungs and decreased vascular endothelial growth receptor 2.

Additional clinical studies of early exposure to combusted cigarette smoke indicate increased incidence of placental abruption, ectopic pregnancy, preterm birth, fetal growth restriction, still birth, infant mortality, sudden infant death syndrome, orofacial clefts, reduced birth weight, bacterial pneumonia, and impaired lung function.[402-409] In addition, prenatal exposure to combusted cigarette smoke was associated with increased likelihood of developing behavioral difficulties (e.g., attention-deficit/hyperactivity disorder)[410] and increased wheezing during childhood.[408,411,412] Very high nicotine levels were detected in neonates of mothers who smoked during pregnancy.[413,414] Also, at least one clinical study of nicotine metabolism found slower metabolism in human fetuses and infants compared to adults, suggesting greater accumulation of nicotine in the fetus or neonate may contribute to the observed toxicities.

Since the NASEM report, several more studies were published addressing developmental and reproductive effects of ENDS exposure specifically, which significantly expand the understanding of this topic. In the Nguyen et al. study, female Balb/C mice were whole-body exposed to ENDS aerosol (0 or 18 mg/mL nicotine) or ambient air (control) for two 15-minute sessions per day for 6 weeks before pregnancy, during pregnancy, and during lactation.[395] Thirteen-week-old offspring of the mothers exposed to ENDS with nicotine aerosol showed short-term memory deficit, and aerosol exposure (regardless of nicotine status) showed reduced anxiety levels and more exploration and locomotor activity compared to sham.[395]

Li et al. (2019) evaluated the impact of the maternal ENDS use on offspring's renal health. This in vivo study sought to determine the effects of (1) replacing combusted cigarette smoke with nicotine containing ENDS and (2) continuous ENDS exposure, during pregnancy and lactation on renal development of male offspring.[415] For replacement studies, a subset of 7 week old female Balb/C mice exposed to combusted cigarette smoke (4 combusted cigarettes/day) for 6 weeks prior to mating, during gestation and lactation were switched to ENDS (0 mg/mL or 18 mg/mL nicotine) until pups weaned. The second set of mice received ENDS with or without nicotine for 6 weeks prior to mating until pups weaned. ENDS replacement improved renal health outcomes, partially restoring renal

development and albuminuria. However, continuous e-cigarette exposure during pregnancy adversely affected renal health, evident by increased markers of ROS, inflammation, and fibrosis in the adult offspring, independent of nicotine.

The impact of ENDS on male fertility is still largely unexplored. Vivarelli et al. (2019) studied the effect of e-liquid aerosol generated from a low-voltage ENDS device on rat testicular functions.[416] Preliminary analyses established a voltage selection of 3.5 V at a resistance of 1.5 Ω, is capable of generating formaldehyde, acetaldehyde and acrolein. Rats exposed 3 hours per day for 28 days to nicotine-free e-liquid exhibited lower testicular weight and high LDH levels. Impaired activity of testicular 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase, key enzymes for testosterone biosynthesis was noted in these animals. This was further associated with down-regulation of sorbitol dehydrogenase, required for maturation of the germinal epithelium of seminiferous tubules and glucose 6 phosphate dehydrogenase essential for gonadal steroid biosynthesis. The pro-oxidative environment in the testicular tissue was reflected by elevated ROS, lipid peroxidation, and protein carbonylation, as well as reduction in the antioxidant capacity. Data from ENDS aerosol exposed animals showed occurrence of double strand breaks in white blood cells, but not in testicular tissue of ENDS exposed rats. Induction of CYP isoforms in particular, the CYP2E1-linked activity doubled in exposed animals. Notably, lipoxygenase, implicated in leukotrienes and ROS generation, and highly expressed in testicular cancer, was up-regulated in ENDS exposed animals. Altogether, the data indicates that ENDS exposure (even at low voltage and nicotine-free conditions) can result in gonadal dysfunction.

In a study by Wawryk-Gawda et al. (2019), male Wistar rats were randomized into three groups and exposed to ENDS aerosol (12 mg/mL nicotine), combusted cigarette smoke or control for 6 weeks.[417] Exposure to ENDS aerosol accelerated the degeneration of testes, reduced fertility with significant malformations in spermatozoa. In addition, ENDS aerosol and combusted cigarette smoke increased apoptosis in testes and induced noticeable degeneration in epididymis. Even though male rat reproductive organs showed a more pronounced effect upon exposure to combusted cigarette smoke, exposure to either tobacco product type led to fertility reduction. Overall, the study found ENDS aerosol exposures are toxic to male reproductive organs, but less so compared to combusted cigarette smoke exposure.

In a study by Chen et al., maternal exposure of female Balb/c mice to ENDS aerosol (KangerTech NEBOX; tobacco flavor, 0 or 18 mg/mL nicotine) for 1 hour/day, 6 weeks prior to mating, during gestation and until the pups were weaned, resulted in increased weight, increased adiposity, and disturbances in the level of central homeostatic control markers in male offspring (NPY and iNOS levels were higher in 0 mg/mL nicotine ENDS compared to sham, and MC4R and Ob-Rb were higher in 0 mg/mL nicotine ENDS compared to 18 mg/mL nicotine ENDS). The authors suggest the effects in male offspring due to maternal exposure to nicotine-free ENDS aerosol may involve inflammation or oxidative stress pathways.[418]

Noel et al. (2020) evaluated lung development and genetic differences among offspring of female BALB/c mice exposed to ENDS aerosol (preconception or during gestation) or to control (filtered air).[419] BALB/c dams were exposed whole body to ENDS aerosols (SCIREQ 3rd generation, 1.5 Ω, 4.2 V; cinnamon

flavored e, 36 mg/mL nicotine; 3 second puff duration, 55-mL puff volume, every 30 seconds, 2 hours/day), for either 12 days before mating plus during gestational days 1 to 19 (preconception group) or only from gestational days 6 to 19 (prenatal group). Dams exposed in the preconception and prenatal groups showed a statistically significant 1.8- and 2.7-fold increases in gene expression of the nicotine receptor (a7nAChR) in the lung, compared to controls. In the offspring, only the preconception exposure group showed a significant 5.6-fold down-regulation of a7nAChR in the lungs compared to air controls. Compared to controls, both preconception and prenatal exposures to ENDS aerosol significantly decreased the offspring birth length and weight. The decreased body weight was sustained through four weeks of age in the offspring exposed prenatally to ENDS aerosol. There was a strong negative correlation between each dam's serum placental growth factor (PIGF) concentration and respective litter birth length, in addition to increased levels of 17-α-estradiol in the serum of the dams. Wingless/integrated (*Wnt*)/β-catenin signaling is essential to lung development, as it is involved in branching morphology and the differentiating of lung cells. Genes associated with *Wnt* signaling were mostly downregulated. The lungs of the prenatal offspring exposed periconceptually to ENDS aerosol, which were in the saccular stage, showed a 3.7-fold downregulation of the Wnt5a gene, and the lung tissue fraction of Wnt5a protein was significantly increased. Using the Ingenuity Pathway Analysis, the downregulated genes were related to decreased growth and proliferation of lung cells. The authors specifically address the usage of cinnamaldehyde, stating that it is a known embryonic and lung toxicant. The authors also suggest that cinnamaldehyde may be primarily responsible for the adverse effects on the developing lung reported in this study.

Conclusions

The NASEM report and subsequently published in vitro and in vivo studies seek to address whether exposure to ENDS aerosols are associated with developmental and reproductive toxicity and adverse health effects. At the time of the NASEM report, very little ENDS-specific data was available. In vivo studies published since reported impaired memory, learning, and motor coordination, and decreased glucose utilization in offspring of female mice and rats exposed to ENDS aerosol. For rats, maternal exposures to ENDS aerosol resulted in reduced maternal uterine artery blood flow and fetal umbilical artery blood flow, decreased expression of genes in the *Wnt* pathway, and decreased body weight and head size were observed in the pups. Male rats exposed to ENDS aerosol showed increased ROS, lipoxygenase, protein carbonylation in testicular tissue, malformations in spermatozoa, and degeneration of the epididymis and testes structure. Other effects such as inflammation, gene expression, ROS, cell dysfunction, and cell death were also observed. Additionally, zebrafish embryos treated with PG, VG, and flavoring compounds exhibited adverse morphological and photomotor behavioral outcomes. Together, these results indicate maternal ENDS aerosol exposure may lead to adverse pregnancy and birth metrics, and alter or impair fetal development, particularly behavioral and neurodevelopment.

Oral

In Vitro and In Vivo Studies

The NASEM report discussed several in vitro studies on the effects of ENDS aerosol exposure on oral toxicity outcomes. Most studies reported on oxidative stress, cytotoxicity, and cell dysfunction after

exposure to ENDS aerosol or e-liquid with varying levels of nicotine on human oral keratinocyte cells. Several studies examined oxidative stress produced by chemicals in the ENDS and indicators of cytotoxicity. Ji et al. (2016) reported increased oxidative stress (measured by decreased glutathione and adenosine triphosphate levels) in human oral keratinocytes.[420] Similarly, studies reported increased ROS, increased Bax expression after 24 hours, increased apoptosis after 48 hours of incubation of gingival fibroblasts with ENDS aerosol with and without nicotine,[421] and increased apoptotic and necrotic cells on cytotoxicity in human gingival epithelial cells from nonsmoking donors.[422] Examining the influence of flavors on cell toxicity, Sundar et al. (2016) reported increased inflammation and DNA damage in a 3D model of human gingival tissues exposed to menthol flavored ENDS aerosol with and without nicotine.[423] A study by Willershausen et al. (2014) found decreased cell proliferation rates and decreased ATP levels in human periodontal ligament fibroblast cells incubated with menthol flavored e-liquid for up to 72 hours, compared to controls (PBS).[424]

In a study by Alanazi et al., human gingival fibroblast cells from never smoker donors were exposed to ENDS aerosol condensate (Smooth Canadian tobacco flavor, 0 or 12 mg/mL nicotine) for 60 minutes a day for 3, 5, and 7 days.[425] Statistically significant dose-dependent decreased cell growth (3 days of treatment) and increased cell death (5 days and 7 days of treatment) decreased fibroblast proliferation, dose-dependent increased apoptosis after 3 days of treatment; and decreased wound healing capacity for treatments with both 0 and 12 mg/mL nicotine ENDS aerosol condensates.[425] Similarly, a study by Rouabhia et al. of Saos2 osteoblast-like cells (a human osteosarcoma cell line) grown on titanium dental implant disks and exposed to ENDS aerosol showed decreased osteoblast growth, decreased F-actin filament networks, decreased alkaline phosphatase activity, increased degradation of mineralized bone tissue, and increased caspace-3 protein—a marker of apoptosis, compared to controls.[426] The authors suggest a potential mechanism leading to dysregulation of osteoblast interactions with the dental implant material may involve the caspase apoptotic pathway.[426]

Human-derived dysplastic oral keratinocytes treated with 10 µM nicotine in vitro showed differing effects of closing of acellular gaps (dysplastic keratinocyte migration) depending on the source of the keratinocytes.[427] Leuk-1 cells taken adjacent to an early invasive tongue squamous cell carcinoma and dysplastic keratinocyte cells taken from a dysplastic tongue leukoplakia from heavy smokers showed increase migration, while normal keratinocytes did not migrate. Nicotine also induced EMT-related changes by increased fatty acid synthase (FASN), which in turn triggers activation of EGFR resulting in migratory phenotype, indicative of EMT.[427] The authors suggest these results are important for current and former smokers that may have oral premalignant lesions.

Ji et al. (2019) evaluated the gene response from ENDS aerosol exposure in normal human oral keratinocytes (NHOK) cells exposed to ENDS aerosol generated from lab-made e-liquid (Mod, 0.5 Ω, 7.5 W; 70% PG, 30% VG, 24 mg nicotine) for four hours.[428] RNA was extracted and used for cDNA microarray analysis and RT-qPCR. Aerosol particle size and morphology was also assessed. The authors identified ~2350 genes to be upregulated and focused on the unfolded protein response (URP) pathway for discussion. The authors speculate how the upregulated genes in this pathway could illicit a toxicological response without supporting data. UPR pathway genes are upregulated at the mRNA level, and these genes are mediated by the PERK - EIF2α - ATF4 and IRE1α - XBP1 pathways, in NHOK exposed to ENDS

aerosol. Although the data is potentially thought provoking, there are many limitations to the study which makes the outcome have limited utility.

In an in vitro study by Cuadra et al. (2019), the growth and viability of four species of oral commensal *streptococci* were determined after exposure to flavorless ENDS aerosol.[429] Peristaltic pumps transported smoke or ENDS aerosol with/without nicotine into chambers containing bacteria on agar plates. The survival and growth of these commensals were assessed as a function of colony counts and sizes. In addition, biofilm formation was examined post-exposure in chambers containing pre-adhered *streptococci* on plastic coverslips. The results demonstrated that flavorless ENDS aerosol had negligible effect on the growth of the species tested. Further studies will determine the impact of flavors on bacterial viability.

Pushalkar et al (2020), utilizing 16S rRNA high-throughput sequencing technology, evaluated the oral microbiota of 119 human subjects, grouped on the basis of smoking habits into combusted cigarette smokers, ENDS users and non-smoker controls.[430] Data indicated the microbial profile in each of the three cohorts was distinct with significantly altered bacterial richness and beta-diversity in ENDS users when compared to never-smokers or combusted cigarette smokers. The salivary microbiome in these cohorts was dominated by eight taxa; *Streptococcus*, *Veillonella*, *Prevotella*, *Neisseria*, *Haemophilus*, *Porphyromonas*, *Rothia,* and *Fusobacterium,* of which abundance of *Veillonella and Porphyromonas* was higher among ENDS users. Analysis of inflammatory markers from the saliva of study participants showed IL-6 and IL-1β to be highly elevated in ENDS users when compared with non-users. To better understand the effect of ENDS use on oral health, human studies were combined with cell culture work. An *in vitro* infection model of premalignant and malignant oral cell lines exposed to ENDS aerosol and challenged by *Porphyromonas gingivalis* and *Fusobacterium nucleatum* resulted in elevated inflammatory response. Taken together, findings from this study suggests ENDS use induces oral environmental shifts resulting in complex heterogeneous microbial biofilms potentially enhancing susceptibility to periodontal disease.

Conclusions

In summary, the NASEM report concluded 1) "there is limited evidence suggesting that nicotine and non-nicotine–containing e-cigarette aerosol can adversely affect cell viability and cause cell damage in oral tissue in non-smokers," 2) "there is limited evidence suggesting that switching to e-cigarettes will improve periodontal disease in smokers," and 3) "there are no epidemiological studies examining the associations between e-cigarette use and incidence or progression of periodontal disease."

Since the NASEM Report was published, additional in vitro and ex vivo studies have been published that provide additional evidence of the impact of ENDS on cell viability and increased oral pathogen infection. The recent studies show compared to controls, exposure to ENDS aerosol extract resulted in increased susceptibility to bacterial infection and microbial biofilms, and increased biomarkers of inflammation and gingival crevicular fluid, as well as peri-implant sulcular fluid. These results suggest ENDS exposure may have new adverse impacts on periodontal health, oral pathogen infection, dental implants, and dental health. Further studies are important to examine the modes of action, short-term

and long-term adverse effects, and human health effects of ENDS exposures on the oropharyngeal system.

Other Physiological Systems

Research in the NASEM Report

The NASEM report and recent in vitro and in vivo studies seek to address whether exposure to ENDS aerosols are associated with toxicity to organ systems other than the respiratory, cardiovascular, and oropharyngeal systems (e.g., ototoxicity, osteotoxicity, and hepatotoxicity). The NASEM report[1] discussed an in vitro study by Rubenstein et al. in which Kupffer cells (liver macrophages) isolated from Sprague-Dawley rats were treated for 48 hours with ENDS aerosol extract (NJOY, OneJoy Traditional Flavor, 1.2% and 1.8% nicotine by volume; and eGo, Desert Sands Flavor, 0, 12, and 18 mg/mL nicotine), combusted cigarette smoke extract (Marlboro Reds), 50 nM of nicotine or lipopolysaccharide (LPS), or controls (not exposed to exogenous compounds). The ENDS aerosol extract treated cells showed increased ROS and inflammatory responses (increased deposition of C1q, C3b, C4d and C5b-9; and increased IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 and IL-13) and increased Kupffer surface cell gC1qR expression compared to controls.[431]

Research Published Since the NASEM Report

Several in vitro studies published after the NASEM report showed exposure to ENDS aerosol or treatment with aerosol condensate: decreased cell viability in middle ear epithelial cells and osteoblast-like cells; increased induction of the collagen osteoblast marker Col1a1 in osteoblast-like cells; and decreased cell viability and metabolic activity, induced morphological changes and inhibited cell proliferation and cell-cell communication in bone marrow derived mesenchymal stem cells. In vivo studies reported increased hepatic steatosis and increased hepatocyte apoptosis in ApoE-/- null mice; and increased ROS, increased inflammation, and reduced wound healing in rats exposed to ENDS aerosols. One more study investigated stress responses in C. elegans and reported no changes from control after ENDS aerosol exposure.[432]

<u>Ototoxicity (in vitro)</u>

Song et al. treated human middle ear epithelial (HMEEC) cells with 0.01–5% dilutions of flavored e-liquids and reported decreased cell viability (for the tobacco and menthol flavors). Cells exposed to e-liquids with nicotine showed further decreases in cell viability. In addition, varying PG:VG ratios may affect viability, as PG content was inversely proportional to overall viability of HMEEC cells.[433] Go et al. (2019) also examined the effect of flavored e-liquids on human middle ear epithelial cells (HMEECs). Their studies demonstrated menthol- and tobacco-flavored e-liquids induced death in HMEECs and increased the levels of inflammatory cytokines.[434] Flavored e-liquids increased the mRNA levels of MUC5AC, MUC5B and MUC4 and decreased the level of genes encoding epithelial sodium channels. Apoptosis and autophagy were induced by menthol- and tobacco-flavored e-liquids in HMEECs, where tobacco-flavored e-liquids caused a greater increase in the autophagosome marker, LC3-II, compared to menthol. Although it provides some insight into the effect of ENDS on middle ear cells, additional experiments are important to elucidate the precise mechanism by which these flavors induce cell death, using physiological doses.

Osteotoxicity (in vitro and ex vivo)

A study by Otero et al. examined exposure to e-liquids as a potential risk factor for poor bone development and osteoporosis. Immortalized osteoblast-like cells, MG-63 and Saos-2, were treated in cell culture for 48 hours with 0.004–4.0% dilutions of 23 commercially available flavored e-liquids with multiple nicotine concentrations (0.001–1.0 mg/mL). With all e-liquids tested, cell viability (MTT assay) decreased in a dose-dependent manner (which was least pronounced in flavorless e-liquids and most pronounced in cinnamon-flavored e-liquids), and cytotoxicity was independent of nicotine. Collagen (Col)1a1, but not runt-related transcription factor 2 (RUNX2) mRNA expression, was upregulated in response to coffee-flavored and fruit-flavored e-liquids. Cells treated with a non-cytotoxic concentration of fruit-flavored (Mango Blast) e-liquid with or without nicotine showed statistically significantly increased Col1a1 mRNA expression compared to culture medium only. Therefore, osteotoxicity may be e-liquid flavor dependent, and increases in Col1a1 expression suggests active remodeling of the matrix tissue. Overall, this study sheds light on osteotoxicity, which was an unexplored endpoint for ENDS use. This study may be important for possible negative effects on bone development with ENDS use in adolescents, given the significant bone development occurs during adolescence and the popularity of flavored ENDS within this age group. The limitations of this study were minor and include ambiguity in experiment replicates and use of immortalized cell lines.[435]

Wavreil et al. (2019) exposed human tumor-derived osteoblast-like MG-63 cells to varying concentrations of e-liquid or ENDS aerosol condensate to evaluate toxicity of ENDS on bone cells.[436] Cells were exposed for either 24 h or 48 h to .004 %, 0.04 %, 0.4 % or 1.0 % of nicotine-free e-liquids or to 0.0025 %, 0.025 %, 0.25 %, 1.0 % or 2.5 % of nicotine-free aerosol condensate (SMOK 220 Watt kit, 60 W; nicotine-free Cinn Candy and Napalm or 50:50 PG:VG (control); 3 second puff with a 27 second interval for 40 puffs).The authors reported reduced cell viability and increased ROS, pronounced with cinnamon-flavored e-liquids. However, no changes in collagen type I protein were observed following exposure to any of the aerosol condensates. Limitations include that the study's exposure methods do not simulate human use and the osteosarcoma cell line is not truly representative, if the study aim was to evaluate the impact of ENDS on bone health. The major takeaway from the study is that cinnamaldehyde produces ROS, as has been reported in other cell types.

In a study by Shaito et al., human bone marrow-derived mesenchymal stem cells (MCS) isolated from healthy individuals were treated with ENDS aerosol extracts (V4L CoolCart, 3.5 Ω; Strawberry flavor, 18 mg/mL nicotine), combusted cigarette smoke extract (3R4F cigarettes, 0.728 mg nicotine/cigarette), or control, and then osteogenic differentiation was stimulated with 50 nM Dexamethasone (Dex) every third day for a total of 14 or 21 days. Treatment with either ENDS aerosol extract and combusted cigarette smoke extracts inhibited MSC proliferation, induced morphological changes, impaired MSC differentiation and inhibited cell-cell communication (decreased N-cadherin and Cx43 mRNA and protein), and showed statistically significant decreased cell growth (trypan blue exclusion assay) and decreased metabolic activity (MTT assay) compared to control. In addition, treatment with ENDS aerosol extract increased ROS production (dihydroethidium (DHE) assay) compared to controls, which was associated with the inhibition of osteoblast differentiation.[437]

Neurotoxicity (in vitro)

Zahedi et al. (2019) used neural stem cells as a model system to study mitochondrial stress response upon exposure to ENDS constituents.[438] E-liquids and aerosols from ENDS induced a stress response in neural stem cells, resulting in mitochondrial hyperfusion and disruption of autophagic flux. The stress induced mitochondrial hyperfusion, presumably serving as a survival response protecting mitochondria from autophagy, was associated with oxidative stress, and mitochondrial DNA aggregation. The study further identified that the mitochondrial changes were mediated by nicotine, and not through volatile organic compounds or solvents. Nicotine-induced superoxide production was suppressed by hindering calcium influx, implicating calcium overload as the contributory factor. The hyperfusion response resulting from ENDS aerosols with low levels of nicotine was associated with increased mitochondrial membrane permeability and motility. In contrast, e-liquids containing higher levels of nicotine induced mitochondrial swelling, with subsequent rupture of mitochondrial membrane, reduced membrane permeability and motility. These data support the notion that the transient survival response mounted by the mitochondria to combat stress may be overwhelmed with chronic exposure. These studies extended to other cell types will demonstrate whether changes in mitochondrial milieu combined with autophagy dysfunction can accelerate cellular aging, leading to morbid outcomes. Chronic and sub chronic exposure studies in vivo and in humans may further validate these observations.

Sifat et al. (2019) studied the effects of nicotine exposure on neuronal glucose utilization employing an in vitro ischemic stroke model.[439] Primary cortical neurons were subjected to oxygen-glucose deprivation by culturing in aglycemic/hypoxic conditions followed by media replacement and reoxygenation to mimic ischemia-reperfusion injury. Short- and long-term nicotine/cotinine exposure decreased neuronal glucose utilization in ischemic conditions. This was associated with attenuated GLUT1 levels, up-regulated α7 nAChR expression and suppressed glycolysis. Reduction in neuronal glucose uptake was reversed by the nAChR antagonist mecamylamine, suggesting a role of this receptor in nicotine-induced modulation of glucose metabolism. Similar results were obtained in ex vivo studies, where brain slices were obtained from six-month-old mice, simulating the aged human population with prior stroke incidence. A seven-day exposure to e-cigarettes significantly reduced glucose uptake in brain slices both under normoxic and ischemic conditions along with down-regulation of GLUT1 and GLUT3 expressions. These findings indicated ENDS could induce a state of glucose deprivation, further compromising the glucose utilization of the ischemic brain, potentially increasing brain injury.

Hepatoxicity (in vivo)

In this study, 8-week-old male Apolipoprotein E null (Apo E -/- null) C57BL/6J mice fed a fat-rich diet were exposed to ENDS aerosol with 2.4% nicotine (Blu PLUS, Gold Leaf Tobacco flavor; puff protocol: 4 s/per puff; 8 puffs/ENDS use episode; with 1 ENDS use episode/30 min for 12 hours) or saline for 12 weeks. Apo E -/- null mice exposed to ENDS aerosol and had higher hepatic fat accumulation (hepatic steatosis). The detrimental effects of ENDS on hepatic steatosis were associated with statistically significantly greater oxidative stress, increased hepatic triglyceride levels, and increased hepatocyte apoptosis—measured by percentage of terminal deoxy-nucelotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL), and cleaved caspase-3 and caspace-9 protein, independent of adenosine monophosphate-activated protein kinase signaling. In addition, hepatic RNA sequencing

analysis revealed 433 genes were differentially expressed in ENDS-exposed mice on a fat rich diet compared with saline-exposed mice. Genes associated with lipid metabolism, cholesterol biosynthesis, and circadian rhythm were most significantly altered in the liver in response to ENDS aerosol exposure. Overall, these results demonstrate adverse effects of ENDS aerosol on the liver and ENDS use may exacerbate the effects of fat rich diet on liver diseases, such as hepatic steatosis. The main limitation of the study was the small sample size (n=6).[440]

Espinoza-Derout et al. (2019) investigated the mechanism through which ENDS aerosols induced oxidative DNA damage in the liver.[441] Male C57BL/6 ApoE$^{-/-}$ mice were exposed to ENDS aerosol (bluCig Plus, Classic Tobacco, 0% or 2.4% nicotine; 4 second puff, 20 second puff interval, 8 puffs/session every 30 minutes, 12 hours/day) or saline for 12 weeks. Hepatocytes of mice exposed to ENDS aerosol with nicotine showed statistically significant increases in DNA damage (assessed a function of apurinic/apyrimidinic sites), reduced NAD+/NADH ratio and increased oxidative stress compared to controls (saline). This was associated with elevated poly (ADP ribose) polymerase (PARP1) activity, reduced Sirtuin 1 levels and stabilization of PTEN-induced kinase 1. Oxidative stress, measured as cellular malondialdehyde concentration, was increased in hepatic cells exposed to ENDS aerosol with nicotine. Mitochondrial DNA, which is sensitive to ROS, showed increased lesions (0.47 lesion/10,000 bases) in mice exposed to nicotine containing ENDS aerosol. TEM studies demonstrated that the hepatocytes of these animals have increased mitochondrial vacuolization and a reduction in cellular organelles. These results suggest that ENDS-mediated NAD+ deficiency may be causally related to hepatic DNA damage and mitochondrial dysfunction. In a follow-up study from the same group, Hasan et al. (2019) investigated if ENDS aerosol exposure can exacerbate hepatic steatosis in a mouse model.[442] ApoE-/- male mice maintained on a western diet were exposed to ENDS aerosol for 12 weeks using an established exposure model system, which delivers nicotine equivalent to that found in human combusted cigarette users. Histological analysis revealed increase in larger lipid droplets and intracellular lipid content, compared with ApoE-/- exposed to saline. Interestingly, ApoE-/- mice exposed to ENDS without nicotine exhibited little or no hepatic lipid accumulation. Mice exposed to nicotine containing ENDS aerosol had significantly higher hepatic triglyceride levels and enhanced oxidative stress. This was associated with increased incidence of hepatocellular apoptosis independent of AMPK signaling. RNA sequencing studies of hepatic tissue identified 433 genes differentially expressed in ENDS-exposed mice. Functional analysis suggested that gene pathways associated with lipid metabolism, cholesterol biosynthesis, and circadian rhythm were significantly modulated. These findings provide insight into the molecular mechanisms involved in ENDS-induced hepatic steatosis.

<u>Systemic ROS, inflammation (in vivo)</u>

A study by Di Biase et al. evaluated the effects of exposure to combusted cigarette smoke (1 cigarette) and ENDS aerosol (Kelvin; 0, 0.6% nicotine; 1 puff/10 seconds) infused into cell medium on peripheral blood mononuclear cells (PBMC) and cells isolated from spleen (splenocytes) and lymph nodes (lymphocytes) from adult Wistar rats.[443] After 24 hours of incubation: 1) ENDS aerosol exposed media caused an increase of nitrites and TBARS, although to a lesser extent than combusted cigarette smoke; 2) the spleen and lymph node cells grown in ENDS aerosol and tobacco smoke exposed medium were able to reduce TBARS but not nitrites present in the medium; 3) PBMC in combusted cigarette smoke

exposed medium were able to reduce nitrites and TBARS more efficiently than spleen and lymph node cells, but released more superoxide anion; 4) combusted cigarette smoke and ENDS aerosol did not influence the PBMC and spleen T-cell subtype populations (CD4+, CD8+); 5) nitrites and TBARS in cells treated with nicotine-free ENDS aerosol gave the same results as unexposed medium, which supports the hypothesis the increase of ROS in ENDS aerosol exposed medium was prevalently due to nicotine. The method of exposure (smoke or aerosol infused into medium) is a limitation of the study, as it may lack relevance to the exposure routes for ENDS and combusted cigarette smoke exposures in humans.

In an ex vivo study evaluating the effects of e-liquids on neutrophils, Hickman et al. (2019) sequentially injected neutrophils isolated from healthy volunteers with 0–5 mM final concentration of the flavoring compounds cinnamaldehyde (cinnamon), ethyl vanillin (vanilla), benzaldehyde (almond or cherry), and isoamyl acetate (banana), followed by 100 ng/mL phorbol 12-myristate 13-acetate (PMA) on a Seahorse XFe24 Flux Analyzer.[444] The effect on neutrophil oxidative burst and phagocytosis was measured. Total oxygen consumption was quantified during oxidative burst, representing the amount of oxygen converted by neutrophils to superoxide. Cinnamaldehyde and ethyl vanillin decreased oxidative burst. Studies using *S. aureus* showed that benzaldehyde, propylene glycol acetal and ethyl vanillin (at 5 mM) impaired phagocytosis. Isoamyl acetate did not affect either measure of neutrophil function. The results supported the notion flavoring chemicals in e-liquids can also form secondary or tertiary reaction products through interactions with various components of the e-liquid, which alter their biological activities and toxicities. In summary, the study provides some data aldehydes in ENDS as flavoring chemicals can negatively impact neutrophil function. Thus, ENDS users may have increased susceptibility to infection and respiratory disease.

In an in vitro study by Zagoriti et al. (2020), mouse 3T3-L1 pre-adipocytes in culture media were exposed to extracts from 48 puffs of ENDS aerosol (EVIC VTC Zenith atomizer, 0.8 Ohm, 16W; 1.2% w/w nicotine), 48 puffs of heated-tobacco aerosol (IQOS regular flavor), 27 puffs of reference 1R6F cigarettes or 3 g/mL of pure nicotine.[445] Combusted cigarette smoke extract exhibited severe adverse cellular effects on pre-adipocyte cell survival in a dose- and time-dependent manner and the ability to differentiate to beige adipocytes, while IQOS and ENDS aerosol exposures exhibited limited or no adverse effects. The results show that IQOS aerosol decreased expression of two adipocyte differentiation genes (Ppar-γ and Resistin). Nicotine solution also did not impact pre-adipocyte differentiation. Thus, only combusted cigarette smoke significantly impacted adipose tissue metabolic function. Combusted cigarette smoke, ENDS, and heated tobacco extracts were not applied at equivalent nicotine concentrations as an equalizing factor even though mentioned in results. In summary, the studied ENDS and IQOS did not impact pre-adipocyte differentiation.

Wound healing (in vivo)

A study Troiano et al. randomized 45 male Sprague-Dawley rats to exposure to ENDS aerosol (Blu, 24 mg/mL nicotine; n = 15) or exposure to combusted cigarette smoke (Marlboro Gold, n = 15) for 30 minutes, twice a day for 30 consecutive days, or negative control (unexposed, n = 15); after 30 days, random pattern dorsal skin flaps were raised and monitored daily for 2 weeks for viability and necrosis.[446] The highest rate of flap necrosis was found in the combusted cigarette smoke exposed group, with a mean (SD) of 68.7% (8.6%), followed by the ENDS aerosol exposed rats, with a mean (SD)

of 65.9% (11.8%); the negative control cohort had the least amount of flap necrosis, with a mean (SD) of 50.8% (9.4%). There was no statistically significant difference in flap necrosis between rats in the ENDS aerosol exposed group and rats in the combusted cigarette smoke exposed group. In summary, there was significantly more dermal necrosis in skin flaps created on the backs of male mice after 30 days of ENDS aerosol or combusted cigarette smoke exposure, suggesting ENDS use and combusted cigarette smoking were equally detrimental to skin wound healing in rats and statistically significantly different than the non-exposed rats.[446]

Conclusions

The NASEM report and subsequently published in vitro and in vivo studies seek to address whether exposure to ENDS aerosols are associated with toxicity to organ systems other than the oropharyngeal, pulmonary, or cardiovascular system. The NASEM report concludes for the in vitro studies of ENDS, "there is substantial evidence that components of e-cigarette aerosols can promote formation of reactive oxygen species/oxidative stress." The recently published in vitro studies provide evidence of ENDS aerosol toxicity in the middle ear, bone cells, and neutrophils, decreased neuronal glucose uptake, and stress responses in neural stem cells. Recent in vivo studies with exposure to ENDS aerosol provide evidence of hepatotoxicity, oxidative stress, and DNA damage in mice. However, ENDS exposures did not significantly impact pre-adipocyte differentiation in mouse adipocytes, and insulin resistance and glucose tolerance in a mouse model. These recent studies present emerging evidence of possible toxicological pathways and adverse effects associated with ENDS exposure.

Conclusions for Section 2.D. Studies Investigating the Physiological Effects of ENDS Use

Several studies indicate the possibility of respiratory effects at both the cellular level (e.g., oxidative stress, ROS formation, cellular dysfunction, cytotoxicity, and inflammation), and the physiological level (e.g., emphysematous lung damage and acute bronchoconstriction). Several in vitro and in vivo studies support the biological plausibility of the impact of ENDS use on hypothesized cardiovascular disease pathways, but further studies are important to determine if ENDS exposure is associated with clinical cardiovascular disease outcomes (e.g., coronary heart disease, stroke, and peripheral artery disease) and subclinical atherosclerosis (e.g., carotid intima-media thickness and coronary artery calcification). Some studies indicate ENDS exposure affects periodontal and dental health, and support the biological plausibility long-term exposure to ENDS aerosol could increase the risk of cancer and adverse reproductive outcomes. Findings suggest maternal ENDS exposure is associated with adverse pregnancy and birth metrics. Recently published studies also indicate evidence of ENDS aerosol related toxicity in ear and bone cells, hepatoxicity in mice, and systemic toxicity in rats. However, more research is important to further understanding in these fields, especially as it relates to humans.

E. STUDIES INVESTIGATING HEALTH EFFECTS OF ENDS USE

Respiratory

Research Published Since the NASEM Report

Since the NASEM report, there have been additional cross-sectional, observational, and case studies related to ENDS use and respiratory diseases, but data on longitudinal respiratory outcomes are still

limited. These more recent studies identify respiratory effects similar to those described in the NASEM report, but they have small sample sizes so conclusions are limited.

There have also been no longitudinal clinical studies on E-cigarette or Vaping Product Use-Associated Lung Injury (EVALI), which was initially recognized in the summer of 2019. A diagnosis of exclusion, EVALI is characterized as an acute or subacute potentially life-threatening pulmonary illness associated with use of ENDS (or vaping products) 90 days or less before symptom onset. Case definition criteria for EVALI have been proposed based upon clinical findings in a cluster of cases reported in July 2019[447,448] and include: a history of ENDS use, use of vaping products, or dabbing within the past 90 days; lung opacities on chest x-ray or computerized tomography (CT) scan; exclusion of lung infection and the absence of a plausible alternative diagnosis. Although vitamin E acetate has been strongly linked to EVALI in patients who vape THC oil,[448] there is currently insufficient evidence to ascertain the potential roles of other chemicals in THC or non-THC products in EVALI cases.[449,450] Additional research is important to establish the definitive etiology and pathogenesis of EVALI, optimal treatment regimens and long-term health outcomes.

In addition to case studies on patients meeting the EVALI case definition criteria, there have been multiple case reports published on the association of previously described respiratory diseases with vaping, including ENDS use. Several studies describe isolated cases of diffuse alveolar hemorrhage (DAH),[451] DAH with bilateral pulmonary emboli, organizing pneumonia, lipoid pneumonia, acute eosinophilic pneumonia, hypersensitivity pneumonitis, respiratory bronchiolitis interstitial disease, giant cell pneumonia, and acute respiratory distress syndrome, following ENDS use.[452] However, since these pathologies have not been seen consistently among EVALI patients in large cluster series and consensus on formal EVALI diagnostic criteria has not yet been reached, these disease processes are currently regarded as alternative diagnoses rather than diverse manifestations of EVALI. Further research is important to elucidate the pathogenesis of these heterogeneous pulmonary injuries and to establish causality between vaping, including ENDS use, and these respiratory diseases.[453]

Polosa et al. conducted a 3.5-year observational cohort study of never smoking ENDS users (n=9) compared to a reference group of never smokers (n=12).[454] Several health measures were obtained including lung function, respiratory symptoms, exhaled breath nitric oxide, and high-resolution CT. No significant changes could be detected over the observation period from baseline in the ENDS users or between ENDS users and controls in any of the health outcomes obtained. While no significant changes were evident, the study was limited by a small sample size and limited duration of ENDS use. The ENDS users in the study were relatively young (mean age 29.7 years) and it is also possible adverse respiratory diseases may manifest in this population over time.[454]

Chaumont et al. reports results for two small controlled trials that evaluated the acute pulmonary effects of "fourth generation" ENDS use (Alien 220 tank box mod with dual coil).[455] The first trial included a within-subjects design of sham ENDS use, PG:VG only, and PG:VG and nicotine using a 60W product. Each condition included single, short-term duration of ENDS use at high wattage. ENDS use (with and without nicotine) induced airway epithelial injury in the small airways and decreased transcutaneous oxygen tension and the effect seems to be driven primarily by PG:VG. The second trial

evaluated smokers exposed to either sham ENDS use or PG:VG (no nicotine) and those exposed to PG:VG had decreased transcutaneous tension of oxygen. These studies were both small, short duration, and did not include flavors. The results indicate the potential for acute pulmonary injury with "fourth generation" ENDS.[455]

Meo et al. conducted a cross-sectional study to evaluate the impact of ENDS on lung function and fractional exhaled nitric oxide (FeNO) among healthy adult males who did not use other tobacco products. ENDS use impaired various lung function parameters and the pattern of impairment exhibited obstructive involvement. The study adds to evidence ENDS use can impact lung function in healthy nonsmokers. Study limitations include small sample size, male gender only, and limited duration of ENDS exposure.[456]

The first published case of acute eosinophilic pneumonia (AEP) was reported by Arter et al. in 2019.[457] An 18-year-old previously healthy female with a two-month history of ENDS use developed fever, cough, difficulty breathing, and respiratory failure. She was ultimately diagnosed with AEP and successfully treated with systemic steroids. In 2020, Antwi-Amoabeng and Islam[458] also reported AEP in a college student (with no significant medical history) only after he vaped cannabis. While studies have reported a relationship between combusted cigarette smoking and AEP, more information is important to further define the relationship between vaping, including ENDS use, and AEP.

Antoniewicz et al. conducted a randomized, cross-over clinical study in 17 healthy, sporadic smokers and found there was an increase in pulmonary flow resistance following use of a nicotine-containing ENDS. This suggests nicotine-containing ENDS use has an acute impact on the conducting airways of the lung. Further investigation is important to replicate the results of this small study and to determine the short- and long-term clinical consequence of these acute changes.[459]

Kerr et al. conducted a randomized, cross-over clinical study in 20 healthy smokers and found peak expiratory flow decreased following nicotine-containing ENDS use, while forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and FEV1/FVC remained the same. The authors postulate the decrease in peak expiratory flow may be a defensive physiologic response against the irritants in the aerosol. While the clinical significance of this finding is unclear, it adds to the evidence ENDS may acutely impact pulmonary function.[460]

Bayly et al., in a cross-sectional study of Florida youth (aged 11–17 years), found secondhand exposure to ENDS aerosol exposure was associated with higher odds of reporting an asthma attack in the past 12 months (consistent with NASEM conclusion 18-5 under harm reduction). The results do not assess causation but also support the NASEM conclusion 11-4 that adolescents who are exposed to ENDS may experience an increase in asthma symptoms. Additional research to assess the association of cough and wheezing for both ENDS users and non-users may further understanding of this issue.[461]

Conclusions

The NASEM report concludes there is insufficient evidence to determine whether ENDS cause respiratory diseases in humans and moderate evidence for increased cough and wheeze in adolescents who use ENDS and an association with ENDS use and an increase in asthma exacerbations. Literature

focusing on ENDS use (rather than dual use) published since NASEM has been consistent with these findings.

The NASEM report concluded (11-1) there is *no available evidence* whether or not e-cigarettes cause respiratory diseases in humans. Since the NASEM report, additional studies, only looking at ENDS use (rather than dual use) have assessed the association between ENDS and pulmonary illness and have been consistent with this conclusion.

Further studies are important to establish causality between ENDS use and pulmonary illnesses including EVALI. Although recent studies strongly link vitamin E acetate with the development of EVALI, the mechanism of EVALI remains unclear and questions about the potential roles for other e-liquid additives persist. In addition, there are currently no formal EVALI diagnostic criteria and the onset of this new syndrome is too recent to assess long term patient outcomes. Further research is important to determine causality between ENDS use and EVALI. Likewise, more studies are important to elucidate any role of ENDS use in the pathogenesis of previously described respiratory diseases.

Cardiovascular

Several recent studies evaluated the effect of ENDS on the cardiovascular system and generally support the NASEM report conclusions. One study[460] did not detect the increase in blood pressure seen by other authors; however, this discrepancy may be due to differences in nicotine exposure and blood pressure assessment protocols. The recent studies also contribute new information to the body of evidence ENDS acutely impact cardiovascular function. Nicotine ENDS use was found to decrease hand microcirculation,[378] increase platelet activation,[462] and increase platelet microparticles.[460] The strength of evidence for these new findings is limited, however, because each study was small.

The following studies are generally consistent with the NASEM report conclusion (9-4 There is *moderate evidence* that diastolic blood pressure increases after nicotine intake from e-cigarettes).

Antoniewicz et al. conducted a randomized, cross-over clinical study in 17 healthy, sporadic smokers and found there was a significant short-term increase in diastolic and systolic blood pressure following use of a nicotine or non-nicotine ENDS.[459] Use of a nicotine-containing ENDS was also associated with a short-term increase in heart rate and arterial stiffness as measured by augmentation index standardized to a heart rate of 75 bpm and pulse wave velocity, while these cardiovascular measures did not significantly change following use of a non-nicotine cigarette. This study provides further evidence ENDS use, particularly with a nicotine-containing e-liquid, has an acute cardiovascular impact; however, the long-term consequences of these short-term effects are unknown.

Kerr et al. conducted a randomized, cross-over study in twenty healthy smokers and found no significant difference in arterial stiffness as measured by augmentation index standardized to a heart rate of 75 bpm.[460] This result conflicts with the findings in the NASEM report and the findings of Antoniewicz et al.[459] Possible explanations for the inconsistency between studies include differences in the nicotine exposure (due to variability in products, e-liquids, and puff protocols), study populations, and measurement protocols. However, in investigating other markers of cardiovascular function, Kerr et al. found the reactive hyperemia index increased after ENDS use, demonstrating that aerosol exposure

effects vasoreactivity. Furthermore, the authors found that platelet microparticles, which are known to be elevated in those with coronary heart disease, increased after ENDS use, adding limited evidence of another short-term cardiovascular impact of ENDS use.[460] Mobarrez, et al. also reported an increase in endothelial and platelet derived extracellular vesicles in healthy volunteers following exposure to ENDS aerosol containing nicotine in healthy volunteers.[463]

Biondi-Zoccai et al. conducted a randomized, cross-over study in twenty healthy smokers and found following nicotine ENDS use, biomarkers of oxidative stress (sNox2-dp, H_2O_2, and 8-iso-PGF2α) and platelet activation (sCD40L and soluble P-selectin) increased, while markers of the antioxidant reserve (vitamin E and HBA) and endothelial function (flow-mediated dilation and NO bioavailability) decreased.[462] Systolic blood pressure also increased following ENDS use. Biondi-Zoccai et al. evaluated the same biomarkers following combusted cigarette and IQOS use. Some of the biomarkers were more negatively impacted by combusted cigarette smoking than by ENDS or IQOS use. This study supports the above NASEM report conclusions and adds new information regarding platelet activation with nicotine-containing ENDS use.[462]

Buchanan, et al. in their review of preclinical and clinical studies on cardiovascular risk associated with ENDS reported ENDS use can induce negative cardiovascular effects through various mechanisms such as oxidative stress, inflammation, DNA damage, arterial stiffness, and altered hemodynamics and platelet activity.[464] These effects suggest pathways leading to cardiovascular disease and corroborate the findings of Biondi-Zoccai et al.[462]

Pywell et al. conducted a cross-over clinical study in seven smokers and eight non-smokers examining the effects of nicotine and non-nicotine ENDS on the microcirculation of the hand.[378] The authors found smokers had significant reductions in superficial and deep hand microcirculation at all investigated time points during and within 20 minutes following use of a nicotine ENDS. Smokers had a significant increase in superficial hand microcirculation, but no change in deep microcirculation following use of a non-nicotine ENDS. In contrast, nonsmokers had no significant change in hand microcirculation following use of either a nicotine or non-nicotine ENDS. The authors postulate reduced nicotine exposure due to inexperience with ENDS may account for this finding of a lack of effect of nicotine on the circulation of nonsmokers. This study demonstrates inhalation of nicotine from ENDS may be responsible for an acute reduction in hand microcirculation in smokers.

Both Antoniewicz et al.[459] and Kerr et al.[460] demonstrated in separate randomized cross-over studies, an increase in heart rate following the use of a nicotine-containing ENDS. These findings support NASEM report conclusion 9-2 (There is *substantial evidence* that heart rate increases after nicotine intake from e-cigarettes). In addition, Antoniewicz et al.[459] and Biondi-Zoccai et al.[462] in two separate randomized cross-over studies, both observed an acute increase in diastolic blood pressure following nicotine-containing ENDS use. These findings support NASEM conclusion 9-3 (There is *moderate evidence* that diastolic blood pressure increases after nicotine intake from e-cigarettes).

Interestingly, Antoniewicz et al. also found an increase in diastolic blood pressure after use of a non-nicotine ENDS, suggesting nicotine is not solely responsible for the effect of ENDS on blood pressure.[459]

In another small cross-over study, Kerr et al. found no change in blood pressure in twenty combusted cigarette smokers following ENDS use,[460] which conflicts with NASEM report conclusion 9-3 and the findings of Antoniewicz et al.[459] and Biondi-Zoccai et al.[462] The discrepancy between the blood pressure results of Kerr et al.[460] and other studies may be due to differences in nicotine exposure and the method of assessing blood pressure.

Conclusions

The NASEM report concluded (9-1) there is *no available evidence* whether or not e-cigarette use is associated with clinical cardiovascular outcomes (coronary heart disease, stroke, and peripheral artery disease) and subclinical atherosclerosis (carotid intima media-thickness and coronary artery calcification) and (9-5) there is *insufficient evidence* that e-cigarette use is associated with long-term changes in heart rate, blood pressure, and cardiac geometry and function. In general, the studies in this section support the NASEM report conclusions regarding ENDS and cardiovascular health effects. However, the findings discussed above were limited by the small sizes of these studies.

Oncology

One oncology study published after the NASEM report supports the NASEM report conclusion (10-3) there is *limited evidence* that e-cigarette aerosol can be mutagenic or cause DNA damage in humans, animal models, and human cells in culture.

Mravec, et al. in their review of ENDS and cancer risk reported potential interconnections between ENDS and cancer, including the stimulatory effect of nicotine containing aerosol on the sympathoadrenal system resulting in cancer induction and progression. In addition, exposure to ENDS aerosol was associated with decreased DNA repair activity and repair proteins in HBEC cultures and mouse lungs.[465] The authors acknowledge further research is important to elucidate potential mechanisms and pathways interconnecting ENDS and cancer.

Further, the NASEM report concluded (10-1) there is *no available evidence* whether or not e-cigarette use is associated with intermediate cancer endpoints in humans. This holds true for comparisons of e-cigarette use compared with combustible tobacco cigarettes and e-cigarette use compared with no use of tobacco products and (10-4) there is *substantial evidence* some chemicals present in e-cigarette aerosols (e.g., formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis. This supports the biological plausibility that long-term exposure to e-cigarette aerosols could increase risk of cancer and adverse reproductive outcomes. There were no additional studies published after the NASEM report evaluating the effects of ENDS use on cancer outcomes to support either conclusion.

Pediatric/Developmental and Reproductive

There were no clinical studies published since the NASEM report evaluating effects of ENDS use during pregnancy or the impact on fetal development reviewed in this section to support either of the following NASEM conclusion statements:

Conclusion 13-1 There is *no available evidence* whether or not e-cigarettes affect pregnancy outcomes.

Conclusion 13-2 There is *insufficient evidence* whether or not maternal e-cigarette use affects fetal development.

Secondhand Exposure

One cross-sectional study published since the NASEM report supports both NASEM report conclusions 18-5 (There is *moderate evidence* that second-hand exposure to nicotine and particulates is lower from e-cigarettes compared with combustible tobacco cigarettes) and 11-4 (There is *moderate evidence* for increased cough and wheeze in adolescents who use e-cigarettes and an association with e-cigarette use and an increase in asthma exacerbations).

Bayly et al. conducted a cross-sectional study of Florida youth (aged 11–17 years) and found secondhand exposure to ENDS aerosol exposure was associated with higher odds of reporting an asthma attack in the past 12 months.[461] The results do not assess causation, but support the NASEM conclusion 11-4 that adolescents who are exposed to ENDSs may experience an increase in asthma symptoms. Additional research to assess the association of cough and wheezing for both ENDS users and non-users may further understanding of this issue.

Oral Health

Since the NASEM Report, few published studies have evaluated the potential impact of ENDS use on periodontal disease. Most were small cross-sectional studies that did not establish a causal relationship between ENDS use and periodontal disease. None specifically evaluated the impact on periodontal disease of switching to ENDS use in combusted cigarette smokers; however, limited evidence suggests the risk of periodontal disease may be lower for ENDS users as compared to combusted cigarette smokers. Higher levels of inflammatory cytokines may suggest an increased peri-implant inflammatory process, which could play a principal role in the progression of peri-implant tissue damage. Additionally, no new clinical studies since the NASEM Report evaluated the effect of nicotine-free and nicotine-containing ENDS aerosol on cell viability and cell damage of oral tissue in non-smokers.

The following studies generally support NASEM report conclusion 12-1 (There is *limited evidence* suggesting that switching to e-cigarettes will improve periodontal disease in smokers).

Mokeem, et al. conducted a cross-sectional study to compare the clinical (plaque index [PI], bleeding on probing [BOP], probing pocket depth [PPD] and clinical attachment loss [CAL]) and radiographic (marginal bone loss [MBL]) periodontal parameters and whole salivary cotinine, interleukin (IL)-1β and IL-6 levels among combusted cigarette-smokers (n=39), waterpipe-smokers (n=40), ENDS users (n=37) and never-smokers (n=38).[466] Clinical and radiographic parameters of periodontal inflammation were poorer in combusted cigarette and waterpipe smokers than ENDS users and never-smokers. Whole salivary IL-1β and IL-6 levels were higher in combusted cigarette- and waterpipe-smokers than ENDS users and never-smokers. Cotinine levels were similar between combusted cigarette, waterpipe and ENDS users suggesting exposure between groups were similar; all user groups' cotinine levels were significantly elevated as compared to never-smokers. There was no difference in PPD, CAL, mesial and distal MBL and whole salivary IL-1β and IL-6 levels among ENDS users and never-smokers.

AlQahtani, et al. compared clinical and radiographic peri-implant parameters (PI, BOP, probing depth (PD), radiographic bone loss (RBL)) and proinflammatory cytokine profiles (tumor necrosis factor-alpha (TNFα), IL-6, and IL-1β in the peri-implant sulcular fluid (PISF) among 40 combusted cigarette smokers, waterpipe-smokers, ENDS users, and nonsmokers and found that PD and RBL were significantly higher for combusted cigarette and waterpipe smokers, as compared to ENDS users.[467] TNFα, IL6, IL-1β were higher for combusted cigarette and waterpipe smokers and ENDS users, as compared to non-smokers; however, it is unclear if there were differences between combusted cigarette and waterpipe smokers and ENDS users.

ArRejaie, et al. compared clinical and radiographic peri-implant parameters (PI, BOP, PD, MBL) and levels of matrix metalloproteinase (MMP)-9 and IL-1β levels in PISF among combusted cigarette smokers (n=32), ENDS users (n=31), and nonsmokers (n=32).[468] MBL was significantly higher in combusted cigarette smokers as compared to ENDS users and nonsmokers ($p < 0.01$). A significant positive correlation was found between IL-1β and MBL in ENDS users ($p = 0.0031$). The authors concluded peri-implant inflammation was more compromised among combusted cigarette smokers than ENDS users and nonsmokers.

Bardellini, et al. evaluated the prevalence and characteristics of oral mucosal lesions (OMLs) in former smokers (n=45) compared to ENDS users (n=45) and found no statistically significant differences in the prevalence of OMLs between former smokers and ENDS users. However, increased prevalence of nicotine stomatitis, a hairy tongue, and angular cheilitis was detected among ENDS users.[469]

Yang et al. systematically reviewed the available research evidence on the oral health impact of ENDS use.[470] Findings from this review suggest switching to ENDS may mitigate oral symptomatology for combusted cigarette smokers, but a wide range of oral health sequelae may be associated with ENDS use. These observations support NASEM conclusions 12-1 and 12-2.

A more recent study by Pushalkar et al. investigated the effect of ENDS aerosol on the oral microbiome and the risk of periodontal infection in ENDS users compared with non-smoker controls and combusted cigarette smokers.[430] Study results demonstrated ENDS aerosols increase pro-inflammatory cytokines (IL-1b, TNF-a, IL-6, IL-8, qPCR) in cells co-infected with periodontal pathogens (*P. gingivalis, F. nucleatum*) suggesting ENDS users have increased susceptibility to infection of these cells and to periodontal disease. These findings appear inconsistent with NASEM conclusion 12-1. However, the study was limited by in vitro experiments done on cell culture models using oral pathogens in lieu of primary cell or 3D oral tissue models. In addition, only three bacteria were used to study the increase in infection after ENDS aerosol exposure.

Karaaslan, et al. also reported findings that appear inconsistent with NASEM conclusion 12-1. Full-mouth clinical periodontal parameters were recorded and gingival crevicular fluid (GCF) samples were collected from 57 subjects divided into three cohorts (combusted cigarette smokers, ENDS users, and former smokers).[471] Combusted cigarette smoking and ENDS use had the same unfavorable effects on the markers of oxidative stress and inflammatory cytokines. Findings for this cross-sectional study were limited by small sample size, self-reporting, and a lack of standardization for ENDS exposure systems.

The studies below support NASEM report conclusion 12-2 (There is *limited evidence* suggesting that nicotine and non-nicotine-containing e-cigarette aerosol can adversely affect cell viability and cause cell damage of oral tissue in non-smokers).

A cross-sectional Turkish study of 81 male volunteers (group 1, n=21 ENDS users) evaluated the effect of ENDS aerosol on voice performance compared with combusted cigarette users (group 2, n=30) and never smokers (group 3, n=30). Subjective (Voice Handicap Index 10 – VHI-10) and objective voice analyses (Praat voice analysis system) found no significant difference in the F0, jitter %, and shimmer % values between groups, but found lower VHI-10 values for e-cig users compared with combusted cigarette users. Overall, the study found the effects of ENDS on voice were mild compared to combusted cigarettes.[472]

Ji et al. investigated the effects of ENDS aerosols on gene expression changes in normal oral keratinocytes (NHOKs). ENDS aerosols induced the unfolded protein response (UPR) in NHOK.[428] This pathway plays a role in restoring homeostasis and assisting protein folding in cells, but can lead to apoptosis under chronic stress and cause cytotoxicity.

The following studies address topics not included in the NASEM Report (i.e., there are no NASEM report conclusions addressing poor dental health among adolescent ENDS users or the efficacy of adjunctive therapies in the treatment of peri-implant inflammation in adult ENDS users).

Cho reported there is insufficient evidence from a single cross-sectional 2016 survey of 65,528 students (n=297 daily users) in South Korea to suggest that when compared to never ENDS users, adolescent daily ENDS users may be at risk for poor dental health (cracked or broken teeth and cheek pain). Differences in 'gingival pain and/or bleeding' were not significant after adjustment for potential confounders.[473]

Al Rifaiy MQ et al. conducted a randomized controlled study of 38 adult male patients, which evaluated the effectiveness of antimicrobial photodynamic therapy (aPDT) as an adjunct to mechanical debridement (MD) to reduce peri-implant mucositis (p-iM) inflammatory response in ENDS users.[474] ENDS users were divided into two groups: Group I receiving MD with aPDT (test group) and Group II receiving MD only (control group). Peri-implant inflammatory parameters including plaque index (PI), bleeding on probing (BoP), and pocket depth (PD) were assessed at baseline and 12-weeks follow-up. The two groups were compared. Scores of peri-implant inflammatory parameters including PI and PD reduced significantly among patients in aPDT group compared with MD alone at 12-weeks follow-up. There was a significant reduction in PI ($p < 0.001$) and PD ($p < 0.001$) between the 2 groups. Results found aPDT was more effective compared to MD alone in treating p-iM in individuals using ENDS.

Additional Findings

The NASEM report did not include studies on mental health or depressive symptoms. In one study, Lechner et al. conducted a longitudinal survey of 2460 subjects (completers) to evaluate the association of ENDS use with depressive symptoms in adolescents in Los Angeles (mean age at baseline = 14.1; 53.4% female; 44.1% Hispanic, who had never previously used combusted cigarettes or ENDS).[475] ENDS and combusted cigarette use was measured by self-reported surveys and compared against depressive symptom scales (Center for Epidemiologic Studies–Depression Scale, CES-D) and covariates

(demographics, other tobacco use, alcohol intake). Descriptive analysis was performed to evaluate at wave 3 the association between the frequency of ENDS and combusted cigarette use vs. the pattern of depressive symptoms. A bi-directional association of depressive symptoms with ENDS use onset across mid adolescence was observed. Elevated symptoms of depression predicted subsequent initiation of both combusted cigarettes and ENDS, as well as dual use of combusted cigarettes and ENDS. Also, sustained ENDS use was associated with an acceleration of growth in depressive symptoms over time.

Biomarkers of Potential Harm

Studies published after the NASEM report exclusively targeting biomarkers of potential harm (BOPH) as clinical endpoints were not identified. Although a number of studies on biomarkers associated with ENDS use have been published since the NASEM report, further ENDS research specifically targeting BOPH is important. In general, BOPH are more closely aligned with health effects than BOE. The NASEM report concluded (7-1, 7-2, and 9-4):

Conclusion 7-1. There is *substantial evidence* that e-cigarette aerosols can induce acute endothelial cell dysfunction, although the long-term consequences and outcomes on these parameters with long-term exposure to e-cigarette aerosol are uncertain;

Conclusion 7-2. There is *substantial evidence* that components of e-cigarette aerosols can promote formation of reactive oxygen species/oxidative stress. Although this supports the biological plausibility of tissue injury and disease from long-term exposure to e-cigarette aerosols, generation of reactive oxygen species and oxidative stress induction is generally lower from e-cigarettes than from combustible tobacco cigarette smoke; And,

Conclusion 9-4. There is *limited evidence* that e-cigarette use is associated with a short-term increase in systolic blood pressure, changes in biomarkers of oxidative stress, increased endothelial dysfunction and arterial stiffness, and autonomic control.

While no studies exclusively studying BOPH were published since the NASEM report, two studies published since the NASEM Report compared both BOE and BOPH levels in exclusive ENDS user and combusted cigarette smoker populations.[281,300]

Oliveri, Liang, and Sarkar (2019) analyzed levels of biomarkers of exposure and biomarkers of potential harm obtained from urine and serum samples from 73 exclusive combusted cigarette smokers and 144 exclusive ENDS users.[281] ENDS users had lower levels of exposure to nicotine, NNAL, acrolein, and carbon monoxide measure than adult combusted cigarette smokers. They also had lower levels of 11-dehydrothromboxane-B2, a biomarker of platelet activation; 8-epi-prostaglandin F2α, a marker of oxidative stress; and soluble intercellular adhesion molecule-1, a marker of endothelial function.

A cross-sectional study by Sakamaki-Ching et al. (2020) compared levels of urinary biomarkers of exposure (cotinine and numerous metals), early effect (metallothionein), and biomarkers of potential harm (8-hydroxy-deoxyguanosine [8-OHdG] and 8-isoprostane) in a cohort of age- and sex-matched exclusive ENDS users (n = 20), non-smokers (n=20), and combusted cigarette smokers (n = 13) from Buffalo, NY.[300] Exclusive ENDS users had 3.3-fold higher levels of metallothionein, 2-fold higher levels of

8-OHdG (a marker of oxidative stress and DNA damage), and 1.8-fold higher levels of 8-isoprostane (a marker of lipid peroxidation) compared to non-smokers, but no statistically significant differences in biomarker levels compared to combusted cigarette smokers. For the oxidative stress biomarkers (8-OHdG and 8-isoprostane), women had significantly higher levels than men, and older participants (≥ 41 years old) had higher levels than younger participants (<40 years old). In ENDS users, urinary cotinine was correlated with increased metallothionein, 8-OHdG, and total metals in urine, and total metals and zinc in urine were correlated with increased urinary 8-OHdG. The authors note increased levels of metallothionein and increases in markers of oxidative damage in the urine of ENDS users suggests the potential for adverse effects from ENDS use. Additionally, the age and sex differences in urinary levels of markers of oxidative damage, suggest ENDS use in older populations and pregnant women may have differential risk.

Synopsis of Adverse Experiences (AEs) Associated with ENDS use:
Additional case reports of AEs indicate ENDS can explode and cause burns. These case reports support NASEM report conclusion 14-1 (There is *conclusive evidence* that e-cigarette devices can explode and cause burns and projectile injuries. Such risk is significantly increased when batteries are of poor quality, stored improperly or are being modified by users) and are discussed in Section 2F "Case Reports of Adverse Experiences (AEs) Associated with ENDS Use: AEs Associated with ENDS Batteries".

Case reports of AEs following intentional and unintentional exposures to e-liquid continue to be reported, which support NASEM report conclusion 14-2 (There is *conclusive evidence* that intentional or accidental exposure to e-liquids (from drinking, eye contact, or dermal contact) can result in adverse health effects including but not limited to seizures, anoxic brain injury, vomiting, and lactic acidosis). Since the NASEM report, conclusion 14-3 (There is *conclusive evidence* that intentionally or unintentionally drinking or injecting e-liquids can be fatal) has been corroborated by case reports on deaths associated with drinking or injecting e-liquids. The case reports reviewed after the NASEM report are discussed in Section 2F "Case Reports of Adverse Experiences (AEs) Associated with ENDS Use: AEs Associated with Exposure to E-liquids".

Additionally, a number of case reports on adverse experiences associated with ENDS use have been published, which were not included in the NASEM report conclusions. These are discussed in Section 2F "Case Reports of Adverse Experiences (AEs) Associated with ENDS Use: AEs Associated with ENDS Use".

Conclusions for Section 2.E. Studies Investigating Health Effects Associated with ENDS Use
Studies do not indicate a direct correlation between ENDS and known respiratory diseases (including EVALI). These findings may change with the increase in reporting of ENDS related lung injuries across the country, but not enough information has been reported thus far to strengthen the correlation between ENDS and respiratory disease. There is also an association between ENDS use and an accelerated growth in depressive symptoms over time in adolescents.

F. CASE REPORTS OF ADVERSE EXPERIENCES (AEs) ASSOCIATED WITH ENDS

AEs Associated with ENDS Use

A number of case reports have been published on adverse experiences associated exclusively with ENDS use. An example is case reports describing seizures following ENDS use.[476,477] Other examples of reported adverse health outcomes include vaping, including ENDS use-associated: epiglottitis (inflammation of the flap at the base of the tongue),[478] cardiac effects (e.g., acute coronary syndrome, acute cardiomyopathy and spontaneous coronary artery dissection[479]), psychosis,[480] and unintentional magnet reversion of an implanted cardiac defibrillator.[481]

AEs Associated with Exposure to E-liquids

In the NASEM report,[1] 19 poisoning cases from oral or dermal e-liquid exposure were identified. Twelve cases were reported as intentional, and six were reported as unintentional. Several of the unintentional exposures involved young children at home. Three deaths from these e-liquid exposures were reported, in addition to non-fatal consequences, including vomiting, lactic acidosis, and other outcomes. The NASEM report also included information from poison control centers and other surveillance centers reporting cases for exposure to e-liquids. For example, from September 2010 to February 2014, U.S. poison control centers recorded 2405 calls related to ENDS exposures, 51% of which involved children age 5 and younger. From January 2012 to April 2015, the number of calls to U.S. poison control centers for ENDS exposures increased by a factor of 15. The NASEM committee found conclusive evidence that oral, dermal, or ocular exposure to e-liquids containing nicotine can cause adverse health effects, including seizures, anoxic brain injury, vomiting, and lactic acidosis, and can even be fatal.[1]

Since the NASEM report was published, additional case reports and studies of e-liquid exposure were published. Wylie published a retrospective analysis of Australian Poisons Information Centers and found the number of calls about ENDS exposures increased considerably from 2009–2016, with the largest increases in 2013 and 2016. The overall call volume was stable from year to year; 38% of the calls were regarding accidental child exposure to e-liquid, and 62% were related to adult use, misuse, and unintentional exposures to e-liquids.[482]

Several published case reports of intentional and unintentional adult e-liquid ingestion were associated with reversible and irreversible adverse health outcomes similar to those previously identified in the NASEM report, including bradycardia, severe weakness, dyspnea,[483] acute heart failure,[484] cardiac arrest, brain hypoxia, and death.[485,486]

Hughes et al. published a prospective study of the Oregon Poison Center, which reported seven unintentional cases of ocular exposure to e-liquids, with all seven reported cases mistaking the e-liquid for eye drops. Mild chemical injury occurred in six of the seven cases, but no systemic toxicity was reported, and symptoms improved or resolved after flushing the eye with water.[487] A similar case of accidental ocular exposure resulted chemical burn of the cornea. The author noted that alkaline (basic pH) burns may be more detrimental to the eye than acid burns because the substances are lipophilic and penetrate faster into the eye.[488]

Demir et al. published a report of a new adverse health effect identified when a 6-year-old child ingested approximately 8.4 mg of nicotine from 7 mL of e-liquid in a bottle. After the child developed nausea and vomiting symptoms, the child received a gastric lavage. About 24 hours after the exposure, the child developed sudden sensorineural hearing loss in both ears, a rare condition in children. After 10 days of treatment, hearing improved but did not fully recover. After 6 months, test results were the same as those from the 10th day.[489]

In summary, the case reports and studies published after the NASEM report was published suggest intentional or accidental exposure to e-liquids may be associated with signs and symptoms related to acute nicotine toxicity. Further information is important on the role of PG and VG as potential contributors to adverse health outcomes associated with e-liquid intoxication.[490] There have also been sporadic case reports of seizures,[491] epistaxis, ocular irritation, allergic dermatitis (allergy to nickel component of an ENDS),[492,493] and sensorineural hearing loss[489] associated with oral, ocular, or dermal e-liquid exposure. Of note, children exposed to e-liquids are 5.2 times more likely to be admitted to a hospital and 2.6 times more likely to experience a severe health outcome.[491]

AEs Associated with ENDS Batteries

The majority of ENDS are powered by a manufacturer-supplied rechargeable or non-rechargeable unit**Error! Bookmark not defined.**.[1] However, based on an analysis of the CTP AE IMAGE database, a greater number of reported ENDS fire and explosion injuries occur via ENDS powered by user-replaceable batteries. The risks of battery overheating, fire, and explosion in ENDS may be mitigated via manufacturing quality control to avoid defects and by battery management systems (BMS) such as protective circuits and controls. Between 2009 and 2016, 195 ENDS incidents of fires and explosions were reported by the media according to the National Fire Data Center (NFDC) of the U.S. Fire Administration, with the rate of occurrence rising sharply with the ENDS sales trends such that over half of the ENDS incidents occurred in 2016 alone.[494] Moreover, data evaluated from the National Electronic Injury Surveillance System (NEISS) showed an estimate of 2,000 emergency room visits in the U.S. between 2015 and 2017 due to battery-related ENDS incidents.[495] The NASEM report[1] suggested there is conclusive evidence of burn and projectile risk posed by ENDS batteries, and this risk is increased with improper user battery practices. Further analysis of recent ENDS literature, as well as user and media reported incidents, suggest there are additional risks of battery failure including fires, explosions, projectiles, and death due to the close proximity of the product to the body during usage, storage of the product in pockets, the likelihood of projectiles during failure, and the accessibility of the core battery cells.

Although lithium ion batteries are generally safe, manufacture-related defects or long-term damage from improper use may lead to battery failure or overheating, resulting in a chain reaction known as thermal runaway. Thermal runaway has been identified as the most immediate threat in ENDS battery AEs , particularly because of the metal enclosure of ENDS batteries typically used, allowing for the dangerous build-up of gases.[496] Additionally, the operating conditions of ENDS pose additional stresses to lithium ion batteries, such as external heat sources that can accelerate failure in cells with defects,[497] or carbon build-up on the atomizer that can cause changes in resistance.

There is ample evidence of battery failure and resulting burn injuries from ENDS in literature, which has been compiled into the NASEM report. A review of 46 case studies in the NASEM report, along with statistics from the NFDC, NEISS, and CTP AE IMAGE database, identifies the following trends regarding ENDS battery-related injuries:

- ENDS or spare battery within a pocket was the leading cause of ENDS incidents (~30%)**Error! Bookmark not defined.**,[497] with some documented to occur from contact with metals such as coins and keys[495]
- ENDS with accessible batteries had higher incidences of acute and serious injuries, despite consisting of only 2% of the 2018 U.S. ENDS market[498]
- 133 of the 195 incidents in the 2017 NFDC report were classified as acute injuries, and 38 as 'severe and requiring hospitalization'[497]
- Burns to the thigh and genitalia were the most frequently reported[499-504]
- There are reports of projectile injuries, including facial trauma[505-507] and two deaths[508,509]
- There are reports of ENDS batteries exploding in a charger and one burn report due to overheating of the coil rather than the battery components of the ENDS[510]

Various classification systems for ENDS related injuries have been proposed to improve patient management, such as classifying by direct injuries to hand, face, waist or groin, and from inhalation, as well as indirect injuries from house fires and smoke inhalation.[511] Four types of ENDS battery-related burn mechanisms have been proposed, consisting of thermal burns with flames, overheat burns, blast injuries, and chemical burns. Chemical or mixed burns are characterized by an increase in pain after rinsing and alkaline pH within lesions, suggesting contamination by lithium-ion deposits.[512] One recent study evaluated the management of oral cavity burns secondary to ENDS explosions. Claes, et al. reported 2 cases of combined flame and chemical partial thickness burns in the oral cavity caused by ENDS.[513] Both patients responded to rapid enzymatic debridement followed by conservative treatment with allografts and dressing changes.

The above trends and sources of risk posed by battery or electrical failures in ENDS have only been analyzed retroactively through case reports. Therefore, additional information regarding ENDS battery safety is important, including unreported or other injury risks, risks to non-users, the brand, health, and user modifications of the ENDS and its batteries during failure events, and product usage behaviors leading to failure.

Conclusions for Section 2.F. Case Reports of Adverse Experiences (AEs) Associated with ENDS

Various adverse experiences have been reported related to ENDS. ENDS use has been associated with case reports of seizures, epiglottitis, and cardiac health outcomes (e.g., acute coronary syndrome, acute cardiomyopathy, and spontaneous coronary artery dissection). Oral, dermal, or ocular exposure to nicotine-containing e-liquids can be fatal or cause adverse health outcomes, such as seizures, anoxic brain injury, vomiting, and lactic acidosis. Studies also indicate a possible association between ingesting e-liquid and pediatric bilateral sudden sensorineural hearing loss, and allergic contact dermatitis after touching nickel-containing components of an ENDS or the e-liquid. Additional cases of ENDS fires or explosions appear to be related to the ENDS batteries, but some cases are caused by the overheating of

the coils. The reported battery incidents often occurred during improper storage of batteries or ENDS (e.g., held in pocket, next to other metal objects) or during charging and some resulted in health outcomes including burns (of varying degrees), projectile injuries (including facial trauma), and even deaths (from projectiles). Many of these incidences may be the result of user practices (e.g., loose batteries held in pocket, incompatible battery used in device, incompatible charger used to charge device) rather than product design issues. Additional research is important to determine how adverse experience case reports relate specifically to ENDS use or specific products and further understand mitigation approaches for such adverse experiences.

SECTION 3. POPULATION HEALTH RISKS OF ENDS

A. PREVALENCE OF ENDS USE

By Population

Youth

ENDS started gaining popularity in the U.S. marketplace around 2007, and since 2014, they have been the most commonly used tobacco product among U.S. youth.[514] ENDS use among U.S. middle and high school students increased 900% during 2011-2015, before declining for the first time during 2015-2017. Among high school students, current ENDS use increased from 1.5% of students in 2011 to 20.8% in 2018 ($p<0.001$).[4] Current ENDS use increased 78% among high school students during the past year, from 11.7% in 2017 to 20.8% in 2018. In 2018, more than 3.6 million U.S. youth, including 1 in 5 high school students and 1 in 20 middle school students, currently use ENDS.[4]

The most recent national estimates of current youth ENDS use come from the 2019 NYTS. Overall, 20.0% (95% CI: 18.6%-21.6%) of middle and high school students are estimated to have used ENDS in the past 30-days. By school type, 27.5% (95% CI: 25.3%-29.7%) of high school students and 10.5% (95% CI: 9.4%-11.8%) of middle school students were current ENDS users in 2019.[515]

Demographics

In NYTS 2019, the proportion of middle and high school students who used ENDS did not vary by sex: 20.0% of female students and 20.1% of male students.[515] Current ENDS use was most common in White, non-Hispanic students (23.1%), followed by Hispanic students (18.7%), Black, non-Hispanic students (13.6%) and Other, non-Hispanic students (13.6%).[515]

Smoking Status

In NYTS 2019, dual use of combusted cigarettes and ENDS is less common in youth than adults. Most students who were current ENDS users were exclusively using ENDS: 63.6% (95% CI: 59.3%-67.8%) of high school ENDS users and 65.4% (95% CI: 60.6%-69.9%) of middle school ENDS users.[516] Among students who use multiple tobacco products, ENDS were the most commonly used product in combination with other tobacco products: 17.2% reported current use of ENDS and cigars, 13.3% reported current use of ENDS and combusted cigarettes, and 9.8% reported current use of ENDS and smokeless tobacco.[515]

Frequency of Use

An estimated 30.4% of middle and high school student ENDS users reported frequent use (i.e., use on ≥20 of the past 30 days).[515] By school type, 34.2% (95% CI, 31.2%-37.3%) of high school student ENDS users and 18.0% (95% CI, 15.2%-21.2%) of middle school student ENDS users reported frequent use.[516] Among current ENDS users, 21.4% of high school users and 8.8% of middle school users reported daily ENDS use.[516]

Adults and Young Adults

Demographics

The most recent national estimates of current ENDS use in adults aged 18 years and older come from the 2018 National Health Interview Survey (NHIS). In 2018, an estimated 3.2% (95% CI: 3.0%-3.5%) of adults have used an ENDS in the past 30-days (i.e., current ENDS users).

By age group, ENDS use in 2018 was most common among young adults aged 18-24 years (7.6%, 95% CI: 6.1%-9.1%).[517] The prevalence of current ENDS use decreases with age: 4.3% (95% CI: 3.7%-4.8%) for those aged 25-44 years, 2.1% (95% CI: 1.8%-2.5%) for those aged 45-64 years, and 0.8% (95% CI: 0.6%-1.1%) for those aged 65 years and older.[518]

By gender, current ENDS use in 2018 was more common in men (4.3%, 95% CI: 3.8%-4.8%) than women (2.3%, 95% CI: 2.0%-2.6%).[518] By race or ethnicity, current ENDS use is most common in Other race or ethnicity groups (5.7%, 95% CI: 3.6%-7.7%), followed by Non-Hispanic Whites (3.7%, 95% CI: 3.3%-4.1%), Hispanics (2.5%, 95% CI: 1.7%-3.3%), Non-Hispanic Asians (2.2%, 95% CI: 1.2%-3.2%), and Non-Hispanic Blacks (1.6%, 95% CI: 1.1%-2.2%).[518]

Smoking Status

The 2018 NHIS found adult current combusted cigarette smokers are more likely than former and never combusted cigarette smokers to use ENDS. For adults 18 years and older, the estimated prevalence of current ENDS use in 2018 among current combusted cigarette smokers is 9.7% (95% CI: 8.5%-10.9%), among former combusted cigarette smokers is 5.5% (95% CI: 4.7%-6.3%), and among never combusted cigarette smokers is 1.1% (95% CI: 0.9%-1.3%).[518] For young adults aged 18-24 years, 22.1% (95% CI: 14.5%-29.7%) of current combusted cigarette smokers, 36.5% (95% CI: 24.0%-49.0%) of former combusted cigarette smokers, and 4.6% (95% CI: 3.4%-5.8%) of never combusted cigarette smokers currently use ENDS.[517] Data from the 2015-2016 National Health and Nutrition Examination Survey also found current ENDS use is highest among combusted cigarette smokers.[519]

When looking at smoking status among current ENDS users, similar findings were observed in a national probability sample from the GfK KnowledgePanel: 40.8% of current ENDS users were current smokers, 32.7% of current ENDS users were former smokers, and 27.3% of current ENDS users were never smokers.[520] Smoking status among ENDS users has not been published for the 2018 NHIS. However, in Wave 3 (2015-2016) of the PATH Study, 58.9% of adult current ENDS users were current combusted cigarette smokers (40.5% were daily smokers and 18.4% were non-daily smokers), 8.0% were former smokers who quit ≤1 year ago, 15.8% were former smokers who quit more than a year ago, and 17.3% were never smokers.[521]

Data suggest most dual users are trying to quit smoking. Among adults aged 25 years or older in Wave 2 of the PATH Study (2014-2015), smoking status of ENDS users was as follows: 48.5% were current smokers that tried to quit in the past year, 19.5% were current smokers who did not try to quit smoking in the past year, 28.4% were former combusted cigarette smokers, and only 3.6% were never combusted cigarette smokers.[522]

Frequency of Use

Dual users are a heterogenous group with differences in frequency of combusted cigarette smoking (daily vs. non-daily), frequency of ENDS use (daily vs. non-daily), and changes in combusted cigarette smoking patterns (decrease in CPD, increase in CPD, no change). That heterogeneity needs to be considered when evaluating dual use. In Wave 1 of the PATH Study, the majority of dual users smoked combusted cigarettes daily and used ENDS some days (69.6%).[523] Less common dual use patterns were some day combusted cigarette smoking and someday ENDS use (14.6%), daily smoking and daily ENDS use (9.9%), and some day smoking and daily ENDS use (5.9%).[523]

Frequency of ENDS use may vary by smoking status. In data collected during 2016 and 2017 from the GfKs KnowledgePanel, daily ENDS use was reported more frequently in current ENDS users who are former smokers (67.1%) than in current dual users (15.3%).[524] In a convenience sample recruited from social media in 2014, former smokers who completely switched to ENDS used ENDS on more days and reported more puffs per day than never smokers or current smokers using ENDS.[525]

Vulnerable Populations

In addition to the vulnerable population of youth, other vulnerable populations to consider for ENDS use could include pregnant women, LGBTQ youth and adults, and people with a history of mental health problems. ENDS may provide benefits and risks to certain vulnerable population groups. Similar to other population groups, the benefits and risks of ENDS for vulnerable groups would include those currently smoking combusted cigarettes or using combusted tobacco products, and non-users.

Pregnant Women

ENDS use among pregnant women appears to be similar to ENDS use among all women of reproductive age. Based on 2017 data from the NHIS women of reproductive age, 3.6% of pregnant women and 3.3% of non-pregnant women reported ENDS use on some days or every day.[526] Additional analyses using this same dataset showed ENDS use among pregnant women is higher among those aged 18-24 years in comparison to those aged 25-44 years, and this pattern was not observed among nonpregnant women.[527] Among pregnant women who smoke combusted cigarettes, 38.9% reported ENDS use somedays or everyday compared to 13.5% of non-pregnant women who smoke.[526] Among pregnant and non-pregnant women who had never smoked, ENDS use somedays or everyday was extremely low; 0.3% of pregnant women and 0.7% of non-pregnant women reported ENDS use.

A study of 34,918 women sampled in 2015-2016 from 30 states found 1.2% of women used ENDS during the last three months of pregnancy, and of those women who used combusted cigarettes during pregnancy, 9.7% also used ENDS, in comparison to 0.5% of those women who did not use combusted cigarettes during pregnancy.[528] In Wave 1 PATH data (2013-2014), 4.9% of pregnant women reported

current use of ENDS.[529] Data collected in 2015-2018 from 1,365 low-income, diverse pregnant woman (aged 16–45 years) who did not use tobacco products other than ENDS or combusted cigarettes showed 4.0% of pregnant women used ENDS, of which 74.0% were dual users of ENDS and combusted cigarettes.[530] Data from the 2015 Pregnancy Risk Assessment Monitoring System for Oklahoma and Texas indicated 7.0% use of ENDS around the time of pregnancy, and 1.4% during the last three months of pregnancy, with 38.4% of pregnant women who use ENDS reporting using nicotine in their products.[531] Overall, these estimates suggest pregnant women use ENDS during pregnancy, with a likely prevalence of ENDS use among pregnant women between 3.6% and 4.9%, and women who smoke combusted cigarettes during pregnancy were more likely to use ENDS, in comparison to their non-smoking peers.

Other samples provide estimates of ENDS use prevalence among pregnant women. An online survey of 445 pregnant women administered through Amazon Mechanical Turk (MTurk) found 6.5% of pregnant women use ENDS solely and 8.5% of pregnant women use both ENDS and combusted cigarettes.[532] Mark et al.[533] surveyed 316 pregnant women sampled from an outpatient clinic in Maryland, of whom 13.0% had ever used ENDS and 0.6% were current daily users of ENDS, finding 43.0% of ever ENDS users and 14.0% of never ENDS users were current combusted cigarette users. In a recent study by Wedel et al.[534] of 85 pregnant, current smokers recruited in Oklahoma from a perinatal center, 5.9% reported concurrent use of ENDS in addition to combusted cigarettes.[534] Out of 103 pregnant smokers screened for a smoking cessation trial from 2012-2016, 53.0% reported having ever tried ENDS with ten women reporting using ENDS during the first trimester.[535] The research suggests dual ENDS and combusted cigarettes use occurs within the population of pregnant women and may be more prevalent than exclusive ENDS use. Pregnant women who smoke combusted cigarettes have higher rates of ENDS use in comparison to their non-smoking peers.

Populations with Substance Abuse and Mental Health Conditions

There are a variety of studies demonstrating increased odds of ENDS use among those with mental health issues, such as internalizing and externalizing behaviors.[536-543] There is limited information, however, on ENDS use among those with a diagnosed mental health condition, such as ADD, depression, bipolar disorder, or schizophrenia or ENDS use among these conditions by smoking status. Similar to combusted cigarettes, those with mental health issues or conditions are likely to be at increased risk of ENDS use.[544]

A study using 2016 data from NHIS of adults, found 0.2% of adults had chronic ADD, bipolar, schizophrenia, or other disorder and 3.1% of all adults in the study were current someday or everyday ENDS users.[545] In the study, there was a higher prevalence of current ENDS use among those with chronic mental illness: 11.7% of those with chronic ADD, bipolar, schizophrenia, or other disorder and 7.7% of those with chronic depression, anxiety, or an emotional problem were current ENDS users. In contrast, current ENDS use was only reported in 2.9% of those who did not have a chronic mental illness, suggesting those with a mental health issues or condition may have 2-3 times higher odds of current ENDS use than those without a mental health issue or condition.[545]

A cross-sectional study examining ENDS use and depression found someday and everyday ENDS users had higher odds of reporting a history of clinical diagnosis of depression compared with participants who never used ENDS.[546] Among never combusted cigarette smokers, current ENDS users had 2.16 times (95% CI, 1.87-2.49) higher odds of reporting clinical depression compared with never ENDS users.[546] There also appeared to be a dose-response between never/someday/everyday use and clinical diagnosis of depression with daily users having the highest odds of current ENDS use.[546]

Research supports an association between tobacco product use and substance use disorders, such as suggested by the Cho et al. study cited above. A meta-analysis of 32 articles published through March 2019 found the cross-sectional association of ENDS use with alcohol use was significant for youth (aged 13.6-18, weighted mean effect size for alcohol use: *OR* =4.50, 95%CI = 3.31 to 6.13, and for binge drinking: *OR* = 4.51, 95% CI: 3.13 to 6.51).[547] This meta-analysis also found a significant association between ENDS use and marijuana use for youth (*OR* = 6.04, 95% CI: 3.80 to 9.60).[547] Further, this meta-analysis found similar effects for adults, albeit with smaller effect sizes (alcohol use: *OR* =1.57, 95% CI: 1.25–1.99; binge drinking: *OR* = 1.63, 95% CI: 1.14 to 2.35, and marijuana use: *OR* = 2.04, 95% CI: 1.53–2.73).[547]

Youth PATH Wave 1 data from 2013-2014 show ENDS users, as well as users of many other tobacco products, have higher odds of use of alcohol, marijuana, Ritalin/Adderall, painkillers/sedatives, and any other drugs in comparison to youth who do not use ENDS.[536] Youth PATH Wave 1 data additionally suggest those youth who are polytobacco users have higher substance use in comparison to single product users.[536] Similar results were found among youth across three waves of the PATH study (data collected 2013-2016), where past 30-day ENDS use exhibited an association with increased substance use disorder symptomatology.[540] In another study using youth data from PATH 2013-2015, past year use of alcohol was associated with ENDS initiation and dual use initiation, whereas past year use of marijuana was significantly associated with initiating dual use of ENDS and combusted cigarettes, but not ENDS alone.[538] National Youth Risk Behavior Surveillance System (YRBS) data from 2017 suggest youth who are dual users or ENDS-only users are more likely than non-tobacco users to engage in alcohol and illicit drug use.[548]

Research on adults similarly suggests people with substance use disorders have high rates of ENDS use. National Epidemiologic Survey on Alcohol and Related Conditions (NESARC-III) 2012-2013 data suggest daily and non-daily ENDS users had increased odds of past year alcohol use and of meeting the criteria for hazardous drinking, with nondaily users having increased odds of alcohol use in comparison to daily users.[549] Data collected in 2015 from 1,127 people at 24 substance abuse treatment centers around the U.S. show that 59.8% have ever used ENDS and 23.6% have current use, with 32.7% of current users being daily ENDS users, of whom 73.6% are dual users.[550] Overall, youth and adults with substance use and psychiatric disorders likely have higher odds of ENDS use.

Active Military and Veterans

There is research suggesting considerable interest in ENDS among military veterans, particularly among those veterans with substance use and psychiatric disorders.[551] Path Wave 1 and 2 data from 2013-2015 suggest past 30-day use of ENDS was higher among veterans (7.2%) in comparison to non-veterans

(5.5%).[552] Among 2467 U.S. active duty service members surveyed in 2015 to 2016, 14.4% had ever used ENDS.[553] Multivariate analyses suggest ENDS use is associated with being younger, enlisted, current or former tobacco users, and having lower perceptions of harm.[553] Having served in a combat unit only was associated with decreased odds of ENDS use; however, serving in both combat and combat support units was associated with increased odds of use, suggesting complexity in the association between combat exposure and ENDS use.[553]

In a 2015 sample of 188 mental healthcare-seeking veterans who currently smoke or quit smoking in the last 30 days in Connecticut, 30.9% used ENDS, with 86.2% of ENDS users reporting dual use patterns and 12.1% reporting ENDS only use.[551] For this sample, the most common motivations for ENDS use was ability to use ENDS in non-smoking areas (64.8%), cost (53.7%), and perception that ENDS use is lower in harm for nearby people (40.7%), with only 12.1% of veterans reporting flavor as an important motivation for use.[551] Furthermore, one trial examined the use of ENDS for cessation among veterans receiving psychiatric services and found that it showed some efficacy.[55] More research is important to further understand ENDS use in the military and veteran populations, including evaluation of efficacy of ENDS for cessation, and work to parse out ENDS use prevalence in populations of veterans and service members with and without psychiatric disorders.

Sexual and Gender Minorities

Research on ENDS use by sexual and gender minority populations generally supports prevalence of ENDS use by sexual minorities is higher than that of the heterosexual population. Additional information is important to further understand ENDS use prevalence and gender minorities.

Overall, sexual minority youth appear to have increased odds of ENDS use in comparison to their heterosexual peers. Analysis of YRBS 2015 and 2017 data show those youth who identify as "not sure" have higher odds of ENDS use in comparison to youth who identify as heterosexual.[554] In analyses stratified by gender, bisexual females and lesbian females had higher odds of frequent ENDS use compared to heterosexual females; similar patterns were not found among males.[554] Data from the 2015 YRBS were used in a latent mixture model to explore substance use patterns broadly, including ENDS use, and results suggest that when controlling for sex, age, and race/ethnicity, lesbian/gay respondents were more likely to belong to a group characterized by high ENDS and marijuana use, those youth who were not sure of their sexual orientation were more likely to belong to a group characterized by polysubstance use, including high ENDS use, and bisexual youth had higher odds of falling into any group other than the non-user group, relative to heterosexual youth.[555] Data from 126,868 youth in Minnesota suggest after adjusting for selected socioeconomic and behavioral characteristics, bisexuals were more likely to report current ENDS use and dual use of ENDS and combusted cigarettes when compared to heterosexuals.[556] However, gay/lesbian identity was found to be associated with higher dual use, but not exclusive ENDS use.[556] In contrast, a nationally representative sample of 3,000 U.S. youth aged 13-17 from 2017 showed that LGBTQ youth had higher odds of perceiving health risks of nicotine in ENDS in comparison to non-LGBTQ participants, although no difference in ENDS use was noted by LGBTQ status.[557]

Similarly, sexual minority adults have increased odds of ENDS use in comparison to their heterosexual peers. No clear conclusion can be reached for gender minority adults. Among adults surveyed in PATH Wave 2 in 2014 and 2015, those people who identify as LGB have higher odds of ENDS use in comparison to adults who identify as heterosexual.[558] Multivariate analyses of PATH Wave 2 data suggest that transgender identity is not associated with using ENDS "somedays or more" in multivariate models, although transgender participants had a higher crude prevalence of ENDS use in comparison to cisgender participants (12.4% vs. 6.5%), and in a sample of which only 0.5% was transgender.[558] Behavioral Risk Factor Surveillance System (BRFSS) 2016 data, collected from 198,057 adults similarly show that LGB identity, but not transgender identity, is associated with ever ENDS use in multivariate models.[559] Of the 0.4% of the sample that identified as transgender, 26.0% ever used ENDS and 27.8% were current ENDS users, in comparison to 18.8% and 19.8% of cisgender participants.[559] Of the 3.3% of the sample that identified as LGB, 36.5% ever used ENDS and 22.3% were current ENDS users, in comparison to 18.1% and 19.7% of heterosexual participants.[559] LGB females had higher odds of ever ENDS use in comparison to heterosexual females and LGB males had higher odds of ever ENDS use in comparison to heterosexual males, which suggests that findings regarding LGB status are present for both men and women.[559] TPRPS 2016 and 2017 data show sexual minorities were more likely to have ever used and currently use ENDS; however, when stratified by combusted tobacco use status, this association remained for current and former combusted tobacco users but not for never combusted tobacco users.[560] An additional study found past 30 day ENDS use, exposure to ENDS content in media, and searching for ENDS in media is higher among LGBT U.S. adults in comparison to non-LGBT U.S. adults.[561] Furthermore, this study noted LGBT adults may have differential exposure to ENDS by media channel, with lower reports of exposure to ENDS content on television and higher exposure on social media.[561] Among 411 men who have sex with men surveyed in San Francisco in 2014, regardless of their sexual orientation, 17.0% used ENDS, and 96.0% of those men who used ENDS also used combusted cigarettes.[562] PATH Wave 1 data from adults aged 18–24 years similarly suggest there may be differences in advertising exposure based on sexual identity.[563]

Sexual minority youth and adults have higher odds of ENDS use in comparison to their heterosexual peers. This association appears to be present for both males and females. Research suggests a high proportion of sexual minority adults and youth who use ENDS may engage in dual use. Furthermore, sexual minority adults may have higher exposure to ENDS marketing, potentially through targeted advertising or due to intentionally searching for information. Gender minority adults exhibited higher prevalence of ENDS use in comparison to cisgender peers, however, analyses adjusted for covariates did not find a significant association between transgender status and ENDS use. Only a small proportion of the U.S. population is transgender, and nationally representative studies may not capture a large enough number of transgender people to characterize ENDS use among this group.

Overall, LGBT groups, including both youth, young adults, and adults appear to report higher rates of ENDS use than non-LGBT groups. Additionally, females who identity as LGBT appear to be at increased risk for ENDS use, especially among youth.

American Indians and Alaskan Natives

Research suggests that ENDS use is higher among the American Indian population in comparison to other racial/ethnic groups, and some analyses show that the American Indian population has the highest prevalence of current and ever ENDS use of any racial/ethnic group.[564] NHIS 2014 data show 20.2% of American Indian adults had ever used ENDS (compared to 12.6% of all adults) and 10.7% of American Indian adults were current ENDS users (compared to 3.7% of all adults).[564] No recent nationally representative study data is available however, most likely due to under sampling of the American Indian/Alaskan Native populations. Data collected from 126,868 youth in Minnesota show American Indian youth have higher odds of ENDS use and dual ENDS and combusted cigarette use in comparison to non-Hispanic white youth.[556] One youth study assessed potential cultural factors associated with the high prevalence of ENDS use, however, this study of 156 American Indian and Alaskan Native Californian youth aged 13-18 years suggests ethnic identity, spirituality, and ceremonial use of tobacco are not associated with ENDS use.[565]

Two recent studies used data collected from 375 American Indian adults who currently smoke who were sampled at a large Cherokee Nation Health Services primary care facility in rural, northwestern Oklahoma to characterize dual use behavior.[566,567] Within this sample, 12.0% of people were dual users of combusted cigarettes and ENDS and 36.0% of people had never used ENDS.[566] One study found dual users, compared to never ENDS users, were younger (18-45 years old), perceived ENDS as low in harm (77.0% of dual users vs. 29.0% of never users), had less uncertainty regarding health outcomes associated with ENDS, and perceived ENDS as helpful for smoking cessation (75.0% of dual users vs. 16.0% never users).[566] Findings from the second study indicate among the 44 dual users in the sample, the most frequently reported reasons for using ENDS were to reduce smoking (79.0%), enjoyment of flavors (78.0%), and to use in place of combusted cigarettes in no-smoking areas (73.0%).[567] Some 11.0% of these dual users reported using e-liquid which did not contain nicotine and the majority reported they did not perceive ENDS use to be more enjoyable in comparison to combusted cigarette use.[567] In another study examining access to ENDS near and on American Indian tribal lands in California at 96 stores surveyed in 2015-2017, stores on tribal lands sold less disposable ENDS (37.7% vs. 69.8%) and less flavored disposable ENDS (28.3% vs. 53.4%) than stores within a 1-mile radius of tribal lands..[568] These findings did not hold for non-disposable ENDS products.[568] Research is beginning to capture information on ENDS use by American Indian adults and youth. However, many nationally representative studies in the U.S. collapse American Indians into an "other" category along with other minority racial/ethnic groups, due to the small number of people who report American Indian ancestry, leading to difficulty tracking ENDS use disparities within this population.

Rural Population

Existing research has not identified differences in rural and urban ENDS use prevalence, appeal, or perceptions. HINTS-FDA data from adults aged 18+ years were used from 2015 and 2017 to create a sample of 4,229 people.[569] No differences were found in ENDS ever use (urban = 23.9%, rural = 23.4%), perception of ENDS as addictive (urban = 11.2%, rural = 14.4%), and perception of ENDS as harmful in absolute (ENDS use is harmful; urban = 7.6%, rural = 9.1%) and relative terms (ENDS use is more harmful than combusted cigarettes: urban: 6.6%, rural: 5.1%).[569] Both populations were equally likely to search

for information on ENDS, although, rural respondents were more likely to trust information from religious entities and, marginally, from tobacco companies, in comparison to urban respondents.[569] A nationally representative sample of 3,000 U.S. youth aged 13-17 years surveyed in 2017 showed no difference in ENDS user status by rural, urban, and suburban place of residence, however, urban participants reported lower odds of perceiving the health risks of nicotine in ENDS in comparison to suburban participants.[557]

Homeless Population

The current evidence supports there is high prevalence of ENDS use among the homeless population; however, these studies used the same sample collected in 2017-2018 of 469 past month tobacco-using youth and young adults (aged 13-25 years) residing in Los Angeles County.[570-572] Data suggest 62.3% of homeless youth and young adults had ever used ENDS, and 32.4% of these ever-users reported past 30 day use;[572] however, adjusting for sampling procedures, adjusted prevalence of past 30-day ENDS use drops to 23.0% of the sample.[571] Some 64.0% of past-month ENDS users reported using ENDS to quit combusted cigarettes, and 29.5% of past-month ENDS users indicated they were motivated to quit in the next 30 days.[571] As these studies relied on the same sample drawn from a bounded geographic region, additional studies are important to further understand ENDS use within the homeless population in the United States.

Comparison Across Different Populations

ENDS use prevalence, patterns, appeal, and harm perceptions may differ by gender, race, and ethnicity. NHIS 2017 and 2018 data provide a broad picture of current ENDS use prevalence among adults and highlight prevalence of use within demographic subgroups continues to shift.[518] Between 2017 and 2018, men increased in reported current ENDS use, further increasing the difference in prevalence of ENDS use for men and women (Men: 2017 = 3.3%, 2018 = 4.3%, Women: 2017 = 2.4%, 2018 = 2.3%).[518] Some racial and ethnic groups additionally noted changes, with an increase in use among Asian adults as the only statistically significant change (Hispanic: 2017 = 1.8%, 2018 = 2.5%, Non-Hispanic White (NHW): 2017 = 3.3%, 2018 = 3.7%, Non-Hispanic Black (NHB): 2017 = 2.2%, 2018 = 1.6%, non-Hispanic Asian: 2017 = 0.9%, 2018 = 2.2%, and Other: 2017 = 4.4%, 2018 = 5.7%).[518] Overall, NHIS 2018 ENDS use prevalence statistics suggest people who identify as men and non-Hispanic White have significantly higher prevalence of current ENDS use.[573] Noted changes in use prevalence between the years highlight there is not yet stability in which demographics exhibit high levels of current use. These data also highlight potential issues with studies examining demographic disparities only considering ever use—use patterns across demographics rapidly change, so ever use statistics may fail to identify the populations currently engaging in disproportionately high levels of ENDS use.

Many publications have highlighted a higher proportion of non-Hispanic White (NHW) youth and adults use ENDS in comparison to other racial and ethnic groups. NHIS data show ENDS ever use increased among U.S. adults from 2014 to 2018 for NHW, NHB, Asian, and Hispanic adults; however, for all years, NHW adults exhibited the highest prevalence of ENDS use.[574] Analysis of the 2014-2015 Tobacco Use Supplement to the Current Population Survey (TUS-CPS) showed among smokers, Hispanic and NHB smokers were less likely to use ENDS daily in comparison to NHW smokers.[575] National YRBS data from 2017 suggest NHW youth have higher prevalence of dual use of ENDS and combusted cigarettes, but not

ENDS alone, in comparison to other racial or ethnic groups.[576] A nationally representative sample of 3,000 U.S. youth aged 13-17 years surveyed in 2017 showed that NHW youth had a significantly higher proportion of people in the current ENDS user group in comparison to the never ENDS user group.[557] PATH Waves 1 and 2 data collected in 2013-2015 from youth (*N* = 8,480, aged 12–17 years) who were nicotine never users at Wave 1 suggested NHB youth had lower odds of ENDS initiation at Wave 2 in comparison to NHW youth.[577] PATH study data from Wave 3, collected in 2015 and 2016, showed open ENDS users are more likely to be NHW in comparison to closed ENDS users (open ENDS: 76.2% NHW, closed ENDS: 65.4% NHW).[521] PATH Wave 2 data shows use of mint/menthol e-liquid flavors was higher among NHB and Hispanic participants than NHW participants.[522] Qualitative interviews conducted in 2015 and 2016 with young, Californian Asian Americans adults aged 19-34 years suggest this subgroup prefers e-liquid flavors of foods commonly included in Asian cuisine, such as lychee, taro, guava, ube, coconut, and mango.[578] Additionally, ENDS marketing may be targeted at other racial or ethnic groups, as data collected from 4,384 college students (aged 20-32 years) in Texas in 2017 showed Hispanic, Asian, and NHB students had higher odds of exposure to ENDS advertisements on social media in comparison to NHW students.[579] However, marketing campaigns, socialization processes, and product characteristics may impact demographic differences in ENDS use.

Analyses suggest complexity exists in understanding health disparities for ethnic/racial groups. For example, Hispanic youth and adults may have higher ENDS use in comparison to NHW youth and adults for some measures of ENDS use and within some geographic regions. ENDS use trajectories were examined using data from 6,258 youth from California and Connecticut sampled in 2013 and 2015, with follow-up data collected one year after baseline.[580] Baseline data (collected in 2013 and 2015) show Hispanic adolescents reported higher ENDS use and dual use in comparison to NHW adolescents, though this pattern reversed for follow-up data (collected in 2014 and 2016), where NHW adolescents exhibited higher prevalence of tobacco product use in comparison to Hispanic adolescents.[580] Furthermore, in this study, ENDS use trajectories differed by ethnicity.[580] NYTS 2014-2015 data suggest that Hispanic youth are more likely to use ENDS prior to other tobacco products in comparison to non-Hispanic youth,[581] supporting the previous study's finding that Hispanic and non-Hispanic youth may have different tobacco product use trajectories. Complementing the initiation product use findings of this study, NYTS 2014-2018 data show among youth who use combusted cigarettes, prevalence of ENDS use exhibits an increasing trend across racial and ethnic groups for ENDS use measures of 10 or fewer, 20 or fewer, and all past 30 day ENDS use in the past 30 days, although Hispanic youth combusted cigarette users did not show increases in ENDS use measures of everyday use or less than/equal to 20 day use in the past 30 days.[582] In another study, data collected from 126,868 youth in Minnesota suggest Asian American and NHB youth have lower odds of ENDS use in comparison to NHW youth, while Hispanic youth have higher odds ENDS use.[556] Monitoring the Future (MTF) data from 2017 suggest NHB youth have lower odds of currently using ENDS with e-liquid containing nicotine and e-liquid without nicotine, while Hispanic youth have lower odds of currently using ENDS with nicotine in comparison to NHW youth.[583] NHANES 2015 and 2016 data show among adults, ENDS ever use is lower in Hispanics in comparison to NHW, however in analyses stratified by lifetime smoking status, non-smoking Hispanic adults were more likely to use ENDS in comparison to non-smoking NHW adults, with no other differences noted by race or

ethnicity.[519] TPRPS 2016 and 2017 data show Hispanic adults were more likely to have ever used and currently use ENDS in comparison to NHW adults.[560]

Many publications also highlight a higher proportion of males use ENDS in comparison to females. National YRBS data from 2017 suggest boys are more frequently ENDS only or dual users in comparison to girls.[576] NYTS 2014-2018 data show among youth who use combusted cigarettes, prevalence of ENDS use less or equal to 10 days in the last 30 days has increased significantly for males, but not for females, although this increasing trend was significant for both males and females for ENDS use measures of less than or equal to 20 days of use or all 30 days of the last 30 days.[582] MTF data from 2017 suggest girls have lower odds of currently using nicotine-containing e-liquid and e-liquid that does not contain nicotine in comparison to boys, suggesting lower odds of ENDS use for girls in comparison to boys is found regardless of nicotine concentration in the e-liquid.[583] A nationally representative sample of 3,000 U.S. youth aged 13-17 years surveyed in 2017 showed girls had higher odds of perceiving health risks of nicotine and toxins or chemicals in ENDS in comparison to boys.[557] NHANES 2015 and 2016 data show among adults, ENDS ever use was higher among men in comparison to women, though analyses stratified by lifetime combusted cigarette use status showed this association held for non-smokers, but not lifetime smokers.[519] Product characteristics may impact use differences among men and women, as PATH Wave 2 data show use of fruit-flavored and candy-flavored ENDS was higher among women.[522] Data from the 2018 Texas population health assessment survey collected from current and former smokers who were ever-users of ENDS suggests men were less likely than women to use ENDS for cessation.[584] These data suggest that a smaller proportion of females use ENDS in comparison to males and health risk factors may play a larger role in ENDS use behavior for females in comparison to males.

Overall, there appear to be potential differential use patterns for racial or ethnic groups and males versus females. However, ENDS are still relatively new tobacco products, in comparison to combusted and smokeless tobacco products, and additional studies are important to further understand potential inequalities among these groups.

By Product Type

Flavored Products

Flavored ENDS use among youth has increased over time.[585] ENDS were the most commonly used flavored tobacco product among students in the 2019 NYTS: 68.8% of middle and high school students who were current ENDS users used a flavored ENDS.[515] Due to the way questions are asked in the 2019 NYTS, it is not possible to disentangle specific flavors used with each product for students who use multiple tobacco products. In the 2019 NYTS, 72.2% of high school student exclusive ENDS users and 59.2% of middle school exclusive ENDS users reported using a flavored ENDS.[516] For high school student exclusive ENDS users, the most common flavors used in 2019 were fruit (66.1%), menthol or mint (57.3%), and candy, dessert, or other sweets (34.9%). For middle school student exclusive ENDS users, the most common flavors used in 2019 were fruit (67.7%), candy, desserts, or other sweets (38.3%), and menthol or mint (31.1%).[516] For ENDS users using other tobacco products, individual flavor types used with ENDS were not reported.

Flavored ENDS use is also common in adults. In PATH Waves 1 and 2, 63.2–64.6% of adult ENDS users reported using flavored ENDS.[586,587] Data from PATH Wave 3 suggests nearly 75% of past 30 day adult ENDS users were using flavors other than "tobacco only", however youth were more likely than adults to use flavors other than tobacco.[588] In that study, popular flavors used among young adult ENDS users ages 18-24 years include fruit, mint/menthol, and among older adult ENDS users were other flavors, fruit, and mint/menthol. The study also found youth current ENDS users were significantly more likely than adult ENDS users to use more than one flavor and use combinations that do not include tobacco flavors. Also, tobacco flavored ENDS use was more common in adults than youth, and fruit flavor was most commonly used, alone or in combination with other flavors for both youth and adults.

Flavor type used may vary by tobacco use status. In Wave 3 of the PATH Study (2015-2016), both youth and adult dual combusted cigarette and ENDS users were less likely than exclusive ENDS users to report use of a non-tobacco flavor versus a tobacco flavor.[588] However, the use of two or more flavor types did not vary by combusted cigarette smoking status. In a study of Texas college students from 2014-2015, the likelihood of currently using non-tobacco flavored ENDS did not vary by combusted tobacco use status.[589] Other studies suggest current use of flavors related to combusted cigarette use (i.e., tobacco, unflavored, mint, menthol) is more common among dual users than complete switchers. In a longitudinal study, young adult ENDS users who, at baseline, had ever-smoked combusted cigarettes, but not in the past month, were less likely to use tobacco or menthol flavored ENDS after a year.[590] In another study, current use of combusted cigarette flavors (i.e., tobacco/unflavored and mint/wintergreen/menthol) was associated with a lower odds of being a complete switcher than a dual user.[524] Similarly, in a social media sample from 2014, current dual users were more likely to report using tobacco flavored e-liquid flavors and less likely to report using caramel, vanilla, chocolate, cream, or fruit flavors.[525]

Product Design and Brands

Data by product type is sparse and the use of different terms to describe product types as well as changes in the marketplace have made it difficult to rely on older data. In the 2019 NYTS, 56.6% of middle and high-school students reported using a closed system product (i.e., disposable or ENDS that used pre-filled pods or cartridges) most often, 34.4% reported using an open system product (i.e., a refillable tank or a mod system) most often, and 8.9% did not know what type of product they used most often. The proportion of closed and open system ENDS users who currently smoked combusted cigarettes were not significantly different (16.8% and 20.3%, respectively).[591]

In the 2019 NYTS, the most common usual brand of ENDS reported by students was JUUL (59.1% of high-schoolers, 54.1% of middle-schoolers).[516] The other brands reported as most commonly used by students (all reported by <10% of students) were SMOK, Suorin, blu, Vuse, and NJOY. About 15% of students reported no usual ENDS brand (13.8% of high-schoolers, 16.8% of middle-schoolers).

In adults, data from PATH Wave 3 (2015-2016) suggest 43.9% of current ENDS users used closed systems and 53.7% of current ENDS users used open systems.[521] compared to closed system users, open system users were significantly more likely to be male, aged 18-24 years, non-Hispanic White, recent former or long-term former smokers, and use ENDS daily. Open system users were significantly less likely to be

current daily smokers or never smokers. Regarding flavor use, open system users were more likely to report using fruit/sweet/spice/alcohol flavor and less likely to report using menthol or mint.[521] In that same study, open system users were more likely to be former combusted cigarette smokers than closed system users. Open system users were also more likely to use fruit flavors and less likely to use tobacco/mint flavors, however no direct comparison was done between former combusted cigarette use and flavor preference.

Some evidence suggests the majority of ENDS users who used ENDS over a 2-year longitudinal study in Texas used rechargeable devices, and those reporting rechargeable device use used on more days per month than disposable users.[592]

Prevalence of use by brand has been most studied for JUUL. Some data suggests ever and current JUUL use is more common among youth ages 15-17 (9.5% ever, 6.1% current) than among young adults age 25-34 (6.0% ever, 3.3% current), however this study did find past 30 day combusted tobacco product users were 5 times more likely to be current JUUL users than current non-combusted tobacco product users.[593] There is also some evidence to suggest youth pod-mod users may use ENDS more days per month and more intensely (more times per day) than other ENDS users,[203] although data from a convenience sample of JUUL users suggests no difference.[594]

Conclusions for Section 3.A. Prevalence of ENDS Use

The 2019 NYTS shows 20.0% (95% CI: 18.6%-21.6%) of middle and high school students are estimated to have used ENDS in the past 30-days. By school type, 27.5% (95% CI: 25.3%-29.7%) of high school students and 10.5% (95% CI: 9.4%-11.8%) of middle school students were current ENDS users in 2019. In the 2018 NHIS, an estimated 3.2% (95% CI: 3.0%-3.5%) of adults used ENDS in the past 30-days (i.e., current ENDS users). By age group, ENDS use in 2018 was most common among young adults aged 18-24 years (7.6%, 95% CI: 6.1%-9.1%), and the prevalence of current ENDS use decreases with age: 4.3% (95% CI: 3.7%-4.8%) for those aged 25-44 years, 2.1% (95% CI: 1.8%-2.5%) for those aged 45-64 years, and 0.8% (95% CI: 0.6%-1.1%) for those aged 65 years and older. In addition to the vulnerable population of youth, other vulnerable populations to consider for ENDS use include pregnant women, sexual and gender minorities, active military and veterans, American Indians, homeless populations, and people with a history of mental health problems. Similar to other population groups, the benefits and risks of ENDS for vulnerable groups would include those currently using combusted tobacco products, and non-users. Flavored ENDS use may vary by tobacco use status, but is common for both youth and adult users. However, flavor preference may vary by age and user status. Currently data for brand or product preference are limited and studies primarily focus on JUUL use, especially for youth and young adults.

B. PATTERNS OF ENDS USE

Initiation

Age and experiences at tobacco product initiation may contribute to continued tobacco product use and incidence or rate of progression to regular use and dependence. Sharapova et al.[595] measured age of first use for ENDS, combusted cigarettes, cigars, smokeless tobacco, and waterpipe among youth in a nationally representative, cross-sectional survey (NYTS, 2014-2016). Among ever users of ENDS,

weighted mean age at first use was 14.1 years; first trying ENDS at age ≤13 years (vs. >13 years) was associated with daily use in the past 30 days and experiencing craving, but not with past 30-day use or earlier time to first ENDS use in the morning (<30 minutes). Age at first ENDS use was somewhat older and associated with fewer dependence metrics compared to other tobacco products, except for waterpipe. Results should be considered in light of several limitations. Although this study has a large sample size, compares a variety of products, and is nationally representative, the findings are based on self-report (subject to recall and reporting biases) and the cross-sectional nature of the data does not allow assessment of temporal and causal relationships between the events. Analyses based on race were not possible for some groups due to small sample sizes and results only apply to youth who already use tobacco.

Several articles examined subjective experience at first tobacco product use by youth. Two cross-sectional surveys examined subjective experiences or symptoms at first use across ENDS and other tobacco products among youth ever users.[176,596] Both studies found more positive subjective experiences and fewer negative subjective experiences at first use of ENDS compared to other products; however, while first use subjective experiences were associated with current use of combusted cigarettes and cigar products, no first use subjective experiences were associated with current use of ENDS.

Flavors

Flavors play an important role in youth ENDS use by attracting youth to initiate the product and reinforcing current use.[544] In a cross-sectional survey of youth and young adult ever-users of ENDS and combusted cigarettes, McKelvey et al.[203] found the first e-liquid used was likely to be flavored (vs. unflavored). Regional longitudinal surveys of youth have found flavoring and nicotine in ENDS pose a risk for progression to regular use among youth. Data from a regional survey in Philadelphia, PA found initial use of a flavored (vs. unflavored) ENDS was associated with progression to current ENDS use as well as escalation in the number of days ENDS were used across 18 months, and initial use of a nicotine-containing ENDS (vs. nicotine-free) was associated with a greater number of ENDS use days at baseline.[597] Use of non-traditional flavors (vs. tobacco, mint/menthol, flavorless) was associated with increased likelihood of continued use and taking more puffs per episode.[598] In contrast, an analysis of PATH data found initiation with a flavored (vs. unflavored) ENDS was associated with progression to current regular ENDS use among young adults and adults older than age 25 in Wave 2; however, these results were not found for the youth sample.[599]

In PATH Wave 1, over 80% of youth, 75% of young adults and 58% of adults over the age of 25 reported their first ENDS used was flavored.[599] In the nationally representative PATH study, a greater percentage of youth, young adults and adults who were new ENDS users between Wave 1 and Wave 2 reported use of a flavored product than a non-flavored product.[600] In PATH Wave 4 (2016-2017), while 93.2% of all youth ever ENDS users reported their first ENDS product was flavored, fewer young adults (83.7%) and adults over the age of 25 (52.9%) reported their first ENDS product used was flavored.[601]

Re-initiation likelihood for Former Tobacco Users

Most data on ENDS use in former tobacco users has been done among former combusted cigarette smokers. Prevalence of ENDS use in NHIS 2018 was higher among former smokers (5.5%) than never smokers (1.1%).[518] One study using a sample recruited from an online national probability panel (GfK KnowledgePanel), found higher current ENDS use among former smokers (range: 5.3–12.9%) than never smokers (range: 3.0–5.0%).[520] However, prevalence of use among former smokers is lower than among current smokers.[518,520] Prospective data also indicates ENDS initiation rates are higher among people who had previously tried a tobacco product (3-year transition probability 3.2% (CI: 2.6%–3.8%), than people who had not (3-year transition probability 1.0% (CI: 0.8%–1.8%).[602] Data among current ENDS users from PATH Wave 3 (2015-2016), and at least one other nationally representative study, also indicate a greater proportion of current ENDS users were former smokers than never smokers.[354,521]

It is important to note some studies have found former smokers who use ENDS were more likely to relapse to combusted cigarette smoking.[602] One study found this was only true of former smokers who had quit for more than 12 months and not those who had quit for less than 12 months.[603] One study comprised mostly of ever combusted cigarette smokers (98% of the sample) found 9.2% of exclusive ENDS users at baseline transitioned to dual use at follow up, but only one participant out of 402 switched back to smoking completely.[200]

Progression from ENDS to Combusted Tobacco Use

There is concern ENDS use among adolescents and young adults with no combusted cigarette smoking history will lead to combusted cigarette initiation and progress to regular smoking. An important consideration is how likely non-smoking youth ENDS users are to start smoking combusted cigarettes.

A total of 21 longitudinal studies have followed adolescents and young adults who were never combusted cigarette smokers to assess whether ENDS use at baseline was associated with combusted cigarette smoking initiation during a follow-up period. A 2017 systematic review and meta-analysis that summarized nine prospective cohort studies found a significantly higher odds of smoking initiation (OR = 3.50, 95% CI: 2.38–5.16) and past 30-day combusted cigarette use (OR = 4.28, 95% CI: 2.52–7.27) among never smoking youth who had ever used ENDS at baseline compared to youth who never used ENDS.[604] Similar associations have been observed in the 11 longitudinal studies published since that review.[605-615]

These studies tend to use ever smoking or past 30-day smoking as the outcome of interest, which could be capturing both transient experimental smoking as well as progression to regular frequent smoking. Similarly, all these studies used ever ENDS use or any past 30-day ENDS use as the main exposure. This definition lumps together youth who tried an ENDS once and youth who are regular ENDS users. Therefore, the studies do not provide information about frequency of ENDS use and initiating combusted cigarette smoking.

The NASEM report concluded there was substantial evidence ENDS use increases risk of ever using combusted tobacco cigarettes among youth and young adults, limited evidence ENDS use increases, in the near term, the duration of subsequent combusted tobacco cigarette smoking, and moderate

evidence ENDS use increases the frequency and intensity of subsequent combusted tobacco cigarette smoking.[1]

In regards to evidence to support ENDS use increases risk of progressing to regular or frequent combusted cigarette smoking, a study by Hammond et al. considered whether ENDS use was associated with initiation of regular smoking, defined as daily smoking for at least 7 consecutive days.[605] In this study, past 30-day ENDS use among never smoking youth at baseline was associated with a significant risk of initiating daily smoking during a 1-year follow-up (OR = 1.79, 95% CI: 1.41–2.28). Additionally, in a PATH Study analysis of youth ages 12-17 who had smoked fewer than 100 combusted cigarettes in their lifetime at baseline (i.e., experimental smokers), ENDS use was associated with established smoking (i.e., smoked ≥100 combusted cigarettes) one year later, but just missed statistical significance after adjustment for smoking risk factors (OR = 1.57; 95% CI: 0.99–2.49).[616] Another study found among baseline never smokers, ENDS users had greater odds of subsequent frequent combusted cigarette smoking, although "frequent" was defined as only smoking 3 or more of the past 30-days.[617] Lastly, two studies that considered ENDS use frequency found more frequent ENDS use at baseline was associated with a higher risk of more frequent and heavy combusted cigarette smoking after follow-up.[618,619]

Association of ENDS Use with Smoking Cessation and Smoking Reduction

A large proportion of adult ENDS users also uses combusted cigarettes. A potential benefit of ENDS could be to promote smoking cessation among established smokers or to reduce the number of CPD.

Smoking Reduction and Complete Switching

The extent to which combusted cigarette smokers use ENDS while continuing to smoke (dual use) or switch completely to ENDS (i.e., complete switching or combusted cigarette smoking cessation) is important to understand the benefits and risks of ENDS. It is not clear if dual use of ENDS and combusted cigarettes is a steady, long-term tobacco use pattern or if dual users discontinue combusted cigarette smoking, ENDS use, or both products. The literature suggests dual use is common, and therefore is important to assess the extent to which combusted cigarette smokers decreased their CPD and biomarkers of exposure when concurrently using ENDS.

Switching from smoking combusted cigarettes to ENDS use, including incomplete switching (i.e., dual use), is an important behavior to understand particularly due to the high prevalence of dual ENDS and combusted cigarette use. Furthermore, the extent to which ENDS can act as a substitute for combusted cigarettes (in dual use or complete combusted cigarette cessation) may reflect their acceptability and abuse liability. ENDS that score higher on subjective effects measures of liking and acceptability and deliver nicotine at similar levels as combusted cigarettes may be more likely to result in complete switching (i.e., from combusted cigarette smoking). However, ENDS with an abuse liability similar to cigarettes may make it difficult for users to achieve complete nicotine cessation, similar to the difficulty of quitting combusted cigarette smoking.

Many studies have examined the extent to which ENDS use may decrease CPD or facilitate complete combusted cigarette cessation. Typically, switching studies require combusted cigarette smokers to replace some (or all) combusted cigarettes with ENDS, which are often provided free of charge to

participants. Switching studies evaluate the reduction in CPD upon ENDS uptake and continued use. In general, studies suggest CPD and exhaled CO (biomarker of combusted cigarette smoking to confirm self-reported CPD) decrease in combusted cigarette smokers who initiate ENDS use.[54,55,141,175,180,620,621] In a study where combusted cigarette smokers intending to quit were encouraged to use a pen-style ENDS for two weeks and refrain from smoking, CPD decreased significantly and nearly half of the participants reported completely switching to ENDS.[141] A separate study evaluated the effects of preferred e-liquid flavor (no flavor, tobacco, menthol, cherry, and chocolate) and nicotine concentration (0 and 18 mg/mL) on switching behaviors. CPD decreased during the six-week study period with ENDS; the effect was significantly greater in participants who preferred menthol-flavored e-liquids,[180] and suggests flavor may impact ENDS substitutability for combusted cigarettes. However, a cross-over within-subjects study found CPD (and nicotine exposure) increased in the dual use condition compared to the exclusive combusted cigarette smoking condition.[51] However, because dual use of ENDS and combusted cigarettes is typically associated with similar nicotine exposures as combusted cigarette smoking, these data suggest some ENDS may deliver sufficient nicotine to effectively replace some combusted cigarettes.

Adult smokers given access to ENDS showed reduced smoking intensity, combusted cigarette reinforcement, and dependence scores.[622] In an analysis of PATH study data, Buu et al.[623] found higher ENDS use frequency was associated with lower smoking frequency, quantity, and dependence symptoms. Similarly, in an analysis of NHIS and TUS-CPS study datasets, Johnson et al.[624] found smokers who reported ENDS use were more likely to have made a past-year combusted cigarette quit attempt and to have successfully quit smoking combusted cigarettes relative to smokers who did not report ENDS use. Conversely, in prospective analyses of UK smokers in the Smoking Toolkit Study, Jackson et al. demonstrated ENDS dual use at baseline was not associated with greater smoking cessation attempts or success at 12 months relative to exclusive smokers,[625] and ENDS dual users were less likely to make a combusted cigarette quit attempt than NRT dual users at 6[626] and 12 month follow ups.[625] Additionally, in a prospective analysis of American Indian smokers, smoking cessation rates and daily combusted cigarette consumption did not differ between participants reporting ENDS use at baseline and ENDS non-users.[627]

Observational studies may also offer insight into switching behaviors and smoking cessation related to ENDS use. For example, an ongoing cohort study has the intent to follow combusted cigarette smokers, ENDS users, and dual users for six years to evaluate participants' switching behaviors and complete cessation;[628-630] data at four years follow-up were recently reported.[628] Among ENDS only users at baseline, 63.6% remained combusted cigarette abstinent at four years; 26.8% of combusted cigarettes smokers and 33.8% of dual users were abstinent ($p<0.05$). Complete tobacco abstinence (no ENDS or cigarette use) was similar among all groups ($p>0.05$). Both baseline dual users and combusted cigarette only users showed significant reductions in CPD. During the four-year period, many participants switched products (37.7%). Twenty percent of participants who used ENDS at least once during the four-year period quit all tobacco product use; 21.7% of participants who did not use ENDS quit all tobacco products. Therefore, ENDS use (at any frequency) did not enhance the quit ratio for all tobacco products.

Longitudinal studies that have looked at trajectories of dual use found ENDS use patterns were highly variable over periods one-year or longer. Among Wave 1 adult dual combusted cigarette and ENDS users in the PATH Study, 44.3% maintained dual use at Wave 2, 43.5% discontinued ENDS use and maintained combusted cigarette smoking, 5.1% stopped combusted cigarette smoking and continued using ENDS, and 7.0% discontinued both products.[261] Another PATH paper looked at longitudinal patterns between Wave 1 and Wave 3, among adults who were past 30-day ENDS users during Wave 1.[631] The analysis found ENDS use was not stable and most adult past 30-day ENDS users at Wave 1 discontinued ENDS use at either Wave 2 or Wave 3. Among Wave 1 young adult (18-24 years) ENDS users who used other tobacco products including combusted cigarettes, 44.8% discontinued ENDS but continued using other tobacco products at Wave 3 and 11.0% discontinued all tobacco products by Wave 3. Only 19.2% had the same tobacco use pattern at Wave 3 and 3.4% switched to exclusive ENDS use by Wave 3.[631] The remaining young adult ENDS users had some combination of discontinuing and then reinitiating ENDS use during follow-up. For adults 25 and older who used ENDS and other tobacco products including combusted cigarettes at Wave 1, 54.1% discontinued ENDS use by Wave 3 but continued to use other products and 8.3% discontinued using all tobacco products. Only 18.8% maintained their tobacco use pattern at Wave 3 and 5.4% switched to exclusive ENDS use by Wave 3.[631] A US study of adult daily smokers who also used ENDS at least once per week and had no intention to quit either product in the next 30 days found that after one-year, 48.8% continued dual use, 43.9% were only smoking combusted cigarettes, 5.9% were only using ENDS, and 1.4% abstained from both products.[632]

In the PATH Study, among Wave 1 youth (12-17 years old) ENDS users who used other tobacco products including combusted cigarettes, 26.3% had the same tobacco use pattern at Wave 3, 26.5% discontinued ENDS but continued using other tobacco products at Wave 3, 17.9% discontinued all tobacco products by Wave 3, and 2.5% switched to exclusive ENDS use by Wave 3.[631] The remaining youth ENDS users had some combination of discontinuing and then reinitiating ENDS or other tobacco products during follow-up. Another study used data collected from California and Connecticut students surveyed in 2013 -2014 and followed them for up to 12 months.[617] Among baseline past 30-day dual users, 51% were dual users at follow-up, 16% became exclusive combusted cigarette users, 15% became exclusive ENDS users, and 18% stopped using tobacco products.

In a sample of young adults aged 18–34 years old enrolled in the Truth Initiative Young Adult Cohort Study (December 2011–July 2015) and surveyed every 6-months for 3 years, after 6-months the probabilities of dual users transitioning to other tobacco use states were as follows: 48.3% remained dual users, 41.5% transitioned to combusted product use only, 7.8% transitioned to ENDS only use, and 2.4% transitioned to no tobacco product use.[633] However, after 3-years of follow-up: 52.3% transitioned to combusted products only, 32.6% transitioned to not using any tobacco products, 7.6% remained dual users, and 7.5% become ENDS only users.

The Truth Longitudinal Cohort surveyed youth and young adults ages 15–21 years every 6-months for 2.5 years (2014-2016). After 6-months, dual users had the following transition probabilities to these tobacco use states: 46.4% remained dual users, 26.6% became combusted product only users, 18.2% stopped using all tobacco products, and 8.8% became ENDS only users.[634] After 2.5 years, dual users had the following transition probabilities: 59.4% probability of using no tobacco products, 21.1% probability

of being a combusted only user, 13.3% probability of remaining a dual user, and 6.2% probability of using ENDS only.

Based on currently published studies, ENDS use patterns are generally not stable over time in adult smokers. Many dual users will discontinue ENDS use over time and the likelihood of transitioning from dual use to exclusive ENDS use is low. More frequent ENDS use increases the likelihood of continuing to use ENDS over time. Similar to adults, dual use in youth and young adults is a transient state with most dual users transitioning to other tobacco use states, especially during follow-up periods of longer than a year. After a year, it's common for youth and young adult dual users to transition to only combusted products or to stop using all tobacco products. A limitation of these studies is they collected data in 2016 and earlier, so these studies do not capture transitions among those using pod-based ENDS products, which are currently the most widely used ENDS by youth and young adults. These estimates do not come from national surveillance datasets (e.g., NYTS, NHIS), so the focus should be on the patterns observed rather than specific estimates from these studies.

Frequency of use

Frequency of using ENDS is an important predictor in the likelihood of continuing to use ENDS over time. One cohort study of current smokers and recent quitters assessed how baseline frequency of past month ENDS use was associated with ENDS use at 1-year of follow-up.[635] Among current smokers at baseline, the prevalence of past 30-day ENDS use at 1-year of follow-up was 75% for baseline daily ENDS users (used 28–30 days of the past 30-days), but only 29% for both infrequent (1–5 day) and intermediate (6–27 days) ENDS users at baseline. Similar findings were observed in an analysis of PATH Wave 1 and Wave 2 data. Among Wave 1 ENDS users (regardless of smoking status), daily ENDS users at Wave 1 were half as likely as non-daily users to discontinue ENDS use at Wave 2 (adjusted prevalence ratio (aPR)=0.49, 95% CI=0.40,0.59).[261] Those who were daily ENDS users at Wave 1 were less likely to discontinue ENDS use at Wave 2 (23.7%) compared to moderate (49.0%, P<0.0001) or infrequent users (62.1%, P<0.0001). Daily ENDS users were also more likely than non-daily users to maintain their same frequency of use at Wave 2. In the International Tobacco Control (ITC) longitudinal study, a higher baseline ENDS use frequency was associated with continued ENDS use at follow-up.[636]

Data collected from 2008-2016 in the ITC Four Country Surveys (United Kingdom, United States, Canada, and Australia) found more frequent smoking was predictive of initiating ENDS use and more frequent ENDS use, but was not associated with ongoing ENDS use over time. Smokers who smoked an average of 30+ CPD vs. 0–10 CPD, had significantly higher odds of starting to use ENDS (OR=1.69, 95% CI: 1.19, 2.39) and using ENDS more frequently (OR = 1.97, 95% CI = 1.36, 2.85). Reporting an intention to quit smoking was also associated with a higher frequency of current ENDS use (OR = 1.48, 95% CI = 1.21, 1.82).[636]

Frequency of ENDS use impacts the likelihood ENDS users will abstain from smoking. Using data from Wave 1 to Wave 3 of the PATH Study, someday combusted cigarette smokers and daily ENDS users at Wave 1 were most likely to completely switch to ENDS by Wave 3 (aOR = 6.19, 95% CI: 3.91-9.79). Dual users who smoked and used ENDS some days were most likely to have completely quit tobacco by Wave 3 (aOR = 3.98, 95% CI = 2.93, 5.40).[523] In a PATH Study analysis using data from Wave 1 to Wave 3, daily ENDS users at Wave 1 had significantly higher odds than non-users of reporting smoking abstinence one-

year and two-years later.[637] However, Wave 1 non-daily ENDS users did not have higher odds of smoking abstinence after one- and two-years in that same study. In another analysis of PATH Study Wave 1 dual combusted cigarette and ENDS users, the odds of smoking abstinence at Wave 2 were higher among dual users who reported everyday (versus someday) ENDS use (aOR: 1.85; 95% CI: 1.18-2.89).[638] In another PATH analysis, daily ENDS users compared to non-daily ENDS users at Wave 1 were more likely to abstain from smoking at Wave 2.[261] Daily ENDS use, compared to no ENDS use, was also associated with a higher likelihood of smoking cessation in another US longitudinal study,[639] the 2016 and 2017 NHIS data,[640] the 2014 and 2015 NHIS data,[641] the 2014–15 TUS-CPS data,[642] and a longitudinal study in France.[643] Non-daily use was not associated with smoking cessation in those studies. In a British Longitudinal study, daily ENDS use was associated with a significant reduction in combusted cigarette consumption after one-year, but not an increased likelihood of cessation.[644] An industry-funded study of smoking cessation among JUUL users found daily use of JUUL and certain flavored pods (i.e., mint or mango flavored pods vs. Virginia tobacco) increased self-reported past 30-day combusted cigarette smoking abstinence at 3-month and 6-month follow-up.[645,646] However, the extent to which these findings are generalizable to other ENDS users and products is unclear due to the differences in nicotine formulation between JUUL and other ENDS and the limitations in study design (e.g., no biochemical verification, selective bias).

The association between ENDS use and quit duration may vary by frequency of use. In 2017, in a sample of ~13,000 European Union citizens ages 15 and older, daily ENDS use was strongly associated with being a recent (≤5 years) former smoker. Compared with never use, current daily ENDS use was associated with being a former smoker of 2 years or less (aPR 4.96, 95% CI 3.57 to 6.90) and 3–5 years (aPR 3.20, 95% CI 2.10 to 4.87). Current daily ENDS use was negatively associated with being a former smoker of 5–10 and >10 years.[647]

Flavors

In young adults aged 18-34 years in the PATH Study who were current combusted cigarette smokers at Wave 1 and ENDS users at Wave 2, those who used non-tobacco and non-menthol ENDS flavors were significantly more likely to reduce combusted cigarette consumption or quit smoking compared to non-ENDS users.[590]

Smoking reduction may vary by flavor type. In a longitudinal laboratory study of smokers assigned to use different flavor types of ENDS in place of combusted cigarettes, the largest drop in combusted cigarettes smoked per day after 6 weeks occurred among those assigned menthol ENDS, while the smallest drop in smoking occurred in those assigned chocolate or cherry flavors.[180]

In GfK KnowledgePanel data collected in 2015-2016, current smokers who used non-tobacco flavored e-liquids (e.g., fruit, dessert, spice) were less likely to report past 30-day smoking abstinence after a year.[648] There was no difference in the odds of quitting for smokers who used menthol/wintergreen/mint ENDS flavors compared to no ENDS use.[648] Similarly, data from the GfK KnowledgePanel collected in 2016 and 2017 found flavor category used at the time of ENDS initiation among smokers did not predict the odds of completely switching to ENDS versus being a dual user.[524] Use of fruit flavored or candy/dessert flavored ENDS at initiation was more common among current dual

users or complete switchers than among former ENDS users. The prevalence of current use of candy/dessert, coffee/alcohol, and spice/other beverage flavored ENDS was similar between dual users and complete switchers.

Variability of flavors may promote continued ENDS use among smokers. In one study, use of two or more flavors at the time of ENDS initiation was associated with higher odds of being a dual user or complete switcher than stopping ENDS use.[524] In a 2016 Netherlands cross sectional study, dual users were significantly more likely to find the availability of a variety of e-liquid flavors more appealing than smokers who completely switched to exclusive ENDS use.[649]

Among dual combusted cigarette and ENDS users in Wave 3 of the PATH Study, there was no association between type of ENDS flavor used and a combusted cigarette quit attempt in the past 12 months, however, dual users who used two or more flavors were more likely than dual users using only one flavor to report a past 12-month combusted cigarette quit attempt.[588] In GfK's KnowledgePanel study, current smokers who used non-tobacco flavored e-liquid (e.g., fruit, dessert, spice) were more likely than non-ENDS users to report a quit attempt in the past 12-months.[648]

Population Differences

PREGNANT WOMEN

A study, which used Waves 1–3 of PATH data for women of reproductive age, examining transitions from combusted cigarette smoking found the majority of pregnant women either quit completely or continued smoking compared to switching to ENDS.[650] In that study, pregnant women comprised 2.8% of those who continued smoking, 1.3% of those who switched to ENDS, and 14.5% who quit altogether.[650]

Pregnant women report using ENDS during pregnancy in order to help them quit using combusted cigarettes. Kapaya et al.[531] reported 45.2% of those using ENDS around pregnancy reported perceptions ENDS might help with quitting or reducing combusted cigarette smoking. Out of 103 pregnant women screened for a smoking cessation trial, 15.0% reported trying ENDS in a cessation attempt, and the subsample who used ENDS during pregnancy had more previous quit attempts in comparison to non-users of ENDS.[535] Wedel et al.[534] found 60.0% of participants were interested in using ENDS during pregnancy to quit or reduce smoking while pregnant, and 63.5% would consider using ENDS after pregnancy to assist in smoking reduction or cessation. Importantly, no difference was noted in willingness to use ENDS vs. NRT during and after pregnancy.[534] Bhandari et al.[651] found 70.5% of pregnant women who self-reported using ENDS stated they used ENDS for cessation and current ENDS users reported higher perceptions in ease of quitting combusted cigarettes in comparison to former ENDS users and non-ENDS users. In a study of pregnant women, Mark et al.[533] found 73.0% of ENDS users reported a benefit of ENDS use was to help with smoking cessation, although no differences were found regarding cessation attempts among those combusted cigarette users who had ever used ENDS and those who never used ENDS. Pregnant women report using ENDS for cessation and perceive ENDS may assist in cessation attempts; however, evidence of successful use of ENDS for cessation is mixed.

Racial and Ethnic Disparity

Cessation behavior may play a role in racial/ethnic differences in ENDS use. Current research overarchingly suggests Non-Hispanic Black (NHB) smokers may have lower odds of fully switching to ENDS use or using ENDS to assist in full cessation of tobacco use in comparison to NHW smokers. Using a subsample of PATH Wave 1 and 2 data of participants who were established combusted cigarette users but did not use ENDS, researchers found NHB and Hispanic combusted cigarette users were less likely to begin using ENDS and switch to exclusive ENDS use, and more likely to believe ENDS are more harmful in comparison to combusted cigarettes in comparison to Non-Hispanic White (NHW) users.[652] Data collected in 2018 in Texas from current and former smokers who were ever-users of ENDS suggests NHB adults have lower odds of reporting using ENDS for cessation in comparison to NHW adults.[584] A 2014 study of 285 current and former smokers residing in Florida found NHB respondents (50.0%) were less likely to report ever ENDS use in comparison to NHW participants (71.0%) and Hispanic participants (71.0%), though no difference was found by race or ethnicity for past-30 day ENDS use.[653] However, among ENDS ever users, NHB participants were more likely to report intentions to continue ENDS use (72.0%) in comparison to White (53.0%) and Hispanic (47.0%) participants and were more likely to use ENDS as a cessation aid.[653] TPRPS 2016 and 2017 data show among former and current combusted cigarette users, NHB adults had the lowest ever and current ENDS use of all racial or ethnic groups.[560] A nationally representative sample of 3,000 U.S. youth aged 13-17 surveyed in 2017 showed NHB youth had lower odds of perceiving health risks due to nicotine and toxins or chemical in ENDS in comparison to NHW youth.[557] More research is important to further understand why NHB smokers may use ENDS for harm reduction at lower rates in comparison to other racial or ethnic groups, but the available literature appears to point at harm perceptions as playing a potential role.

Systematic Reviews

The most recent systematic review and meta-analysis on ENDS use and smoking cessation was published by El Dib et al. in 2017.[654] The meta-analysis combined results from two Randomized Controlled Trials (RCTs)[655,656] and found an increased likelihood of smoking cessation after 6-months with the use of nicotine-containing ENDS compared to the use of non-nicotine ENDS, but it was not statistically significant (RR=2.03, 95% CI 0.94 to 4.38). For reduction in CPD, the two RCTs found no difference between the nicotine-containing ENDS group and the non-nicotine ENDS group (RR=0.97, 95% CI = 0.57 to 1.66). A limitation of these RCTs is they used older generation ENDS, which may differ from newer generation ENDS (e.g., nicotine delivery capability). The current RCT literature is lacking information about the efficacy of more recent generations of ENDS, such as pod-based ENDS, for smoking cessation.

The other comprehensive systematic review was by Hartmann-Boyce et al. in 2016.[657] This review was the updated Cochrane Review on Electronic Cigarettes for Smoking Cessation. The same two RCTs included in the El Dib et al. review[654] were included in this review. However, the Hartmann-Boyce et al. review included participants with missing smoking data in the analysis and coded them as still smoking. When participants with missing data were included, using ENDS with nicotine was associated with a significantly higher likelihood of cessation at 6-months compared to using ENDS with no nicotine (RR=2.29, 95% CI = 1.05 to 4.96).

Several RCTs on ENDS and smoking cessation have been published since the El Dib and Hartmann-Boyce reviews, however the findings have been mixed. Three RCTs found cessation rates were higher for smokers assigned to ENDS use versus smokers assigned to NRT,[170,620,658] however, two RCTs did not find a higher cessation rate in ENDS users versus NRT use or standard care.[659,660]

Randomized Controlled Trials (RCTs)

Cessation studies are best conducted as randomized controlled trials (RCTs) where combusted cigarette smokers are randomized to several cessation options, including ENDS use, NRT, and standard care (which may involve behavioral counseling), with biochemically verified cessation outcomes. Currently, the impact of ENDS on cessation outcomes in RCTs is unclear: while four recent studies have found ENDS do not impact cessation beyond NRT or standard care,[659-662] two studies that examined combusted cigarette smoking cessation at three months and one year found ENDS use was associated with greater cessation rates compared to usual care or NRT.[170,620] However, Hajek et al.[170] noted complete nicotine cessation was higher among the NRT group (91%) compared to the ENDS group (20%). These contradictory findings may be due to the populations studied, ENDS used (and their ability to deliver sufficient nicotine to replace combusted cigarettes), comparators tested, and the specific definitions of cessation evaluated. Moreover, the Halpern et al.[660] and Masiero et al.[620] studies published preliminary findings, and final results may differ from these preliminary data. Interpretation of the Hajek et al. (2019) study[170] is limited because it was conducted in the UK where ENDS regulations differ from the US, limiting the types of ENDS (and e-liquids) used in the study. Although absolute combusted cigarette cessation did not differ among groups in either study, Hatsukami et al.[661] and Lucchiarri et al.[662] both demonstrated significant reductions in combusted cigarette smoking in participants randomized to ENDS substitution groups relative to NRT or control treatments. These results should be interpreted with caution, as Hatsukami et al.[661] provided bonus payment for combusted cigarette abstinence (negative CO sample) in the ENDS group, and the Lucchiarri et al.[662] study was conducted in Italy and limited older smokers (55+ years).

One of the studies that found an association compared the effectiveness of a refillable ENDS with NRT for smoking cessation.[170] The 1-year abstinence rate was 18.0% in the ENDS group and 9.9% in the NRT group (RR=1.83; 95% CI: 1.30-2.58). Participants using ENDS were encouraged to experiment with e-liquids of different flavors and nicotine strengths, so it's not clear if the use of flavors impacted the results. Another RCT of adult smokers who were ENDS never users and motivated to quit, randomized participants to receiving either nicotine patches, patches plus a nicotine-containing ENDS, or patches plus a nicotine-free ENDS.[658] At 6-months, 7% of participants in the patches plus nicotine-containing ENDS group had CO-verified continuous abstinence compared with 4% in the patches plus nicotine-free ENDS group (RR = 1.75, 95% CI: 1.02-2.98) and 2% of people in the patches only group (RR = 2.92, 95% CI: 0.91-9.33). Preliminary findings from an ongoing RCT of 210 combusted cigarette smokers who smoked for at least 10 years and were randomized to receive nicotine-containing ENDS, non-nicotine-containing ENDS, or telephone counseling only, found after 3-months both ENDS groups had a significantly higher rate of smoking abstinence compared to the counseling only group.[620] Although all groups reported a reduction in daily combusted cigarette consumption, the nicotine-containing ENDS group had the largest reduction.

Nationally Representative Studies

Several studies not considered in the systematic reviews used nationally representative data to look at the association between ENDS use and smoking cessation. Two of these studies also assessed how frequency of ENDS use may influence the association.

Cross-Sectional

Several papers have been published on the association between ENDS use and smoking cessation using data from the nationally representative TUS-CPS and NHIS studies. The main limitation of these data was they were cross-sectional and asked about past-year quit attempts and past-year cessation. Therefore, there is the potential for recall bias related to ENDS use, quit attempts, and cessation. Given these were cross-sectional studies, reverse causation (i.e., started using ENDS after cessation to prevent relapse) cannot be ruled out.

Data from the 2014-2016 NHIS and the 2014-2015 TUS-CPS found a higher prevalence of self-reported past 12-month smoking cessation in current ENDS users compared to those not using ENDS.[624] Another analysis using 2014-2015 TUS-CPS data found more frequent ENDS use was positively associated with past year cessation.[642] Similar results were observed in the 2014-2015 NHIS data; the prevalence of smoking cessation was significantly higher among daily ENDS users compared to those who had never used ENDS or only used ENDS on some days.[641]

In addition to frequency of ENDS use, duration of smoking cessation may impact the effect of ENDS use on smoking cessation. Data from the 2016 and 2017 NHIS were pooled to look at associations between current ENDS use and quit duration. Current ENDS use was inversely associated with being a former smoker when quit duration was not considered (aPR = 0.64, 95% CI = 0.59 to 0.69), but was positively associated with being a former smoker for less than one year (aPR = 1.44, 95% CI = 1.12 to 1.84) or 1–3 years (aPR = 1.21, 95% CI = 1.03 to 1.42).[640]

Longitudinal

Findings from longitudinal observational studies that have assessed the association between ENDS use and smoking cessation and reduction have been mixed. It should be noted cessation and smoking reduction findings from observational studies have the limitation that outcomes are self-reported and lack biochemical verification, therefore recall bias and misclassification are potential issues that may impact results. There are differences in the measurement of ENDS exposure; some studies look at ever use while other studies look at current past 30-day use. Also, some studies include smokers who are not using ENDS to quit smoking, which may impact findings and possibly underestimate the effectiveness of ENDS for smoking cessation. However, observational cohort studies have the potential to inform how effective ENDS are for reducing smoking rates in a real-world setting since not all smokers who use ENDS are necessarily trying to quit smoking.

The El Dib meta-analysis included 9 prospective cohort studies of current combusted tobacco users regardless of their intention to quit.[654] Results from the meta-analysis suggested smokers who use ENDS

are less likely to quit smoking than smokers who do not use ENDS (OR = 0.74, 95% CI: 0.55-1.00; p=0.05). The review concluded the observational studies provided very low-certainty evidence about the link between ENDS use and smoking cessation because participants were not necessarily using ENDS as a cessation device or may not have intended to try to quit smoking.

Additional observational studies of ENDS use and cessation have been published since the El Dib review. Berry et al. used data from Waves 1 and 2 of the PATH Study to assess the role of ENDS initiation in cigarette cessation or smoking reduction.[663] The analysis was limited to current smokers aged ≥25 years who were not ENDS users at Wave 1. Smokers who started using ENDS between waves had a significantly higher odds of reporting past 30-day cigarette cessation at Wave 2 compared to smokers not using ENDS. Frequency of ENDS use was an important factor. Compared to smokers not using ENDS, smokers who started using ENDS every day and were not able to quit smoking had higher odds of reducing their daily combusted cigarette use by at least 50%. Less frequent ENDS use was not associated with cigarette cessation or smoking reduction. In another PATH Study analysis of Wave 1 combusted cigarette smokers who reported an attempt to quit smoking between Wave 1 and Wave 2, using ENDS to quit combusted cigarettes increased the probability of persistent abstinence (≥30 days) at Wave 2, but ENDS use was not associated with reductions in combusted cigarette consumption among smokers who attempted to quit smoking who relapsed.[664]

In a US community sample of adult daily smokers, there was a significantly higher prevalence of smoking abstinence after 1-year among baseline dual users (8.0%) compared to baseline exclusive smokers (1.9%).[632] However, in a community sample of Minnesota current smokers attempting to quit, the prevalence of smoking abstinence one year later was slightly higher than never ENDS users, but the difference was not statistically significant: 20.0% in never ENDS users vs. 26% in infrequent ENDS users and 29% in daily ENDS users.[635]

Several studies have found dual users are able to reduce their daily combusted cigarette consumption. Baseline data from a cessation trial investigating the efficacy of a self-help intervention to promote cessation were used for a retrospective analysis of changes in combusted cigarette consumption.[199] Dual users were asked to recall their daily combusted cigarette consumption pre- and post-ENDS use. Based on that retrospective recall, dual users were able to significantly reduce their CPD from an average of 19.2 to 11.2 after they started using ENDS at least once per week. In a French study, dual-users of ENDS and combusted cigarettes were significantly more likely than exclusive smokers to reduce their combusted cigarette consumption by at least 50% over a 6-month follow-up period, although there were no differences in 7-day cessation rates between the groups.[665] A small (n=18) group of smokers who were given ENDS to help quit smoking were able to reduce their smoking by more than 50% after 6 and 10-weeks.[666] Among Dutch ENDS users who completed a social media online survey in 2016, dual users had started using ENDS an average of 22 months ago and reported an 82% reduction in average daily combusted cigarette consumption.[667] In a French longitudinal study, ENDS use in smokers was associated with a significantly larger reduction in CPD compared to smokers not using ENDS.[643]

In contrast, some studies did not find a reduction in combusted cigarette consumption among dual users. A prospective cohort study in England found after 12-months, smoking quit rates were not

significantly different in dual combusted cigarette and ENDS users compared to exclusive combusted cigarette smokers or dual combusted cigarette and NRT users.[625] In the Tobacco User Adult Cohort, daily smokers who also used ENDS daily or some days were not more likely than exclusive smokers to reduce their combusted cigarette consumption or report smoking abstinence after 18-months.[668] In an ongoing cohort study in Italy, after four-years of follow-up there were no significant differences in smoking abstinence rates or smoking reduction between dual users and exclusive smokers.[628] An analysis using data from the GfK KnowledgePanel collected in 2015 and 2016 found the odds of quitting smoking after 1-year were significantly lower for smokers who used ENDS at baseline compared to non-users of ENDS, even among daily ENDS users.[648]

Conclusion

The NASEM report concluded evidence about the effectiveness of ENDS for smoking cessation is limited. There are only a few RCTs that have looked at this association and the observational studies looking at this association have the following methodological issues: 1) they do not account for ENDS product characteristics (e.g., device type, flavors, and nicotine concentration; 2) most studies do not look at frequency of ENDS use or duration of use; and 3) many studies do not assess interest in quitting smoking. Based on these factors, the NASEM report came to the following conclusions:

- Overall, there is limited evidence ENDS may be effective aids to promote smoking cessation.
- There is moderate evidence from RCTs that ENDS with nicotine are more effective than ENDS without nicotine for smoking cessation.
- There is insufficient evidence from RCTs about the effectiveness of ENDS as cessation aids compared with no treatment or to FDA-approved smoking cessation treatments.
- While the overall evidence from observational trials is mixed, there is moderate evidence from observational studies that more frequent ENDS use is associated with an increased likelihood of cessation.

These conclusions are mirrored in the 2020 Surgeon General's 2020 Report on Smoking Cessation:[669] which stated:

"E-cigarettes, a continually changing and heterogeneous group of products, are used in a variety of ways. Consequently, it is difficult to make generalizations about efficacy for cessation based on clinical trials involving a particular e-cigarette, and there is presently inadequate evidence to conclude that e-cigarettes, in general, increase smoking cessation."

There remains limited evidence from RCTs that ENDS are effective for smoking cessation or smoking reduction. Findings from observational studies are mixed, although they suggest more frequent ENDS use may be associated with higher odds of smoking cessation.

Conclusion for Section 3.B. Patterns of ENDS Use

Subjective experiences at ENDS initiation use may contribute to continued ENDS use and progression to nicotine dependence. There is concern ENDS use among youth and young adults will lead to initiation of

combusted cigarettes and progression to regular smoking. Although a number of studies have investigated this concern, their design limitations impact interpretations of their findings on ENDS role in smoking initiation and progression. Evidence on the effectiveness of ENDS for smoking cessation also remains limited. When switching from combusted cigarette smoking to ENDS use, combusted cigarette consumption decreases, likely to maintain preferred nicotine concentrations. Conclusions from the few available RCTs are mixed: some studies found ENDS do not impact smoking cessation beyond NRT or standard care and two studies found ENDS are associated with greater smoking cessation rates compared to NRT or usual care (one of these two found NRT to be more effective than ENDS at complete nicotine cessation). Findings from observational studies are also mixed, although study findings suggest more frequent ENDS use may be associated with higher odds of cessation. Additional RCTs and observational studies are important to determine the ability of ENDS to facilitate complete switching from combusted cigarettes, and the impact of ENDS use on cessation outcomes.

C. INFLUENCE OF PRODUCT CHARACTERISTICS AND MARKETING ON ENDS APPEAL

Flavor

Tobacco non-user

A review of the use and appeal of flavored ENDS indicates non-traditional flavors (i.e., flavors other than tobacco and menthol) are typically used by most youth at ENDS initiation.[670] A national sample (*N* = 1,125) collected from a 2014-2015 study found youth (aged 13–17 years) were more likely to report interest in trying ENDS offered by a friend if they were flavored like menthol (OR = 4.00, 95% CI [1.46, 10.97]), candy (OR = 4.53, 95% CI [1.67, 12.31]) or fruit (OR = 6.49, 95% CI [2.48, 17.01]).[671] In a discrete choice experiment conducted in 2015 among 515 U.S. youth (aged 14–17 years), 465 of which were non-users, fruit, sweet, and beverage flavors increased the probability of choosing ENDS ($p < .01$), but not ever-users ($p < .10$); and menthol flavor increased the probability of choosing ENDS relative to tobacco flavor ($p < .05$).[672] Data collected in 2016 indicates flavor was reported as a common reason for ENDS initiation in 29.5% of current ENDS users (*N*=1,492) and fruit flavors were more likely to motivate young adults aged 18–24 years to initiate ENDS use compared to adults aged 35–44 years ($p < .001$).[673]

Flavors may also play a differential role in harm perceptions among youth. Multiple youth studies have examined users and non-users together. A large US survey (*N* = 1,125) collected in 2014-2015 among youth (aged 13–17 years) found most respondents had never used combusted cigarettes (89.0%) or ENDS (85.0%) and only 4.0% used combusted cigarettes and 5.0% used ENDS. Among this group, most survey respondents believed fruit-flavored ENDS were less harmful than tobacco-flavored ENDS.[671] PATH data from Waves 2 and 3, collected between 2014-2016, indicate 21.2% of youth who had never used tobacco (aged 12–17 years) perceived flavored ENDS were easier to use than tobacco flavored ENDS.[674] Among these youth who had never used tobacco, 41.0% were susceptible to ENDS use, and 10.6% initiated ENDS use a year later. The Texas Adolescent Tobacco and Marketing Surveillance system survey (Wave 1) was collected during the 2014-2015 academic year among 3,704 youth in 6th, 8th, and 10th grades. This data indicates never users were more likely to report flavored ENDS were not less harmful than non-flavored ENDS, whereas ever and current ENDS users thought flavored ENDS were less harmful (OR = 2.84, 95% CI [1.91, 4.21]). [675]

Data from these studies show flavors may play a differential role in both harm perceptions and appeal in youth who had never used tobacco. In general, if a product is perceived as less harmful, it may be more appealing.

Current Tobacco Product Users

Data from Waves 1[586] and 4[601] of the PATH Study indicate most youth's first ENDS use was with a flavored ENDS, and studies have found flavor is among the most important factors in determining if youth will try ENDS.[673,676,677] PATH data from Wave 2 found concurrent use of multiple flavors of ENDS was more common among youth and young adults than older adults and the availability of multiple flavors was the leading reason for ENDS use among youth and young adults.[522] Using NYTS 2016 data, the availability of flavors was the second most commonly selected reason for ENDS use (31.0%).[678] Other research indicates young adults (aged 18–24 years) are more likely than older adults (aged 55+ years) to cite flavor as a reason they tried ENDS,[679] and young adults (aged 18–24 years) were more motivated to initiate ENDS use because of flavors compared to older adults (aged 35–44 years).[673]

Focusing on specific flavors, several studies have found the most common or preferred ENDS flavor among youth and young adults is fruit, often followed by candy and other sweet flavors.[183,522,587,589,680-682] Among a small sample (N = 60) of youth and young adults (aged 16–20 years), a high flavor concentration of menthol (3.5%) in e-liquids, compared to no menthol in e-liquids was associated with greater appeal ($p < 0.001$).[147] Another survey of youth found the use of fruit-flavored, dessert-flavored, and alcohol-flavored ENDS was associated with more frequent ENDS use.[183] A study of young adults (aged 18–22 years) who tried either combusted cigarettes or ENDS found those who "preferred vaping" favored fruit and candy flavors over tobacco flavor, while those who "preferred smoking" favored tobacco flavor.[683] Other research conducted with young adults found fruit and mint were the most commonly used flavors in cartridge-based ENDS and other ENDS, followed by candy flavors.[684] Additionally, in multiple lab studies of young adult ENDS users, sweet flavors were more appealing than non-sweet and flavorless solutions.[685,686]

Among adults, while some studies have found the most common or preferred ENDS flavor is fruit, often followed by candy and mint/menthol,[183,522,525,587,589,681,687] others have found the most common or preferred ENDS flavor for adults is mint/menthol[587] or tobacco.[103,522,682,688] In a 2017 discrete choice experiment among adult smokers who also used ENDS (N = 1,154), smokers were not interested in menthol flavored ENDS unless they already used menthol combusted cigarettes.[689] Some of these inconsistent study findings might be explained by longitudinal research in older adults (N = 383, aged 45+ years) collected from 2012-2019 in two waves. This research found flavor preferences have changed over time among ENDS users.[631] Respondents who preferred tobacco, mint, or menthol ENDS flavors switched to candy/sweet flavors, with fruit ENDS flavor preference remaining stable and preference for other ENDS flavors increasing slightly. Patterns of results from the studies described above indicate younger adults were likely to switch to candy/sweet ENDS flavors, and exclusive ENDS users likewise had a stronger preference for candy/sweet flavors compared to dual users.

PATH Wave 2 data indicates tobacco-flavored ENDS was higher among older adults,[682] while youth and young adults were more likely to use fruit-flavored and candy-flavored ENDS or use multiple ENDS flavors concurrently than older adults.[522] Similarly, PATH data from Wave 2 also indicated first use and past 30-day use of ENDS among all age groups were likely to be fruit, sweet, or menthol/mint flavored ENDS. A high use prevalence of these ENDS flavors was found among all age groups.[600] Additionally, using PATH Wave 2 data, use of fruit-flavored and candy-flavored ENDS was higher among women, while use of mint/menthol ENDS was higher among Non-Hispanic Black and Hispanic participants than non-Hispanic White participants.[522]

Two qualitative studies[544,690] and two survey studies[691,692] suggest adult smokers perceive the availability of flavors as an appealing or important aspect of completely switching to ENDS, while a study of youth and young adults found using two or more ENDS flavors mixed together was associated with a greater likelihood of quitting smoking.[693] Another study found menthol ENDS was perceived among adult smokers as having greater potential to help smokers quit combusted cigarettes[694] and a one-year longitudinal study found mint/menthol/wintergreen ENDS users were more likely to report a combusted cigarette quit attempt than non-users of ENDS or users of tobacco-flavored and unflavored ENDS.[648] However, actual cigarette cessation rates did not differ between mint/menthol/wintergreen ENDS users and non-users, and users of tobacco-flavored, unflavored, and "other-flavored" (e.g., fruit, dessert, spice) ENDS were less likely to quit combusted cigarettes than non-users of ENDS.

Longitudinal data from PATH Waves 1 and 2 found most new ENDS users, regardless of age, used flavored ENDS.[599] Experimentation with flavored ENDS at Wave 1, compared to non-flavored ENDS, was associated with young adult and adult regular use at Wave 2 (about one year later). Results from another nationally representative sample, the 2016 and 2017 Tobacco Products and Risk Perceptions Survey (TPRPS), indicated using multiple flavors at ENDS initiation, as well as using mint and menthol flavors, leads to longer exclusive ENDS use rather than dual ENDS and combusted cigarette use.[524]

Research on the general impact of flavors on different aspects of ENDS use is somewhat mixed. For example, one lab study found flavors reinforced young adults desire to use nicotine in ENDS.[181] Another study found preferring a higher number of flavors was associated with more frequent ENDS use among youth.[183] Finally, a study using 2014 NYTS data found flavored ENDS use was associated with higher intentions to initiate combusted cigarette use and lower intention to quit ENDS use.[695] Thus flavors may impact several different aspects of ENDS use and appeal.

Overall, research suggests flavor may generally make ENDS appealing. The appeal of fruit and sweet flavored ENDS among youth may be due to a direct preference for the flavor, and may also be impacted by ENDS harm perceptions, as multiple studies found youth perceived fruit and sweet flavored ENDS to be less harmful than non-flavored ENDS. Flavored ENDS have been found to be appealing to all age groups, not only youth. Most new users report initiating with flavored ENDS and fruit and sweet flavors are more likely to be used by individuals who have never used combusted cigarettes rather than by smokers trying to quit smoking cigarettes. However, some smokers reported the flavors in ENDS to be an incentive to switch from combusted cigarettes to ENDS.

Former Tobacco Users

In general, the prevalence of ENDS use by recent former smokers is higher than the prevalence of ENDS use by long-term former smokers, and according to the 2014 data, it is very uncommon for adult long-term former smokers to report currently using ENDS.[262] Current literature indicates although prevalence and incidence of ENDS use is higher among former combusted cigarette users than never smokers, it is also lower among former smokers than current smokers.[517] According to 2017-2018 NHIS data, the prevalence of adult former combusted cigarette smokers reporting current use of ENDS increased significantly from 4.2% of former combusted cigarette smokers (i.e., smoked 100 combusted cigarettes in lifetime but not currently smoking) using ENDS in 2017 to 5.5% in 2018. However, the NHIS data are cross-sectional and did not assess the timing of quitting combusted cigarettes and initiation of ENDS for former smokers. Therefore, it is unclear whether these former smokers were smoking combusted cigarettes when they first started using ENDS and ENDS may have aided in cessation; or if, conversely, these former smokers began using ENDS after quitting combusted cigarette smoking.

There is limited data available on ENDS appeal in this group. In a nationally representative study (*N* = 1,814), a little under 15.0% of former tobacco users believe flavored ENDS are safe; however, information on beliefs about non-flavored ENDS was not provided.[524] In the same study, former tobacco users were less likely, compared to current dual users, to have initiated ENDS use with flavors such as spice or beverage flavors (excluding alcohol or coffee flavors). A similar pattern emerges when examining the number of flavors at initiation with former tobacco users being more likely than current smokers to have only used one flavor. Most of these former tobacco users (92.8%) reported they had used ENDS containing nicotine.[524]

Product Design

Tobacco never users

Some ENDS product characteristics are described as appealing, especially to youth, such as sleek designs, easy-to-use products, and small products, which are easy to conceal, if desired.[3] However, most data available on the appeal of product design is from current users, so product characteristic appeal data specific to the tobacco never users is limited. One discrete choice experiment among youth (aged 14–17 years) non-users (*N*=465), found the ability to modify an ENDS was associated with choosing an ENDS, even when given the option to not choose any tobacco products ($p<.05$).[672] Additional information in this area would further understanding of what the tobacco never users may find appealing.

Current Tobacco Product Users

A wide variety of ENDS designs are available, and users have different preferences for product designs, which may influence product appeal and use. A preference for reusable or rechargeable products has been found in studies of youth,[681] young adults [683] and adults,[263] although these preferences may be changing among youth due to changes in product availability.[696] Research also indicates modifiable products are preferred relative to "cig-a-like" products among both youth[672] and adults.[697] Experienced users of ENDS cited the ability to customize products as one of the most important aspects of their

device[698] and often switch to customizable products from more basic products, which are easier to use.[699] Indeed, adult ENDS users identified ease of use as an important product feature for initial ENDS use.[697] For example, a 2019 qualitative study of 13 experienced adult ENDS users in Atlanta explored ENDS modification behaviors. Respondents mentioned they modified coils, batteries, and e-liquids.[135] This was done for a variety of reported reasons, from producing large clouds and experiencing different throat hits to changing nicotine levels and flavors. Although some ENDS are designed to be modified by the consumer, respondents indicated they also modify products not meant to be reused, such as pods.

Disposable ENDS, which are low-power products designed with no replaceable parts, recently emerged. These disposable ENDS have many similar appealing qualities to reusable pod-style ENDS, such as being user friendly, sleek in design, and easy to conceal. There is indication these products are easier to acquire than pod-style ENDS (such as JUUL), due to price and lax age verification.[700] Further, the popularity of disposable products coincides with a number of brands reported as having a similar taste, a variety of flavors, and some brands even last longer than JUUL products.[696]

A review of the literature focused on youth ENDS use has found the sleek design, ability to use the products discreetly if desired (i.e., "stealth" ENDS use), and user-friendly nature makes pod-style products appealing.[3] Several studies noted among the most appealing aspects of an ENDS among youth and young adults is the ability to easily conceal the products (e.g., similar aesthetics to other personal electronics) and the ability to use the products discreetly (e.g., "stealth" vaping, little detectable evidence of use).[701-705] This allows ENDS to be used in public and can prevent authorities (e.g., parents, teachers) from being aware of use.[704] These findings are supported by a study that found younger adults (aged 18–24 years) were more likely than older adults to have ever used a USB-shaped ENDS, and a much higher proportion of ever users transition to become regular users if they are young adults, rather than older adults.[706]

A small, qualitative study of why and how current and former combusted cigarette smokers used ENDS found they were used to reduce combusted cigarette use and as a cessation strategy. Study participants agreed a positive attribute of ENDS was the ability to use when combusted cigarettes were not allowed and a common negative attribute was that ENDS are habit forming.[707] PATH Wave 2 data demonstrated 58% of dual users of ENDS and combusted cigarettes had used ENDS in smoke-free places in the past month.[708] These respondents were more likely to use ENDS to cut down on combusted cigarettes or to replace smoking completely than respondents who had not used ENDS in smoke-free places. A national, cross-sectional discrete choice experiment of adults aged 18+ years that have ever used ENDS found the 8th and 9th ranked choices (out of 9) of preferred ENDS attributes, were product design (7.2%) and modifiability (4.6%), respectively. However, when looking at importance scores of each attribute, use as a cessation aid was ranked third (12.6%).[709] A separate study of current combusted cigarette, cigar, little cigar, or cigarillo and ENDS users (aged 18–54 years), collected in 2018, found the most common reason for using JUUL, was as a cessation aid (37.0%).[710] This data contradicts the previously mentioned study findings, in which experienced ENDS users stated customizing products was one of the most important aspects of their product;[698] however, this data was collected in 2012-2013. Similarly, other work on the modification of ENDS states fewer users currently engage in these behaviors compared to previous

years.[135] This may indicate experience with ENDS may change desired modifiability over time or that preferences have changed in previous years, leading to divergent results.

Nicotine

Tobacco never users

Research on the appeal of nicotine in ENDS among individuals never used tobacco is currently limited because most nicotine appeal in ENDS research focuses on current users. Research with a youth sample, containing 95% tobacco never user respondents and 5% current ENDS users, collected in 2014-2015, found some youth did not believe ENDS contained nicotine (14.6%) or did not know whether ENDS contained nicotine (3.6%).[671] Similarly, in another study of youth and young adults (aged 15–24 years), only 25% of non-users who recognized JUUL were aware JUUL always contains nicotine.[711] The American Heart Association Tobacco Regulation and Addiction Center collected a nationally representative sample of youth (aged 13–17 years) in 2017. This data found non-users of ENDS were more likely to perceive nicotine in ENDS might cause health problems compared to current ENDS users.[557] Additional research regarding perceptions of nicotine would provide better insight into differences between tobacco never users and current ENDS users.

Current Tobacco Product Users

Awareness of nicotine is important because research has found associations between believing ENDS are less addictive than combusted cigarettes and ENDS use among youth and adults.[675,712-715]

In a study of individuals aged 15–24 years, only 37.0% of past-30-day users of JUUL were aware JUUL always contains nicotine.[711] Other research indicates some consumers do not classify ENDS as tobacco products. In a study of youth ENDS users (*N* = 1,589, aged 15–17 years) 17.0% of non-nicotine users and 34.0% of nicotine users understood the nicotine in ENDS was derived from tobacco;[716] most youth thought the nicotine was artificial, potentially indicating a belief this nicotine is "safer," and about one-third of ENDS users with (33.8%) and without (36.4%) nicotine believed firsthand aerosol is just water vapor. Similarly, in a Californian sample of students (*N* = 786, grades 9th-12th), 19.1% believed the aerosol from ENDS was just water and 23.0% believed ENDS were not a tobacco product.[717]

Although a number of false beliefs around nicotine in ENDS exists, some research suggests the nicotine content in ENDS is an appealing attribute that may impact current tobacco use.[702,709] In a national discrete choice experiment of product attributes, the second most important attribute was nicotine content, reported by 13.0% of respondents who had ever used ENDS, while harm perceptions was rated first by 49.0%.[709] Qualitative studies of young adults have found ENDS appealing in part because they allow choice in how much nicotine you consume and can provide a better "buzz" than combusted cigarettes,[702] with some noting cartridge-based systems with a high nicotine content have distinctive psychoactive effects.[704] Another study which was weighted to match current United States census distributions found the leading reason for ENDS use was 'to deliver nicotine' (30.7%).[706]

Regardless of beliefs about ENDS containing nicotine, most ENDS have nicotine e-liquid solutions. Research indicates use of high nicotine concentrations is associated with greater frequency of past-30-day combusted cigarette and ENDS use, as well as greater per-use intensity of both products, six months

later in youth ENDS users.[718] Similarly, in another youth sample, always using nicotine in ENDS (versus sometimes) was associated with more frequent ENDS use.[680] In a longitudinal study of Finnish youth, experimentation with nicotine containing ENDS at baseline predicted daily combusted cigarette use at follow-up two years later. This same pattern of results was not found for experimentation with non-nicotine ENDS.[719] PATH data found over time, 15.7% of non-nicotine ENDS users transitioned to using both nicotine and nicotine-free products, while 17.8% transitioned to using ENDS containing nicotine exclusively.[720]

Other research suggests the experience of the "throat hit" from ENDS, affected by product voltage, nicotine concentration, and the nicotine formulation (i.e., nicotine salts are more effective in delivering an intense experience), impacts individuals' interest in quitting combusted cigarettes; how "pleasant" the individual perceived the ENDS experience to be was positively associated with participants' interest in quitting combusted cigarettes.[721] In a study with a sample of Canadians aged 16+ years, use of low nicotine ENDS was associated with reduced harm perception and greater perceived quit efficacy.[722] JUUL is known to have a high nicotine content (i.e., 5.0%) compared to other products. A 2018 nationally representative AmeriSpeak sample of U.S. adults between 18–54 years, found among current tobacco users, 15.0% had tried JUUL. These users reported the most common reason for trying the product was "to quit smoking cigarettes" (37.0%).[710]

Overall, research suggests many youth are not aware ENDS can contain nicotine, and beliefs about the addictiveness of ENDS are associated with whether youth and young adults use ENDS. Additionally, while the presence of nicotine in ENDS is associated with more frequent ENDS and combusted cigarette use among youth, it may be associated with a greater likelihood of interest in quitting combusted cigarettes among adults.

Product Promotion

The ways ENDS are promoted may influence product uptake. Kantar media data shows ENDS advertising expenditure for radio, print (magazines and newspapers), television, internet (standard and mobile devices, but does not fully capture social media), and outdoors was $133 million in 2014, $57 million in 2015, $72 million in 2016, $48 million in 2017, and $110 million in 2018.[723] A systematic review of literature examined ENDS marketing practices and impact via 124 relevant articles published before June 2017.[724] Studies found exposure to marketing is associated with greater intention to use ENDS and lower perceived harm of ENDS use. Furthermore, longitudinal studies support exposure to ENDS advertisements may be associated with increased odds of ENDS initiation among youth who had never used tobacco. Less evidence is available to characterize the association between marketing and dual use or cessation outcomes, and no studies have analyzed marketing's impact on current and former tobacco users.

Traditional Marketing

Nationally representative study data suggest most youth are exposed to ENDS marketing. Data from the NYTS 2014 indicate, 68.9% of middle and high school students (18.3 million) were exposed to ENDS advertisements from at least one source.[725] For both middle and high school students, exposure was

highest at retail stores (52.8% and 56.3%, respectively), followed by online (35.8% and 42.9%, respectively), television and movies (34.1% and 38.4%, respectively), and newspapers and magazines (25.0% and 34.6%, respectively).[725] NYTS 2019 data indicate 69.3% of middle and high school students (18.26 million) were exposed to ENDS advertisements from at least one source.[515] For both middle and high school students, exposure was highest at retail stores (58.4%), followed by online (44.6%), newspapers and magazines (34.8%), and television (including streaming services and movies, 26.2%).[515] NYTS data also show exposure to ENDS advertising through any source rose from 68.9% in 2014 to 78.2% in 2016, with statistically significant increases found for 2014-2015, 2015-2016, and 2014-2016.[726] Taking the 2019 NYTS statistics into consideration, it appears exposure rose between 2014 and 2016, but may have decreased from 2016 to 2019. Consistent with NYTS data, U.S. youth (aged 16–19 years) data from the 2017 ITC Youth Tobacco and Vaping Survey (*N* = 12,064) show the majority of youth (81.0%) report exposure to ENDS advertisements in the past 30 days.[727]

Qualitative interviews suggest youth ENDS users and non-users may receive information on ENDS from multiple sources, including peers, advertising, family, school (for non-users only), television, social media, and other online sources.[728] Advertisements can be disseminated through different communication channels. From 2014-2016 NYTS data, youth ENDS advertisement exposure through retail stores, the most common avenue for exposure in the NYTS data, rose from 54.8% in 2014 to 68.0% in 2016, while no change in exposure through internet and television occurred, and exposure through newspapers and magazines , the least common avenues for exposure, dropped from 30.4% in 2014 to 23.9% in 2016.[726] Nationally representative data collected in 2014-2015 from youth (aged 13–17 years, *N* = 1,124), young adults (aged 18–25 years, *N* = 809), and adults (aged 26+ years, *N* = 4,186) provide estimates on exposure to ENDS advertising through different channels, of which television was the most commonly reported communication channel for all three age groups (74.9%, 70.7%, and 66.9%, respectively).[729] Youth and young adults were more likely than older adults, to be exposed to ENDS advertising through television and digital marketing.[729] Older adults were more likely to report exposure through radio, print, and retail environment, compared to youth.[729] Taken together, these data suggest all age groups have high levels of ENDS advertising exposure through retail and television, and high exposure for all channels overall. Adults may be preferentially reached through print and radio and youth and young adults may be reached through digital channels. Furthermore, ENDS marketing expenditure differs by communication channels, with print advertisements representing the largest proportion of advertising expenditure across 2014-2018.[723] JUUL Labs advertising accounts for $73 million of the $110 million spent on ENDS advertising in 2018.[723] Furthermore, 91% of 2018 expenditures were on print and radio advertising (statistics do not fully capture social media marketing expenditure). This suggests JUUL primarily used print and radio venues.[723]

Both experimental and observational research has shown exposure to ENDS advertisements is associated with subsequent ENDS use among youth, and some studies have found an increased likelihood of use of other tobacco products as well. An RCT with youth who had never tried ENDS found exposure to four ENDS television advertisements resulted in greater likelihood of trying ENDS soon, trying ENDS in the next year, and trying ENDS if a best friend offered one, as well as lower perceptions of

risk.[730] In another experiment, exposure to television ENDS advertisements caused youth (aged 13–17 years) never users to perceive ENDS as cooler, more fun, healthier, and more enjoyable.[731]

Experimental data is in line with nationally representative data about marketing exposure. Studies using NYTS data have found exposure to point-of-sale advertising was associated with greater odds of having higher curiosity about ENDS,[732] exposure to ENDS marketing was associated with ever and past-30-day use of ENDS,[712,733] and exposure to ENDS advertisements is associated with non-smoking youths' intention to use ENDS, but not associated with smoking youths' intention to use ENDS.[734] This suggests advertisements are attracting youth who had never used tobacco rather than causing tobacco-using youth to switch from combusted cigarettes to ENDS. Additional research conducted in 2015 found less than 1.0% of youth in eight high schools in Connecticut reported not seeing any ENDS advertisements, and higher socioeconomic status was associated with greater recent advertising exposure, which in turn was associated with greater frequency of ENDS use.[735] NYTS 2015 cross-sectional data show combined exposure to ENDS and non-ENDS tobacco product advertisements is associated with youth ENDS use, yet exposure to non-ENDS tobacco product advertisements solely is not associated with higher odds of ENDS use.[736] This suggests advertising exposure and its impact on youth may be specific to tobacco product type.[736]

Longitudinal research indicates advertisement exposure may be associated with future ENDS use. Data from Waves 2 and 3 of the PATH study concerning youth (aged 12–17 years, *N* = 8,121) and young adult (aged 18–24 years, *N* = 1,683) never tobacco users indicate youth and young adults who were exposed to ENDS marketing at Wave 2 had higher odds of experimenting with ENDS at Wave 3 compared to youth and young adults who were not exposed (youth AOR = 1.53, 95% CI: 1.07-2.17; young adult AOR=2.73, 95% CI: 1.16-6.42).[737] PATH data also indicates ENDS advertising has greater receptivity in comparison to other tobacco product advertising for never users of tobacco, and subsequently, ENDS advertising exposure in Wave 1 was associated with ENDS use at Wave 2, for PATH participants aged 12–17 years at Wave 1.[738] A structural equation model of longitudinal data collected in 2014-2016 from 1,553 Southern Californian youth including a latent variable of ENDS marketing including exposure to internet, print, in-store, outdoor, and television advertising found this variable to be independently associated with youth ENDS initiation, controlling for baseline tobacco use, social environmental factors, and demographics.[615] One study examined passive exposure to ENDS media (including, but not limited to advertising) using data from a nationally representative survey collected in 2014–2017 from youth and young adults (mean age = 18.39 years, *N* = 3,212) and found passive exposure to media that mentions ENDS is associated with subsequent ENDS use, and this association is partially mediated by norm perceptions of ENDS use and interpersonal conversations mentioning ENDS.[739]

Additional research supports ENDS marketing is associated with initiating ENDS use. A recent systematic review corroborates these conclusions, where seven out of nine studies reviewed found increased intention to use ENDS following exposure to advertising.[740] Furthermore, three out of five longitudinal studies noted ENDS initiation was higher among participants who recalled or liked advertisements at earlier timepoints.[740]

Specific marketing practices and communication channels may have independent impacts on youth and young adult ENDS intention to use, initiation, and use. One study using NYTS data found higher frequency exposure to ENDS advertisements from the internet, newspapers and magazines, stores, and television and movies were each independently associated with current use of ENDS.[669] Longitudinal youth (aged 12–17 years, *N* = 2,288) and young adult (aged 18–29 years, *N* = 2,423) never ENDS user data from a Texas cohort study show youth and young adults who recalled retail ENDS advertising or marketing at baseline had higher odds of ENDS initiation 2.5 years later (youth AOR=1.99, 95% CI: 1.25-3.17; young adult AOR=1.30, 95% CI: 1.05-1.61) compared to people who did not recall.[741] In the same study, young adults who recalled ENDS advertising/marketing on television had a higher odds of ENDS initiation 2.5 years later (AOR = 1.29, 95% CI: 1.03-1.63) compared to those who did not recall.[741] A longitudinal study conducted from 2014-2015 of youth enrolled at alternative high schools (a high-risk population for substance use) found exposure to ENDS commercials and likeability of ENDS commercials were associated with subsequent ENDS use at follow-up, controlling for tobacco use, and this association was stronger among females compared to males.[742] However, other analyses of these same data employing a different methodology showed males aged 16–18 years exhibited an association between frequency of exposure to ENDS commercials on television and internet and ENDS use, but this association was not observed in males aged 15 or 19–20 years or in females.[743] Other research has found ENDS advertising volume within a half-mile of one's school was significantly associated with being a past-month ENDS user.[744] Each communication channel used for marketing an ENDS may have independent effects on ENDS use.

Distributing coupons for ENDS may be an effective marketing practice used by tobacco companies in conjunction with advertising to increase ENDS use in youth and adults. PATH Waves 1 and 2 youth data show engagement with online tobacco marketing and past six-month receipt of a tobacco product coupon were associated with subsequent past 30-day ENDS use, although only for some user groups.[745] Receipt of a free ENDS sample was associated with ever use, past 30-day use, and new ENDS use among youth and adults in PATH Wave 3 cross-sectional data.[591]

Few analyses explored if exposure to ENDS advertisements increases use or intention to use ENDS among smokers. NYTS data from 2014 support ENDS advertisement exposure is associated with intention to use ENDS for non-smokers, yet not for smokers.[734] However, this study is focused on youth smokers, rather than adult established users of combusted cigarettes.

Furthermore, few studies examined the association between exposure to advertisement and cessation behavior of smokers. U.S. National Consumer Survey data from 2013-2015 suggest television advertising for ENDS may be associated with combusted cigarette use cessation among adults.[746] Further, this study found ENDS advertising through television, but not magazines, is associated with successful quit attempts among adult smokers, with no effect on failure rate of quit attempts, and the association between television marketing exposure and successful quit attempts may be strongest among young adults aged 18–34 years.[746] One online experimental study of adult smokers, of whom the majority had used ENDS at least once, found those participants who received messaging of harm reduction or the ability to use ENDS anywhere had lower odds of smoking in comparison to those participants who received control messaging, although no differences were found for quit intentions.[747] One study, which

examined cessation behavior among young adult smokers aged 18–29 attending college in Texas, found exposure to ENDS and combusted cigarette advertising was not associated with combusted cigarette smoking behavior six-months later.[748] However, this study found exposure to ENDS displays was associated with reduced tobacco abstinence, although combusted cigarette point-of-sale advertising exposure increased the odds of using ENDS for cessation or reduction at a six-months follow-up.[748] Given the mixed research findings, the association between cessation and exposure to advertising and marketing highlighting cessation is unclear at this time.

There is also evidence ENDS use leads to higher marketing exposure as evidenced by a longitudinal study of college students in Texas where exposure led to future ENDS use and ENDS use led to increased marketing exposure.[749] ENDS use may increase exposure to advertisements through many mechanisms, including increased sensitivity to ENDS cues in the environment and marketing practices targeting current ENDS users. Additional research suggests ENDS users have higher exposure to ENDS advertisements (82.8% exposed) in comparison to non-users (77.9%), with a similar pattern noted for users (82.7%) vs. non-users (77.6%) of tobacco products, generally.[726]

Users of other tobacco products may have increased exposure to ENDS marketing. NYTS 2014 data show exposure to ENDS marketing is associated with higher odds of ever and current use of combusted cigarettes, cigars, waterpipe, and polytobacco products,[750] and in other analyses exposure to tobacco marketing in general was associated with use of tobacco products, with single, dual, and poly users having higher exposure in comparison to non-users, and dual and poly users having higher exposure in comparison to single product users.[751] From nationally representative data, older adults who are current smokers (including lifetime smokers) have increased odds of exposure to ENDS marketing in comparison to nonsmokers.[729] Poly-tobacco product users have a higher risk of having peers who use tobacco and receptivity to tobacco advertising in comparison to non-users and single-product users. In a sample of youth from North Carolina, 12.0% were single tobacco product users and 13.0% used two or more tobacco products.[752] Of this sample, 52.0% of the single product users were ENDS users and 76.0% of the poly-tobacco product users used ENDS.[752] In sum, exposure to ENDS advertising differs by tobacco user group status.

The content of ENDS advertisements may play a role in the association between exposure to advertisements and perceptions, appeal, and use of ENDS. Static ENDS advertisements (19.8% from print sources, 80.0% from online sources) shown to the U.S. public from 2015-2016 highlighted taste (22.9%), device features (24.7%), ENDS as an alternative to combusted cigarettes (13.1%), included vapor (18.9%), health disclaimers (10.2%), cartoons (11.5%), financial incentives (54.9%, primarily on opt-in emails), people (17.2%) and rarely included harm reduction or cessation messaging (<2.0% of all advertisements).[753] Content analysis of tobacco advertisements (*N* = 131 for ENDS) from 2016 suggest ENDS advertisements were geared toward new users, included descriptions of ENDS characteristics, and showed how to use ENDS, but did not focus on specific marketing themes.[754] Some researchers note many advertisements carry promotions of ENDS for cessation.[724] An eye-tracking study of 30 young adults aged 18–26 years (80.0% of sample between 18 and 21 years) recruited from a midwestern U.S. city suggests adults fixate faster on and spend more time looking at people in ENDS advertisements in comparison to logos or descriptors.[755] Qualitative interview data collected in 2017 from 59 young adult

(aged 18–29 years) Californians who had used at least two tobacco products in the past 30 days (*n* = 52 used ENDS) identified young adults prefer ENDS advertisements that feature people similar to them in demographics and peer crowd status (such as hipster or main stream) and value authenticity in advertisements, although participants exhibited heterogeneity in which advertisements they perceived as inauthentic.[756] ENDS advertisements use myriad appeals, including visual features, informative content, and marketing strategies to attract diverse consumer groups.

Specific populations, including age and tobacco user groups, may perceive and interpret advertisements differently. One online study of 765 U.S. and UK adults suggests dual users, single product users, and non-users may develop different perceptions of ENDS upon viewing the same ENDS advertisement.[757] Key study results suggest for combusted cigarette users, ENDS users, and dual users, advertisement exposure resulted in increased or unchanged perceptions of ENDS as desirable, socially acceptable, and healthy, while generally increasing negative perceptions about combusted cigarettes.[757] However, dual users increased their perceptions of combusted cigarettes as healthy, suggesting ENDS advertisements could decrease harm perceptions of combusted cigarette use for some populations.[757] Among PATH Wave 2 ENDS users, 14.2% of youth (aged 12–17 years), 15.2% of young adults (aged 18–24 years), and 15.2% of adults (aged 25+ years) indicated appealing ENDS advertisements was a reason for their use of ENDS.[682] Sussman[758] posits youth and young adults view marketing material similarly regarding favorability, but young adults may process messages with more critical thought in comparison to youth, albeit with an open perspective of lifestyle choices. Therefore, while all age groups may report similar appeal of advertisements, characteristics that make the advertisement appealing or thought processes that result in increased positive attitudes toward ENDS and increased intention to use ENDS may differ between populations.

Digital Marketing

Studies on ENDS marketing and communications content have heavily focused on online communication channels, including social media, likely due to the cross-industry shift in marketing practices mirroring changes in consumer media use. In 2016, spending on digital advertising, which includes digital video, such as video streaming services, for all industries in the U.S. surpassed spending on television advertising, with respective costs of $72 billion and $67 billion.[759] American shopping habits have changed as well. A Nielson 2019 report states American online purchases have increased by over 24.0% in the past two years.[760] Online marketing can be achieved through multiple communication channels. Industry-generated content on social media is effective, in conjunction with other marketing activities, to increase purchasing behavior and form relationships between customers and brands.[761] Online advertisements, specifically, banner advertisements, have been shown to increase offline purchasing behavior as mediated by increased product website visits.[762] Traditional advertisement channels remain effective, however industry and consumer generated content on social media both contribute to marketing outcomes alongside traditional channels, and following with the two-step flow model in communications (which suggests content from mass media sources passes through opinion leaders to their family, friends, and peers), traditional advertisements may spur consumer-generated or shared social media content which can further the reach of advertisements.[763]

Online advertisements and websites highlight product features and contain marketing features that may motivate people generally or specific user groups to engage in ENDS use. Of 21 mobile websites for ENDS captured in November 2017, seven had no warning present and 11 had a warning present on all pages, although the majority of these required scrolling to view the warning.[764] In this study, 17 sites allowed users to sign up for emails or text messages, and mobile websites included videos, prizes, internal social networking features, and links to external social media.[764] All 21 mobile websites offered product descriptions and features, and the majority offered discounts, user reviews, and store locator tools.[764] Another study using data from December 2014 found most manufacturer (77.0%) and retailer websites (65.0%) made health-related claims about ENDS, including potential modified risk claims (70.5% and 46.9%, respectively).[765] An additional study of website content captured in 2012 found 95.0% of ENDS retail websites made explicit or implied health claims, 64.0% had a smoking cessation-related claim, 22.0% featured doctors, and 76.0% claimed the product does not produce secondhand smoke; 88% stated the product could be used anywhere, and 71.0% mentioned using the product to circumvent clean air policies; and many websites included images or claims related to modernity (73.0%), social status (44.0%), romance (31.0%) or use by celebrities (22.0%).[766] Furthermore, research conducted in 2016-2017 with demographically diverse U.S. participant coders suggests tobacco company websites, including websites for ENDS may present different material based on user demographics.[767] Therefore, ENDS websites may be dynamic and highlight ENDS features marketers have identified to be most appealing to the current user's demographic group. One study found nearly all (97.9%) of ENDS' online marketing promote the product flavor(s).[768] Another study found ENDS were presented in video and banner advertisements as an alternative to combusted cigarettes when an individual cannot smoke (33.0% of advertisements), as reducing harm from tobacco use (37.5%), or as helping with cessation (20.8%).[769] Both advertisements and websites use marketing content, which contains health and potential modified risk claims.

The digital marketplace may enable marketing ENDS with implied health claims, whether the claims were intended by the seller or not. In a digital forensic analysis, two out of the four advertisements assessed included cessation claims, linked to affiliate websites that made a variety of health or cessation claims, and linked to a website that sold ENDS (but did not make health or cessation claims).[770] Data are not currently available on how marketing schemes use advertising technology to potentially obfuscate attribution of health claims to industry. Data to support that this practice is still occurring are not available. Alternatively, major retailer website may mislead consumers. Even if a product description does not include language about cessation, the product category in which an online retailer places an ENDS may communicate product purpose to consumers. In May 2019, researchers identified Amazon, Walmart, and eBay all had ENDS in categories for smoking cessation on their websites, which could mislead consumers to believe these products can be used for cessation.[771]

Social media is frequently used by tobacco companies for marketing but is also used by the public to communicate unsponsored, user-generated, novel content. Findings from a systematic review supported conclusions people use social media to learn, communicate, and make decisions about ENDS use.[740] A different systematic review found posts highlighting ENDS for combusted cigarette cessation were frequently posted on social media by accounts including personal and industry accounts; however,

the authors noted one study suggested reasons for ENDS use shared by users online have changed from cessation to social image from 2012-2015.[724] Research suggests celebrity endorsement and communication of social advantages of ENDS on social media can increase use intentions.[740] The majority of studies exploring ENDS marketing on social media focused on Twitter or Reddit (likely due to open application programming interfaces [APIs] offered by these social media services) and identified social media content includes broad themes, ranging from discussions concerning harmfulness to policy, motivations in use, and sensations from ENDS use, with a plurality of the studies finding positive sentiment toward ENDS use.[772]

ENDS marketing content is prominent on social media. Other work suggests people increasingly search for ENDS information online; most online content is positive or neutral; and much content on social media is generated by organizations and individuals involved in the ENDS industry.[724] In 2019, 80% of 35 major ENDS brands had pages on 3 or more social media platforms, with presence on platform ranked from highest to lowest as Instagram (82.9%), Facebook (80.0%), YouTube (80.0%), Twitter (77.1%), Pinterest (60.0%), and Tumblr (37.1%).[773] In this study, less than half of brand pages included health warnings (0–44.8% across platforms), more than half of ENDS brand social media bios contained links to brand websites (30.8–100%), and young people were visible on the homepage or recent posts for many brands.[773] Furthermore, this study found pages additionally included features such as links to buy specific products, mentions of flavor, videos, pictures of the product alone, in a hand, or being used by a person, and hashtags, including hashtags related to a brand, ENDS, or unrelated to tobacco.[773] In a study of ENDS content, which used healthy food descriptors on Twitter posted from January to March 2017, researchers found Tweets presenting ENDS use as harmless (28.0% of Tweets) or focused on the sensations experienced while using ENDS were more likely to be authored by marketers (e.g., manufacturers) than non-marketers (e.g., users).[774] From 2017-2018, the volume of JUUL-themed tweets was 3,715,539 Tweets, compared to 10,421,752 Tweets about ENDS excluding JUUL-themed Tweets. JUUL-themed Tweets increased in quantity and decreased in diversity of content from 2017-2018 while ENDS-themed Tweets decreased in quantity and had no change in diversity of content.[775] YouTube videos of ENDS tricks were characterized as 48.0% originating from industry accounts and 53.0% containing marketing content.[776] Instagram posts from 2018 sampled on JUUL-related keywords and JUUL official and related accounts found about a third of posts contained marketing content, over half of posts had youth-related content, and 57.0% of posts contained lifestyle content, with 71.9% of marketing posts containing lifestyle content.[777] Roughly 15.0% of Reddit submissions, from a corpus of submissions posted from 2017-2018 which included at least one ENDS-related keyword, were algorithmically identified as party to a group of vendor/sales accounts.[778] ENDS forums host marketing content as do Instagram and Pinterest.[772] Research in one study highlights potential industry manipulation of Twitter, which has many posts from potential social bots on ENDS topics, which may impact the overarching conversation on ENDS and perceived popularity of conversation topics.[779] However, attribution of bots from industry for marketing purposes remains a technical challenge.

ENDS social media content reaches youth and young adults. In a study of JUUL's reach on Twitter, researchers found that approximately one-in-four Twitter users who retweeted Tweets from JUUL's official account were youth (aged 17 years or less).[780] Of followers with at least one public Tweet of

JUUL's @JUULvapor Twitter account, 80.6% were predicted to be under the age of 21 years.[781] Data from 2019 on ENDS major brands social media pages showed median page follower counts ranged from 61,400 for Instagram to 90 for Pinterest; interaction content suggests social media users engaged with pages through "likes", comments, and sharing posts; and brands interacted with social media users by responding to user comments and reposting content shared by other accounts.[773] Focus groups of young adults aged 18–24 years in Wisconsin (12 groups, n=69) were asked to view e-liquid marketing posts on Instagram and discuss those elements they found appealing and unappealing. Participants preferred posts they deemed trustworthy, had unpaid, user-generated content, were visually appealing, and contained products with flavor and nicotine level options. Participants disliked content focused on ENDS use culture or which made light of nicotine addiction.[782] Studies have highlighted that ENDS companies use lifestyle content for social media marketing.

Research supports exposure to ENDS social media content is associated with appeal, intention to use, and use of ENDS. U.S. youth who are exposed to ENDS content on social media have more positive attitudes toward ENDS.[783] New Jersey Youth Tobacco Use Survey 2018 data (*n* = 4,183) showed 8.6% of youth (in grades 9-12) liked or followed a tobacco brand on a social media platform, and these students had higher odds of being current and frequent ENDS users.[784] Higher exposure to social media was found to be associated with greater willingness and intention to try ENDS, higher perceptions of ENDS use as socially normative, more positive attitudes toward ENDS, and lower danger perceptions of ENDS among youth aged 13–18 years in California.[785] Further, this same study found high exposure to ENDS content designed to appear as social media posts in an experimental environment was associated with intention to use and positive attitudes toward ENDS, in comparison to light exposure to ENDS material, suggesting a dose-response effect.[785] Content presented as advertisements resulted in greater willingness, intention, positive attitude, and perceptions of ENDS use as socially normative in comparison to content presented as user generated posts.[785] Data collected from college students suggests viewing peer posts on social media featuring ENDS in the past six months is associated with current and lifetime ENDS use and viewing ENDS advertisements in the past six months on social media is associated with lifetime ENDS use in a cross-sectional study.[786] Data collected from 4,384 college students (aged 20–32 years) in Texas in 2017 show 20.1% of participants saw at least one ENDS advertisement on social media is the past 30 days.[579] Furthermore, single and dual tobacco product users reported higher exposure to and higher engagement with ENDS advertising on social media in comparison to non-users.[579] Many students in this study reported engaging with tobacco product messaging on social media (22.7% of the sample); however the majority of these engagements are represented by the 12.9% of the sample who posted content to discourage tobacco use, although 3.2% of respondents reported using social media to encourage others to use tobacco.[579] More ENDS users in this study (14.0%) vs. non-users (6.1%) reported pro-tobacco social media engagement.[579] Lastly, interacting within an ENDS online community may lead to reduced likelihood an ENDS user will engage in complete tobacco product cessation. Those current ENDS users (36.2% dual users) who are members of online ENDS communities, socially identify with ENDS users, or have high subjective norms of ENDS use have lower intentions to quit ENDS in comparison to other ENDS users.[787] ENDS social media content exposure and interaction may impact ENDS use for many tobacco user groups.

Exposure and Appeal Mitigation Strategies

Many potential strategies have been suggested to reduce the impact of marketing on those populations who are not currently using tobacco products, mainly youth. While some of those strategies focus on limiting access and restricting youth's ability to purchase the products, others focus on tailoring advertising plans to minimize youth exposure to ENDS advertising and limiting product appeal to youth.

Despite age restrictions on youth purchase of ENDS, many youth have found ways to access ENDS. YRBSS 2017 data show 19.8% of youth ENDS users obtain access through retail stores and retail store access increases the relative risk of daily use.[788] Data collected in 2017 for Wave 1 of the ITC Youth Tobacco and Vaping Survey from the 385 U.S. youth (aged 16–19, 9.4% of the U.S. sample) who purchased ENDS in the past 12 months either while under the legal age (*n* = 135) or of legal age (*n* = 250) show reported location of ENDS purchase was at a vape shop (underage = 58.5%, legal age = 68.9%), online (underage = 24.8%, legal age = 17.6%), retail (underage = 36.8%, legal age = 33.0%), and other (underage = 4.4%, legal age = 2.4%).[789] A 2014-2017 study of California youth found home neighborhood density of tobacco retail stores is associated with initiation of alternative tobacco product use, which includes ENDS use in a combined variable along with use of smokeless tobacco, pipe tobacco, cigars, cigarillos, and waterpipe.[790] Convenience stores may be perceived as associated with ENDS use by youth. Data from a longitudinal study of 1060 youth enrolled in alternative high schools in California suggest having a spontaneous association of gas stations, convenience stores, and liquor stores with tobacco products in a data collection task was associated with increased odds of belonging to a latent class characterized by ENDS use.[791] User posts on the "UnderageJuul" subreddit (which is now banned by Reddit) suggest youth have multiple ways of obtaining JUUL, including through official JUUL and other websites (sometimes using fake or borrowed identity documents), buying products from other Reddit users, using replacement codes for JUUL shared by other Reddit users, and local stores.[792] This study highlights youth use social media to share tactics for obtaining ENDS. In 2018, JUUL was available for purchase from eBay, with 87.8% of JUUL listings containing no age restrictions.[793] Following actions in 2018 to remove JUUL from eBay, JUUL can still be found on eBay and sellers changed the spelling of JUUL to obscure product listings.[793] Data suggest youth and ENDS sellers have both found ways to skirt age restrictions on youth purchases of ENDS.

Some ENDS brand websites use age-gating techniques, but it's unclear if these techniques are effective. Mobile websites for 21 ENDS products captured in November 2017 showed no website required an age-verified account for site entry, 18 required click-through verification of age or birthdate/state legal age entry, and of those mobile websites that sell ENDS products (*n* = 20), five allow ordering without an age verified account and 9 sell branded merchandise.[764] Other work conducted in 2015-2016 with demographically diverse U.S. participants serving as coders found of four major ENDS brands, all included click-through age-gating.[794] Data collected in 2019 on social media pages of major ENDS brands found the majority of brand pages on social media did not use age-gating (83.8–100% no age-gating across platforms) and the majority used no age statements in social media bios or posts (59.1–100% no age statement across platforms).[773] Age-gating for mobile apps has also been considered by researchers—in 2017, one brand sponsored app for ENDS was available on Google Play or Apple iTunes US online stores, and this app is intended to be paired with a Bluetooth-enabled Vuse ENDS.[795] However,

now Google Play bans apps encouraging the use or purchase of tobacco products by minors and Apple bans apps encouraging tobacco product use.[795]

Qualitative work supports that people learn about and are influenced to try ENDS through family and friends, in addition to advertisements.[740] Wave 1 PATH data suggest someone offering or asking someone for tobacco products were the primary sources of tobacco products for youth, with 56.7% current ENDS using youth stating this is how they attained their ENDS.[796] Just under 40% of US youth who had used ENDS in the past 30 days obtained their ENDS through a social source only, while roughly another 15% used social and commercial sources, using data collected in 2017 for Wave 1 of the ITC Youth Tobacco and Vaping Survey.[789] Longitudinal studies found associations between peer ENDS use and subsequent ENDS use.[740] For example, the third top reason selected by current JUUL users sampled from four high schools in Connecticut for using JUUL was friends' JUUL use.[177] However, in regression models, peer influence was negatively associated with the number of days the participant used JUUL in the past month.[177] The authors suggest the inverse association may be due to friend use resulting in experimentation but not sustained use.[177] Social influence may impact ENDS initiation, particularly among youth, and marketing campaigns, which capitalize on this phenomenon, may be particularly effective at increasing youth initiation. Studies suggest mitigation strategies focusing on reframing youth's conversations about ENDS with their peers, including reducing suggestion of ENDS as a social lubricant, may impact ENDS use prevalence among youth.

Purchase location of ENDS may be associated with adult ENDS use patterns. U.S. data from 2014 and 2016 suggest young adults aged 18–24 years tend to buy ENDS on the internet and not at "Vape Shops," which are more frequently used by adults aged 25–44 years, or "Smoke Shops," which are patronized by adults aged 45 years and older.[760] This study also found "Vape Shop" and internet ENDS purchasers were more likely to be former smokers and more likely to report daily ENDS use in comparison to retail and "Smoke Shop" customers.[760] ENDS sales in specific store types may impact ENDS use patterns.

Several studies have examined the use of warning labels for ENDS. For example, in an online experiment of young adults (aged 18–29 years), perceived warning effectiveness of researcher-generated warnings was higher for warnings related to the impact of nicotine on youths' developing brains and the presence of harmful chemicals compared to a warning about the addictive qualities of nicotine.[797] Warnings related to the relative harm of ENDS compared to combusted cigarettes were perceived as less believable and credible and were less frequently recalled. Research in U.S. adults conducted in 2018 suggests including warnings on ENDS packaging increases user motivation to quit and does not motivate use of combusted cigarettes.[798]

Warning label efficacy may be impacted by advertisement messaging and graphical layout. In an experiment assessing a blu advertising campaign in which the company included fake warnings conveying positive messages (e.g., "IMPORTANT: Contains flavor," "IMPORTANT: Less harmful to your wallet"), participants who viewed the fake warnings were less likely to recall real warnings than those who did not see the fake warnings. One study looked at the effects of exposure to positive and negative news headlines on perceptions of ENDS harm and found participants who viewed negative news headlines had higher perceptions of harm and lower perceptions of benefits in comparison to those

exposed to positive, conflicting, or no headlines. In subgroup analyses, this finding was significant for non-users of ENDS, but not for ENDS users.[799] Furthermore, exposure to conflicting news headlines did not result in differed perceptions from those exposed to positive, negative, or no headlines for main analyses; however, for subgroup analyses, non-ENDS users exposed to conflicting news headlines had lower perceptions of benefits of ENDS in comparison to those who viewed positive headlines.[799] These studies suggest warning labels may have different impacts on ENDS users and non-users, and advertisement characteristics and context may alter the interpretation of warning labels.

Conclusions for Section 3.C. Influence of Product Characteristics and Marketing on ENDS Appeal
Different factors may contribute to ENDS appeal, including product characteristics and product marketing and advertising. Overall, research suggests ENDS flavor in general, and fruit and sweet flavors in particular, may make ENDS more appealing to youth, while flavors like tobacco and menthol may be more appealing to adults than youth. Additionally, some evidence suggests fruit and sweet ENDS flavors may be more appealing to never smokers and tobacco ENDS flavors may be more appealing to combusted cigarette smokers. Research suggests many adolescents are not aware ENDS often contain nicotine. Additionally, while the presence of nicotine in ENDS is associated with more frequent ENDS and combusted cigarette use among adolescents, it may be associated with a greater likelihood of attempting to quit smoking combusted cigarettes among adults.

D. PERCEPTIONS OF RISK ASSOCIATED WITH ENDS USE
While not a direct measurement of product use, individuals' perceptions of risks associated with ENDS use can help inform an understanding of the likely users of ENDS. Using nationally representative data from middle and high school students from the 2014 NYTS, among ever users and never users of combusted tobacco products, higher levels of perceived absolute harm and comparative harm were associated with lower levels of curiosity about ENDS.[732] Additionally, among youth, using both PATH Study[800] and NYTS data,[712,801] research found perceiving ENDS as having less absolute risk and relative risk (compared to combusted cigarettes) was associated with ENDS initiation. Multivariate analyses of Truth Longitudinal Cohort Waves 7 (*N* = 14,379) and 8 (*N* = 12,114) data from youth and young adults (aged 15–34 years) suggest among ENDS never user Wave 7 participants, those who perceive ENDS as similar or greater in harm relative to combusted cigarettes had lower odds of current, but not ever, JUUL use at Wave 8.[802] Furthermore, longitudinal data on harm perceptions may provide insight on the association between ENDS use and future use of combusted cigarettes among youth. PATH Waves 1 and 2 data show youth (aged 12–17 years) initiation of ENDS use at Wave 1 was associated with decreased perceived harm of combusted cigarettes (OR: 1.50; 95% CI: 1.04, 2.16) and increased willingness to try combusted cigarettes (OR: 9.61; 95% CI: 5.67, 16.3).[803]

Harm and risk perceptions are also associated with changes in ENDS use in adults. A longitudinal study of young adults (mean age = 24.1 years) found those who perceived ENDS as less harmful in comparison to combusted cigarettes in 2010-2011 were more likely to have used ENDS in 2011-2012.[804] Longitudinal analyses of Wave 1 and Wave 2 adult (aged 18+ years) PATH data show Wave 1 users and non-users of tobacco products who perceived ENDS as lower in harm relative to combusted cigarettes had higher odds of Wave 2 ENDS use in comparison to peers who did not perceive ENDS as lower in harm relative to combusted cigarettes (OR = 1.97, 95% CI 1.74–2.22).[805] Waves 2 and 3 PATH data support adult dual

users who perceived ENDS to be less harmful relative to combusted cigarettes, in comparison to dual users who perceive ENDS as equally or more harmful relative to combusted cigarettes, had higher odds of switching to ENDS use only and maintaining dual use, lower odds of switching to combusted cigarette only use, and no difference in odds of complete cessation of tobacco use.[806] Similarly, in a large study of U.S. adults, higher positive affect toward ENDS was associated with lower perceived risks of ENDS, which was in turn associated with higher odds of being a current ENDS user.[807] Furthermore, research supports perceiving ENDS as less addictive relative to combusted cigarettes is associated with trying ENDS.[714]

Research supports all user groups, including both users and non-users, perceive ENDS as less harmful relative to combusted cigarettes.[808] Harm and risk perceptions of ENDS have changed from the early to late 2010s.[808-810] Studies from the early 2010s generally find people perceive ENDS as lower in harm in comparison to combusted cigarettes; however, publications from the late 2010s report most user groups perceived ENDS as equal in harm to combusted cigarettes.[808] TPRPS data from 2012-2015 show, among smokers, perceptions of ENDS as 'equally or more harmful' relative to combusted cigarettes rose from 11.7% to 35.1% and perceptions of ENDS as addictive increased from 25.3% to 56.7%.[810] This trend may be exacerbated by recent reports of EVALI (e-cigarette or vaping use-associated lung injury). For example, young adults (aged 18–35 years) in the U.S. surveyed before and after news reports of EVALI in 2019 had increases in perceptions ENDS may result in early or premature death with frequent use and increases in perceptions ENDS are more or as harmful as combusted cigarettes among non-users, combusted cigarette users, ENDS users, and dual users.[811]

Overall, ENDS users perceive ENDS as lower in harm compared to other tobacco products.[812] Dual users are generally found to perceive the lowest relative risks of harm of ENDS use. However, a study focused on ENDS users found dual users were more likely to perceive ENDS as higher risk relative to combusted cigarettes in comparison to ENDS users that never formally smoked.[812] In another study, smokers and tobacco non-users had higher likelihood of perceiving ENDS as containing dangerous chemicals, lower likelihood of viewing ENDS as relatively less harmful in comparison to combusted cigarettes, and lower likelihood of viewing ENDS as helpful with cessation, in comparison to exclusive ENDS users and dual users.[813] Conversely, in a sample of U.S. adult current smokers, no differences were noted in ENDS use and health risk perceptions of ENDS; however, smokers were more likely to perceive ENDS as less harmful in comparison to combusted cigarettes.[814] Differences in study findings may be due to different study methodologies such as question wording and inclusion of covariates in multivariate models. In general, ENDS users perceive lower harm in ENDS use compared to ENDS non-users, including those that use combusted cigarettes.

Perceptions of Health Risks by Population

Age may be associated with risk perception, where older adults perceive higher risk in ENDS use in comparison to younger adults. An inverse relationship between age and perception of ENDS as safe compared to combusted cigarettes has been found, suggesting younger adults may be at increased risk of initiation to ENDS use.[813] Research supports young adults (aged 18-24 years) were more likely to rate ENDS as "less risky" than combusted cigarettes than older young adults (aged 25–34 years).[815]

Youth and Young Adults

Several studies have shown youth generally perceive ENDS to be less risky than combusted cigarettes,[816] with youth ENDS users being more likely than non-users to perceive ENDS as less risky.[675,713] MTF data indicate 20.9% of youth who use no tobacco products, including ENDS, perceive great risk in ENDS use, in comparison to 9.5% of youth who use no tobacco products and use e-liquid without nicotine, and 9.1% who use any tobacco product, including e-liquid with nicotine.[817] Qualitative findings from interviews conducted with 34 youth in New York further support that youth perceive ENDS as less harmful relative to combusted cigarettes and recognize using ENDS is not healthy, though they have uncertainty regarding the harmfulness of ENDS use.[728]

NYTS 2015-2018 data show harm perceptions of ENDS use differ across youth user categories, with percentages of youth reporting no harm for ENDS use varying from 9.2% for non-users to 47.0% for high frequency dual users, with combusted cigarette only users and ENDS only users reporting 23.5% and 26.8%, respectively.[818] NYTS 2014-2015 data suggest youth perceptions differ by initial tobacco product used for (1) perceptions all tobacco products are dangerous (ENDS-first user group had no overlap in percentages with any other group) and (2) perceptions harm is caused by breathing smoke (ENDS group significantly different from non-initiators and those who could not remember what product they first used).[581] MTF data (national samples of 8th and 10th grade students) from 2014-2015 support the NYTS findings, with evidence youth who use ENDS solely view ENDS as less harmful in comparison to combusted cigarette smokers and non-users of tobacco, with dual users exhibiting the lowest perception of harm associated with ENDS use of all 4 groups.[819] Analysis of a nationally representative sample of 3,000 U.S. youth aged 13–17 years surveyed in 2017, found perceptions nicotine in ENDS might cause health problems and perceptions toxins or chemicals in ENDS might cause health problems differed by user group (71.4% and 70.1 for never ENDS users, 49.6% and 47.7% for current ENDS only users, 43.7% and 37.7% for dual users, and 59.0% and 57.4% for former ENDS users, respectively).[557] Regional studies of youth in Florida[820] and North Carolina[752] of ENDS users and tobacco product users, respectively, similarly suggest harm perceptions may differ by user group.

Similar harm perceptions patterns across user groups have been found among young adults. Young adult ENDS users were more likely than non-users to perceive ENDS as less risky, as shown by data from 5,203 college students aged 18–29 in Texas.[715] Additionally, a study of 348 young adults aged 18–24 years who had smoked combusted cigarettes non-daily for at least 6 months found ENDS use frequency was positively associated with perceiving ENDS as less harmful than combusted cigarettes.[821]

Adults

Adult data support tobacco product user group status is associated with harm perceptions. TPRPS 2012-2015 data show adults who have ever used ENDS are more likely to perceive ENDS as lower in harm relative to combusted cigarettes in comparison to adults who have never used ENDS, and current smokers, in comparison to never smokers, are more likely to perceive ENDS as equally or more harmful than combusted cigarettes, in a multivariate model including ENDS use.[810] TPRPS data from 2012-2017 support adult ENDS users and dual users perceive ENDS as less harmful in comparison to combusted cigarettes when compared to never smokers, former smokers, and current smokers.[809] However, data

from TPRPS 2017 and 2018 show a growing minority of adults perceive ENDS as equally harmful or more harmful relative to combusted cigarettes.[822] TPRPS 2017 and 2018 data show perceptions of ENDS as relatively more harmful increased in current and former smoker subgroups.[822] Furthermore, among all adults in TPRPS 2017 and 2018 data, perceptions of ENDS as equally harmful as cigarettes increased (2017 = 36.4%, 2018 = 43.0%) and uncertainty of relative harm decreased (2017 = 25.3%, 2018 = 19.3%).[822] Estimates of perceptions of ENDS as equally harmful as cigarettes in 2018 were 44.7% for never smokers, 41.5% for former smokers, 39.3% for current smokers, 26.1% for current ENDS users, and 32.7% for dual ENDS and combusted cigarette users.[822] Using data from 1,736 respondents of Health Information National Trends Surveys (HINTS)-FDA2, perceived health risks of combusted cigarettes were higher in comparison to perceived health risks of ENDS for current, former, and never ENDS users.[823] The patterns above found in nationally-representative data are supported by other studies.[813,824-826]

Adults report perceptions of lower relative harm as a motivation to use ENDS. Of the 2,051 dual users identified in PATH Wave 2 adult data, 79.8% selected they used ENDS because they thought ENDS was lower in relative harm in comparison to combusted cigarettes.[708] A 2018 survey of Minnesota adults found of the reasons for using ENDS among ever users, perceiving ENDS as less harmful than other tobacco products was reported by 47.7% of daily smokers, 58.1% of occasional smokers, 66.4% of recent former smokers, 36.7% of long-term former smokers, and 39.6% of never smokers.[827] However, from this same study, health concerns of ENDS were selected as a reason for never trying ENDS by 26.9% of daily smokers and 19.0% of occasional smokers, and as a reason for discontinuing ENDS by 28.9% of daily smokers and 23.7% of occasional smokers.[827] A 2016 sample of 660 U.S. adults aged 18+ years who had used ENDS at least once identified the attribute of 'ENDS as less harmful relative to cigarettes' as the most important attribute of ENDS in a discrete choice experiment, suggesting the perception of ENDS as a product for harm reduction is an important factor for many ENDS users.[709] Lower harm perceptions of ENDS relative to combusted cigarettes were also found to be a potential factor for selecting ENDS over combusted cigarettes in a similar discrete choice experiment conducted in 2017 with a national sample of 1,154 adult (aged 18+ years) combusted cigarette lifetime users (76% daily smokers) who were either dual users with ENDS or were uncertain if they may use ENDS in the future (77% ENDS ever users, 47% dual user).[689] Among 1,432 U.S. adult current ENDS users aged 18–64 years surveyed in 2016, 31.9% endorsed "healthier than other products" as a reason to start using ENDS.[828] Younger participants had lower perceptions of ENDS use providing a health benefit in comparison to older participants, which may be due in part to older participants reporting higher use of ENDS for smoking cessation.[828]

Research suggests people have high levels of uncertainty about health risks associated with ENDS use. ENDS users may have higher knowledge of traditional tobacco product-related health risks in comparison to ENDS-related health risks.[812] Findings from focus groups held in 2017 in Atlanta, Georgia of primarily African American males aged 25+ years suggested people perceive health harms of ENDS remain unknown, while they perceive health harms of combusted cigarettes have less uncertainty surrounding them.[829] In another qualitative study, U.S. young adults (aged 18–34 years) voiced uncertainty regarding the possibility of harmful chemicals in e-liquids.[830] Furthermore, quantitative

research supports that adults perceive uncertainty regarding the health effects of ENDS. In a sample of 1,872 U.S. adults aged 18+ years who either used ENDS, combusted cigarettes, or both, awareness of health risks of ENDS use was low.[831] In a sample of adults, 22.0% selected "unknown effects of chemical" as a potential harm associated with ENDS use, which was more than the 15.7% of the same sample that selected cancer.[813] TPRPS data from 2012-2017 support uncertainty regarding health risks associated with ENDS appears to be decreasing as ENDS are on the market longer.[809]

Pregnant Women

Pregnant women perceive ENDS as lower in risk relative to combusted cigarettes and perceive ENDS may be helpful tool for cessation, yet have uncertainty regarding health risks of ENDS, mirroring findings in the larger U.S. adult population. Fallin et al.[832] conducted two focus groups in Kentucky with 12 Medicaid eligible pregnant or newly postpartum women who smoked within three months of, or during, pregnancy. Themes that arose were using ENDS for harm reduction (cessation or reducing combusted cigarette use), lack of clarity regarding health risks associated with ENDS, mixed preferences for characteristics of ENDS vs. combusted cigarettes, and dual use and relapse to combusted cigarette use.[832] Kahr et al.[833] conducted 11 focus groups in Houston with 87 pregnant women from three prenatal care clinics, of which no information on tobacco use status was available. Themes that arose included perceptions of relatively lower harm of ENDS use in comparison to combusted cigarette use, perceptions of health risks of using ENDS during pregnancy, stigma surrounding using ENDS during pregnancy, and perceptions of benefits of using ENDS for cessation during pregnancy.[833] Focus groups of women who were pregnant or planning to become pregnant conducted across four cities relayed similar themes such as uncertainty regarding health risks of ENDS use, perceptions of ENDS as less harmful in comparison to combusted cigarettes, flavor appeals, and experiences of using ENDS for cessation with the result of becoming a dual user.[834] Uncertainty regarding health risks of ENDS use and patterns of cessation attempts/harm reduction using ENDS including dual use were prominent reported themes across studies. Pregnant women generally perceived ENDS use during pregnancy was less risky in comparison to combusted cigarette use. Pregnant women additionally perceived ENDS to be useful as a cessation device, although some women who attempted to use ENDS for cessation were unsuccessful and became dual users. Prominent themes from focus groups are mirrored in quantitative findings.

Pregnant women may perceive ENDS use as less risky to their health and the health of their child. In a study of pregnant women Mark et al.[533] found 78.0% of pregnant ever ENDS users and 31.0% of pregnant never ENDS users perceived ENDS to be less harmful than combusted cigarettes for their personal health; however, 62.0% of pregnant ever ENDS users and 60.0% of pregnant never ENDS users perceived ENDS as addictive. Wagner et al.[532] also found pregnant women view ENDS as safer in comparison to combusted cigarettes, and ENDS users and dual users were more likely to view ENDS as relatively safer than combusted cigarettes overall and during pregnancy, in comparison to women who did not use tobacco during pregnancy. From 382 pregnant women sampled from an obstetrics clinic waiting room, Bhandari et al.[651] report current ENDS users were less likely, in comparison to non-ENDS users, to agree with risk perception statements regarding ENDS use ("E-cigarettes cause lung cancer", "If a pregnant woman smokes e-cigarettes, her baby may be harmed", and "The exhaled smoke from e-cigarettes can cause harm to others nearby"), and more likely to agree ENDS can help with quitting

combusted cigarettes and it is easy to stop using combusted cigarettes. In general, pregnant women perceive ENDS as relatively less risky in comparison to combusted cigarettes, yet qualitative work supports pregnant women perceive risks associated with ENDS use and are uncertain of their health risks. Those pregnant women who have used ENDS are more likely to perceive ENDS as relatively less risky than combusted cigarettes, in comparison to their peers who have not used ENDS.

Perceptions of Health Risks Associated with Flavors

Flavors may play a differential role in harm perceptions for all age groups. In qualitative interviews with U.S. young adult (aged 18–34 years) current dual users of combusted cigarettes and ENDS, who had used ENDS to reduce combusted cigarette use, some participants mentioned flavors such as menthol or tobacco were perceived as more harmful in comparison to other flavors.[830] A national U.S. survey (*N* = 1,125) found youth (aged 13–17 years) believed fruit-flavored ENDS were less harmful than tobacco-flavored ENDS.[671] A systematic review found among youth, fruit and candy flavors are perceived as lower in harm in comparison to tobacco flavors, and among user groups, ever and current ENDS users are less likely to view flavored ENDS as harmful, in comparison to non-users.[835] In another study, among youth in 6th, 8th, and 10th grades in Texas surveyed in 2014-2015, ever and current ENDS users were more likely to report flavored ENDS were "less harmful" than non-flavored ENDS than never users.[675] Similarly, analyses of the 1,814 participants with history of both combusted cigarette and ENDS use from TPRPS data collected in 2016 and 2017 showed perceptions of harm of flavors in ENDS differs by user group, with percentages of those agreeing flavors are "safe" as 22.2% among dual users, 11.6% among current combusted cigarette users who formally used ENDS, 33.2% for complete switchers from combusted cigarettes to ENDS, and 13.7% for those who stopped using both ENDS and combusted cigarettes.[524]

Perceptions of Health Risks Associated with Product Design and Brands

Current literature suggests preferred product design or brand may be associated with harm perceptions in young adults, but not youth. In a study examining differences between JUUL and other ENDS users, no difference was found in choosing the motivation "healthier than smoking" between JUUL and other ENDS users, or in perceived harm or perceived addictiveness of JUUL/cartridge-based ENDS.[836] Data from JUUL-aware U.S. youth aged 13–17 years collected in 2018 suggest 45.9% of youth think using JUUL everyday would cause a lot of harm, although this dropped to 28.9% when asked about using JUUL on some days but not all.[837] Current and former JUUL users, and current and former smokers, had higher odds of reporting daily use of JUUL would result in no harm in comparison to never JUUL users and never smokers, respectively.[837] Some 17.3% of youth thought it is was very unlikely or somewhat unlikely to become addicted to JUUL, with current and former JUUL users reporting higher percentages.[837] Regarding relative harm, this study found 39.3% of youth perceived JUUL as lower in harm relative to combusted cigarettes, and 39.2% perceived it as equally harmful. Importantly, current JUUL users perceived JUUL use as lower in relative harm in comparison to combusted cigarettes (60.4% of current users) and less addictive than combusted cigarettes (49.8% of current JUUL users).[837] One study also suggests young adults may perceive cartridge-based ENDS as lower in harm in comparison to other ENDS. This study of 445 Californian young adults (aged 17–24 years) surveyed in 2019, found

70.8% of the *n* = 193 participants who ever used cartridge-based ENDS reported a reason they used cartridge-based ENDS is because "they are healthier than other vapes".[838]

Perceptions of Health Risk Associated with Nicotine Content

An association between ENDS use and harm perceptions may exist regardless of the presence of nicotine in e-liquid. 2017 MTF data were used to generate a subsample of youth with no history of nicotine or marijuana use to characterize differences between youth who used ENDS with nicotine free e-liquids and youth who did not use ENDS.[839] This study found youth who engaged in past 12-month and past 30-day flavor only ENDS use were less likely to perceive nicotine ENDS use as risky in comparison to their non-using peers.[839]

Perceptions of Addiction Risk

Perceptions of ENDS as addictive have increased over time, as illustrated by 2012-2015 TPRPS data which show a doubling in the percentage of adults who perceive ENDS as addictive (32.0% to 67.6%).[810] Adult ENDS users and non-users responding to an open-ended response question on health risks associated with ENDS use mentioned addiction more frequently than any other health effect across user groups (36.5% for total sample), though less than 40.0% of all user groups mentioned specific health effects as associated with ENDS use.[813] HINTS-FDA2 data from 2017 show perceived addiction risks of combusted cigarettes was higher in comparison to perceived addiction risks of ENDS for current, former, and never ENDS users.[823] Among 4,091 U.S. adults aged 18–40 years considered ever tobacco product users surveyed in 2016, 55.0% believed it was easy to become addicted to ENDS and 66.0% thought ENDS were as likely to cause addiction as combusted cigarettes.[840] Overall, adults increasingly perceive ENDS as addictive in both absolute and relative terms. However, research in U.S. adults who use ENDS, combusted cigarettes, or both suggests health related risks are more likely to discourage ENDS use in comparison to addiction-related risks.[831]

Awareness of nicotine is important because research has found associations between believing ENDS are less addictive than combusted cigarettes and ENDS use among youth and adults.[675,712-715] Youth perspectives captured in qualitative research in New York suggest youth perceive ENDS as addictive;[728] however, some quantitative research suggests youth are not knowledgeable about the nicotine content and addictiveness of ENDS. Nationally representative data collected in 2019 from U.S. youth (aged 13–17 years, *N* = 4,860) who are JUUL aware showed 38.0% were aware JUUL always contains nicotine.[841] Among U.S. ENDS youth and young adult users aged 16–19 years who completed the ITC Policy Evaluation Project Youth Tobacco and E-cigarettes survey in 2017, only 76.1% knew if the ENDS they used contained nicotine or not, and 60.2% perceived the possibility of addiction to ENDS to be at least somewhat likely.[842] In a study of youth ENDS users, only 17.0% of non-nicotine users and 34.0% of nicotine users understood the nicotine in ENDS was derived from tobacco;[716] most youth thought the nicotine in ENDS was artificial, potentially indicating a belief this nicotine is "safer," and about one-third of ENDS users with (33.8%) and without (36.4%) nicotine believed firsthand aerosol is just water vapor. Similarly, in a sample of high school students, 19.1% believed the aerosol from ENDS was just water and 23.0% believed ENDS were not a tobacco product.[717]

Adults may similarly have little knowledge regarding the nicotine content of ENDS. Nationally representative data collected in 2019 from young adults (aged 18–24 years, *N* = 3,746) and adults (aged 25+ years, *N* = 5,000) who are JUUL aware showed 51.5% and 45.9%, respectively of each age group, were aware JUUL always contains nicotine.[841] In a study of 445 Californian young adults (aged 17–24 years) surveyed in 2019, of the young adults who had heard of cartridge-based ENDS, a plurality ("about half") reported they do not know the amount of nicotine contained in cartridge-based ENDS, by brand.[838]

Conclusions for Section 3.D. RISK PERCEPTIONS ASSOCIATED WITH ENDS USE

Overall, research shows perceiving ENDS as posing less risk than combusted cigarettes is associated with an increased likelihood of ENDS use. However, the research does not indicate whether those who are affected by such perceptions are never-tobacco users initiating with ENDS or current combusted cigarette users considering quitting with ENDS. Furthermore, product characteristics such as flavors, or population characteristics such as age or tobacco use history, may be associated with differences in perceived harms and risks of ENDS use. Importantly, harm perceptions of ENDS appeared to have changed over the 2010s and may continue to fluctuate.

E. HEALTH RISKS ASSOCIATED WITH ENDS USE

Respiratory Disease

The NASEM report reviewed studies of ENDS use and respiratory outcomes and did not find studies that examined the long-term effects of ENDS use and the development of chronic respiratory symptoms due to the newness of the products at the time. Studies have shown ENDS with nicotine can have short-term effects on lung defense mechanisms such as mucociliary clearance, urge to cough, and cough sensitivity. The NASEM report found moderate evidence of increased cough and wheeze among adolescent ENDS users and an association between ENDS use and an increase in asthma exacerbations. It also found limited evidence from animal and in vitro studies of adverse effects of ENDS exposure on the respiratory system.

Since the NASEM report was published, eight observational studies[843-850] showed a significant positive association of ENDS use and respiratory health outcomes. The majority of these studies were based on cross-sectional surveys, so reverse causation could have occurred.

Two studies using the pooled 2016 and 2017 BRFSS data examined the relationships of ENDS use and self-reported asthma and COPD. For the asthma study, the association between ENDS use and asthma was examined among never smokers to minimize potential confounding by combusted cigarette smoking.[850] In that study, compared to never ENDS users, exclusive ENDS users had 39% higher odds of self-reported asthma (OR=1.39, 95% CI: 1.15-1.68); occasional ENDS users had 31% higher odds (OR=1.31, 95%CI: 1.05- 1.62) and daily ENDS users had 73% (OR=1.73, 95%CI: 1.21-2.48) higher odds of self-reported asthma. However, this study has some limitations. First, since this study utilized BRFSS, which is a cross-sectional survey, temporality cannot be established. It will be difficult to know whether people developed asthma before or after starting to use ENDS. Second, asthma status was self-reported, so it may have been under-reported, but it's not clear if reporting differs by ENDS use status. Third, the authors did not assess family history nor childhood asthma.

For the COPD study, the authors observed an association between ENDS use and self-reported COPD (i.e., chronic bronchitis, emphysema, or chronic obstructive pulmonary disease) stratified by combusted cigarette smoking status.[849] Among never smokers, current ENDS use (OR= 1.75, 95% CI: 1.25-2.45), occasional ENDS use (OR=1.51, 95% CI: 1.03-2.23), and daily ENDS use (OR=2.64, 95% CI: 1.43-4.89) was associated with higher odds of self-reported COPD compared to never ENDS use. Dual users of ENDS and combusted cigarettes had the highest odds of self-reported COPD compared to never users (OR=6.89, 95% CI=6.29-7.55). This study has limitations. First, since this study utilized BRFSS, which is a cross-sectional survey, temporality cannot be established. It will be difficult to know whether people developed COPD before or after they started using ENDS. Second, COPD was self-reported, therefore it may have been under-reported. Third, although smoking is a major risk factor for COPD, other tobacco use information including combusted cigar use, waterpipe use, and smoking variables such as smoking duration, smoking intensity, and time since quit smoking (if former smokers) could be confounders not adjusted for.

One study[848] used the 2017 BRFSS data to examine the associations between ENDS use and respiratory symptoms (i.e., daily cough, sputum production or breathlessness during the past 3 months) by smoking status (current, former quit ≤1 year, former >1 year, and never). Among never smokers, young ENDS users (18-34 years old) had 1.36 times higher prevalence of respiratory symptoms (PR=1.36, 95% CI: 1.08- 1.70) compared to never ENDS users. However, the prevalence of respiratory symptoms was not significantly higher in older age groups. Due to the nature of cross-sectional survey design, it cannot be determined whether ENDS use occurred before symptom onset. The use of other combusted tobacco products was also not adjusted for.

Cardiovascular Disease

The NASEM report concluded the possibility of cardiovascular effects from ENDS use due to exposure to substances such as nicotine, fine particular matter, and metals is a cause for concern but also found epidemiological and even clinical data on cardiovascular disease endpoints and intermediate outcomes were very limited. The report stated, "Relatively few studies have investigated the cardiovascular effects of e-cigarette products. In particular, there are no epidemiological studies evaluating clinical outcomes such as coronary heart disease, stroke, or atherosclerotic peripheral artery disease, or established subclinical outcomes of underlying atherosclerosis such as carotid intima-media thickness or coronary artery calcification". As such, the report concluded, "There is no available evidence whether or not e-cigarette use is associated with clinical cardiovascular outcomes (coronary heart disease, stroke, and peripheral artery disease) and subclinical atherosclerosis (carotid intima-media thickness and coronary artery calcification)".

Since the NASEM report was published, four cross-sectional studies were published that examined the relationship of ENDS use on cardiovascular health outcomes (e.g., myocardial infarction). Two studies[851,852] used the National Health Interview Survey (NHIS) to examine the relationships between ENDS use and risk of myocardial infarction (MI) and between ENDS use and risk of coronary heart disease (CHD). Alzahrani et al.[852] pooled 2014 and 2016 NHIS data to examine the relationship between ENDS use and risk of MI. They found that daily use of ENDS is significantly associated with increasing risk of MI (OR=1.79, 95% CI: 1.20-2.66) compared to never tobacco users, however, former and someday

ENDS use was not associated with MI risk. Farsalinos et al.[851] used 2016 and 2017 NHIS data to examine the relationship between ENDS use and risk of MI and CHD and found non-daily ENDS use was significantly associated with increasing odds of MI in 2017 (OR=2.11, 95% CI: 1.14-3.88); and daily ENDS use was significantly associated with an increasing odds of CHD in 2016 (OR=1.89, 95% CI: 1.01- 3.53). Additionally, two studies[383,853] used the BRFSS to examine the relationship between ENDS use and risks of stroke and CVD. Parekh et al. examined the association between ENDS use and odds of stroke among young adults aged 18 to 44 years using the BRFSS.[853] They found compared with nonsmokers, exclusive ENDS users did not have a different odds of stroke risk (AOR=0.69, 95% CI=0.34, 1.42). However, odds of stroke were lower for exclusive ENDS users versus exclusive combusted cigarette users (AOR=0.43, 95% CI=0.20, 0.93). Osei et al. examined the association between ENDS use and CVD (including stroke, MI, and CHD) risk. They found that current ENDS users who never smoked had no different risk of CVD (OR=1.04, 95% CI: 0.63- 1.72) compared to never ENDS users, but dual users of combusted cigarettes and ENDS had a significantly higher odds of CVD compared never users (OR=1.59, 95% CI= 1.20- 2.08).

There are a number of limitations in these studies. Although these studies controlled for combusted cigarette smoking, with the majority of ENDS users being former smokers, there may have been some residual confounders due to the effect of past smoking on MI risk. Additionally, these studies were cross-sectional, thus temporality of ENDS use and CVD cannot be assessed. Another major limitation is the lack of sufficient sample size to examine these associations among exclusive ENDS users who never smoked. One study[383] was able to examine exclusive ENDS users who never smoked, although this group may be too young to develop any CVD conditions.

An additional paper on ENDS use and MI risk that used PATH Study data was retracted by the journal due to major methodological issues.[854]

Oral Disease

The NASEM report found no epidemiological studies on ENDS use and periodontal disease. It found limited evidence ENDS aerosol can affect cell viability and cause cell damage in oral tissue, although there was some evidence switching to ENDS use can improve periodontal disease among smokers.

Since the NASEM report, four observational studies examined the association between ENDS use and oral health outcomes.[855-858] In Wave 1 of the PATH Study, ENDS users were more likely than never tobacco users to report treatment for gingival disease (OR=2.3, 95%CI: 1.3-4.1),[857] however, this estimate did not adjust for combusted cigarette smoking. Also using PATH Wave 1 data, dual ENDS and combusted cigarette use, compared to never combusted cigarette or ENDS use, was associated with increased odds of a past-year diagnosis of dental problems in adolescents (POR = 1.7; 95% CI: 1.24-2.38), but no association was observed for exclusive ENDS users.[855] In the 2016 BRFSS, daily ENDS use was associated with high odds of self-reported poor oral health (e.g., tooth removed because of tooth decay, or gum disease) (OR=1.79, 95%CI: 1.39-2.30), although no increased risk was observed for intermittent ENDS use.[858] Atuegwu et al.[856] conducted a longitudinal analysis using PATH Study Wave 1 to Wave 3 data to examine the incidence of gum disease among ENDS users who reported no history of gum disease at Wave 1. They found compared to never tobacco users, ENDS users had significantly increased

odds of being diagnosed with gum disease (OR=1.76, 95%CI: 1.12-2.76) and bone loss around teeth (OR=1.67, 95%CI: 1.06-2.63) during follow-up.

Cancer

The NASEM report reviewed available studies related to cancer and ENDS use and found them to be very limited in number and relevance and generally lacking in methodological rigor. The NASEM report found there were no available epidemiological studies on the potential association between ENDS use and cancer or intermediate cancer endpoints in humans that would allow for conclusions. The NASEM report found there was substantial evidence some chemicals found in ENDS aerosols such as formaldehyde and acrolein can cause DNA damage and mutagenesis, although it was not clear if they were present at levels that would cause cancer in humans. No additional observational studies published after the NASEM report related to ENDS use and cancer risks were found.

Developmental and Reproductive Effects

The NASEM report found no epidemiological studies on ENDS use and pregnancy health and outcomes. Although fetal exposure to combusted cigarette smoking has been linked to conditions such as SIDS and ADHD, the report found insufficient evidence to determine if ENDS use adversely affects fetal development. No additional observational studies published after the NASEM report related to ENDS use and developmental and reproductive health risks were found.

Injuries and Poisonings

The NASEM report stated there is conclusive evidence ENDS can explode and cause burns and projectile injuries. Such risk is significantly increased when batteries are of poor quality, stored improperly, or modified by users. The NASEM report also stated there is conclusive evidence intentional or accidental exposure to e-liquids (from drinking, eye contact, or dermal contact) can result in adverse health effects including but not limited to seizures, anoxic brain injury, vomiting, and lactic acidosis and intentionally or unintentionally drinking or injecting e-liquids can be fatal.

Since the NASEM report, additional ENDS adverse experiences have been raised, including burn events related to battery explosions and poison events related to e-liquid nicotine exposure.[495,859-862] Studies used National Emergency Injury Surveillance System (NEISS) and National Poison Data System (NPDS) to estimate these burdens. From 2013 to 2017, an estimated 4,745 poisoning cases related to e-liquids among children under age five were treated in US hospital emergency departments (EDs);[860] in 2018, 885 children under age 5 were treated in the EDs.[859] From 2015 to 2017, 2,035 ENDS battery explosion and burn injuries were presented in the US hospital EDs.[495,861]

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