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Attorneys for Defendant JUUL LABS, INC.

UNITED STATES DISTRICT COURT

CENTRAL DISTRICT OF CALIFORNIA

WESTERN DIVISION

TIMOTHY MALANEY; and BRENDAN GORMAN,

Plaintiffs,

v.

JUUL LABS, INC.; and PAX LABS, INC.,

Defendants.

CASE NO. 2:18-CV-09605

**NOTICE OF REMOVAL OF CLASS ACTION**

(Los Angeles County Superior Court Case No. 18STCV02948)

Action Filed: October 26, 2018
Trial Date: None Set

**TO THE CLERK OF THE UNITED STATES DISTRICT COURT FOR THE CENTRAL DISTRICT OF CALIFORNIA, AND TO PLAINTIFFS TIMOTHY MALANEY AND BRENDAN GORMAN AND THEIR COUNSEL OF RECORD:**

**PLEASE TAKE NOTICE THAT**, pursuant to the Class Action Fairness Act of 2005, 28 U.S.C. §§ 1332, 1367, 1453, and 1711, Defendant JUUL Labs, Inc. ("JUUL Labs" or "Defendant") hereby removes the above-captioned action—with reservation of all defenses and rights—from the Superior Court of the State of California for the County of Los Angeles, Case No. 18STCV02948, to the United States District Court for the Central District of California, Western Division. Removal is proper on the following grounds:

**TIMELINESS OF REMOVAL**

1. Plaintiffs Timothy Malaney and Brendan Gorman (collectively, "Plaintiffs") filed a putative Class Action Complaint against JUUL Labs in Los Angeles Superior Court, State of California, Case No. 18STCV02948, on October 26, 2018. Pursuant to 28 U.S.C. § 1446(a), true and correct copies of the (a) Class Action Complaint, (b) Civil Cover Sheet, (c) Summons, (d) Notice of Case Assignment, and (e) Minute Order are attached as Exhibits A–E to the Declaration of Austin V. Schwing ("Schwing Decl.") filed concurrently herewith.

2. Plaintiffs served JUUL Labs, through JUUL Labs' agent for service of process with the Summons and Complaint on November 6, 2018. Schwing Decl., ¶ 7. This notice of removal is therefore timely pursuant to 28 U.S.C. § 1446(b) because it is filed within 30 days after service was completed. *See* 28 U.S.C. § 1446(b); Fed. R. Civ. P. 6(a)(1).

**SUMMARY OF ALLEGATIONS AND GROUNDS FOR REMOVAL**

3. Removal is proper pursuant to 28 U.S.C. §§ 1441 and 1453 because this Court has subject matter jurisdiction over this action and all claims asserted against

JUUL Labs in this action pursuant to the Class Action Fairness Act of 2005 ("CAFA"), 28 U.S.C. § 1332(d).

4. CAFA applies "to any class action before or after the entry of a class certification order by the court with respect to that action." 28 U.S.C. § 1332(d)(8). This case is a putative "class action" under CAFA because it was brought under a state statute or rule, namely California Code of Civil Procedure § 382, authorizing an action to be brought by one or more representative persons as a class action. *See* 28 U.S.C. § 1332(d)(1)(B); *see also* Schwing Decl. Ex. A ("Compl."), ¶ 25.

5. In their Complaint, Plaintiffs assert eleven counts against JUUL Labs: (1) Negligence; (2) Strict Liability; (3) Failure to Warn; (4) Negligent Misrepresentation; (5) Fraudulent Misrepresentation; (6) Breach of Implied Warranty; (7) Breach of Express Warranty; (8) Intentional Infliction of Emotional Distress; (9) Negligent Infliction of Emotional Distress; (10) Equitable Relief: Medical-Monitoring Program; and (11) Punitive Damages. *See* Compl. ¶¶ 30-81.[1]

6. Among other things, Plaintiffs allege that putative class members are entitled to general, special, and punitive damages, restitution and disgorgement of profits, the establishment of a medical monitoring program to study the health effects of JUUL Labs products and notify users of potential harms, and attorneys' fees. Compl. ¶ 81.

7. Under CAFA, removal of a class action is proper if: (1) there are at least 100 members in the putative class; (2) there is minimal diversity between the parties, such that at least one class member is a citizen of a state different from that of any defendant; and (3) the aggregate amount in controversy exceeds $5 million, exclusive of interest and costs. *See* 28 U.S.C. §§ 1332(d), 1441.

8. JUUL Labs denies any liability in this case. JUUL Labs expressly reserves all of its rights, including, but not limited to, its right to file motions to compel

[1] Two of these "counts" are actually requested remedies (medical monitoring and punitive damages), and not actually claims.

arbitration and motions challenging the pleadings. JUUL Labs also intends to oppose class certification and believes that class treatment is inappropriate under these circumstances in part because there are many material differences between the named Plaintiffs and the putative class members Plaintiffs seek to represent. JUUL Labs expressly reserves all rights to oppose class certification and to contest the merits of all claims asserted in the Complaint. However, for purposes of meeting the jurisdictional requirements for removal *only*, JUUL Labs submits on a good-faith basis that the allegations in Plaintiffs' Complaint identify a putative class of more than 100 members, meet the minimum diversity requirement, and put in controversy, in the aggregate, an amount that exceeds $5 million. *See* 28 U.S.C. § 1332(d)(2), (d)(5)(B), and (d)(6).

**A. The Proposed Class Consists of More than 100 Members**

9. Based on Plaintiffs' allegations, this action satisfies CAFA's requirement that the putative class action contains at least 100 members. *See* 28 U.S.C. § 1332(d)(5)(B).

10. Plaintiffs' proposed class consists of "[a]ll persons who were never smokers, or who were not regular traditional tobacco smokers, who purchased or used or consumed in the United States JUUL e-cigarettes or pods and suffered personal injury to their pulmonary, cardiovascular, neurological, or behavioral health." Compl. ¶ 25.a.

11. The Complaint estimates that this "class will be in the hundreds of thousands to potentially millions." Compl. ¶ 27. Accordingly, while JUUL Labs denies that class treatment is permissible or appropriate, based on the Complaint's allegations the proposed class plainly consists of more than 100 members.

**B. JUUL Labs and A Member of the Class Are Not Citizens of the Same State**

12. Under CAFA's minimum diversity of citizenship requirement, the plaintiff or any member of the putative class must be a citizen of a different state from any defendant. *See* 28 U.S.C. § 1332(d)(2)(A).

13. Plaintiff Brendan Gorman alleges that he is a "resident of Alabama." Compl. ¶ 2. As such, Plaintiff Gorman is a citizen of Alabama. Further, Plaintiffs' proposed class definition includes purchasers or users of JUUL Labs products "consumed in the United States," Compl. ¶ 25.a, and therefore would include citizens of states other than California.

14. Plaintiffs allege that JUUL Labs "is a Delaware corporation [whose] principal place of business is in San Francisco, California." Compl. ¶ 4. As such, JUUL Labs is a citizen of Delaware and California. *See* 28 U.S.C. § 1332(c)(1). Accordingly, at least one Plaintiff is a citizen of a different state from that of JUUL Labs.

**C. The Amount in Controversy Exceeds $5 Million**

15. CAFA requires that the amount in controversy in a class action exceed $5 million, exclusive of interest and costs. 28 U.S.C. § 1332(d)(2). In calculating the amount in controversy, a court must aggregate the claims of all individual class members. 28 U.S.C. § 1332(d)(6).

16. The Ninth Circuit applies "a preponderance of the evidence" standard to determine whether removal under CAFA is proper. *Rodriguez v. AT&T Mobility Servs. LLC*, 728 F.3d 975, 981 (9th Cir. 2013); *Guglielmino v. McKee Foods Corp.*, 506 F.3d 696, 699 (9th Cir. 2007). A defendant seeking to remove under CAFA need only "provide evidence establishing that it is more likely than not that the amount in controversy exceeds [the jurisdictional] amount" of $5 million. *Guglielmino*, 506 F.3d at 699 (internal quotation marks omitted). To satisfy this burden, a defendant may rely on a "reasonable" "chain of reasoning" that is based on "reasonable" "assumptions." *LaCross v. Knight Transp. Inc.*, 775 F.3d 1200, 1201 (9th Cir. 2015).

17. Plaintiffs' allegations—if accepted—would place in excess of $5 million in controversy, exclusive of interest and costs. *See Lewis v. Verizon Commc'ns, Inc.*, 627 F.3d 395, 399 (9th Cir. 2010) ("In determining the amount [in controversy], we first look to the complaint."). In assessing whether the amount in controversy has been

satisfied, "a court must 'assume that the allegations of the complaint are true and assume that a jury will return a verdict for the plaintiff on all claims made in the complaint.'" *Campbell v. Vitran Exp., Inc.*, 471 F. App'x 646, 648 (9th Cir. 2012) (quoting *Kenneth Rothschild Tr. v. Morgan Stanley Dean Witter*, 199 F. Supp. 2d 993, 1001 (C.D. Cal. 2002)). In other words, the focus of the Court's inquiry must be on "'what amount is put "in controversy" by the plaintiff's complaint, not what a defendant will actually owe.'" *Roth v. Comerica Bank*, 799 F. Supp. 2d 1107, 1117 (C.D. Cal. 2010) (quoting *Korn v. Polo Ralph Lauren Corp.*, 536 F. Supp. 2d 1199, 1205 (E.D. Cal. 2008)).

18. Although JUUL Labs denies that Plaintiffs' claims have any merit or that Plaintiffs have suffered any harm or damages, JUUL Labs avers, for purposes of meeting the jurisdictional requirements for removal *only*, that if Plaintiffs were to prevail on every single claim and allegation in their Complaint on behalf of the putative class, the requested monetary recovery would exceed $5 million. This can hardly be disputed since Plaintiffs seek damages for personal injury, restitution and disgorgement, medical monitoring, punitive damages, and attorneys' fees, all on behalf of a class that allegedly consists of "hundreds of thousands to potentially millions" of people. Compl. ¶ 27.[2]

Allegations of Personal Injury

19. Plaintiffs allege that JUUL Labs unlawfully designed, manufactured, marketed, advertised, and distributed JUUL Labs products. *See, e.g.*, Compl. ¶ 31. Plaintiffs further allege that JUUL Labs' alleged conduct caused the putative class members a wide variety of personal injuries, in the form of physical, mental, and

[2] JUUL Labs reserves the right to present evidence establishing the amount placed in controversy by each of Plaintiffs' claims should Plaintiffs challenge whether the jurisdictional amount-in-controversy threshold is satisfied. *See Dart Cherokee Basin Operating Co.*, 135 S. Ct. 547, 554 (2014) ("Evidence establishing the amount is required by § 1446(c)(2)(B) only when the plaintiff contests, or the court questions, the defendant's allegation [that the amount in controversy exceeds the jurisdictional threshold].").

emotional harms as well as medical, hospital, pharmaceutical, and other expenses. *See, e.g.*, Compl. ¶¶ 15, 19-21, 25, 28-29, 31-32, 34-36, 40, 49, 52, 56.

20. For example, Plaintiffs allege that the putative class members' injuries include "inflammatory lung disease, COPD [Chronic Obstructive Pulmonary Disease], restrictive airway disease, hypersensitivity pneumonitis, vascular disease including myocardial infarction, nicotine addiction, and other diseases and injuries affecting pulmonary, cardiovascular, neurological, or behavioral health, including cancer." Compl. ¶ 29.l. They further allege that their proposed class consists of "hundreds of thousands to potentially millions" of individuals. Compl. ¶ 27.

21. Given Plaintiffs' allegations that "potentially millions" of people are entitled to recover damages for serious medical conditions, Plaintiffs' personal injury claims puts more than $5 million in dispute. This is especially true given that Plaintiffs also seek punitive damages. Compl. ¶ 81.iv.

Restitution and Disgorgement

22. Plaintiffs also seek restitution and disgorgement. Compl. ¶ 81.v. Plaintiffs allege that JUUL Labs has 70% of the $5.5 billion domestic e-cigarette market. Compl. ¶ 12. Thus, Plaintiffs' restitution and disgorgement request puts more than $5 million in dispute.

Medical Monitoring

23. Plaintiffs have also put more than $5 million in dispute by seeking equitable relief in the form of a medical monitoring program, "including notifying purchasers and users of the defects and the potential medical harm; funding of a program [to] monitor and measure injury, including to the lungs, brain, and cardiovascular systems; funding a study of the long-term effects of using the products; funding research into possible cures of the detrimental effects of using JUUL e-cigarettes and pods; gathering and forwarding to treating physicians information relating to the diagnosis and treatment of injuries that may result from using the

product; aiding in the early diagnosis and treatment of resulting injuries; and providing funding for diagnosis and preventable [sic] medical treatment." Compl. ¶ 77.

24. The creation of a medical monitoring program of the kind demanded by Plaintiffs, for a proposed class consisting of "hundreds of thousands to potentially millions" of people, likely would cost more than $5 million.

Attorneys' Fees

25. Plaintiffs' demand for "attorneys' fees," Compl. ¶ 81.vii, places an additional amount in controversy. *Fritsch v. Swift Transp. Co. of Ariz., LLC*, 899 F.3d 785, 794 (9th Cir. 2018) ("such future attorneys' fees are at stake in the litigation, and must be included in the amount in controversy" for CAFA purposes).

26. Under Ninth Circuit precedent, the benchmark commonly used for the award of attorneys' fees is 25% of the common fund. *See Hanlon v. Chrysler Corp.*, 150 F.3d 1011, 1029 (9th Cir. 1998); *but see Fritsch*, 899 F.3d at 796 (rejecting a "per se rule" of 25%).

27. JUUL Labs denies that any such attorneys' fees are owed to Plaintiffs or the putative class, and reserves the right to contest the application of the 25% benchmark in this case. However, for purposes of this jurisdictional analysis *only*, JUUL Labs relies on Plaintiffs' allegations that the attorneys' fees are owed. Applying the 25% benchmark to the damages alleged in the Complaint, Plaintiffs' request for attorneys' fees places a significant additional amount in controversy.

28. Plaintiffs' allegations therefore place more than the requisite $5 million in controversy. The jurisdictional amount-in-controversy requirement is met, and removal to this Court is proper under CAFA.

**THIS COURT HAS JURISDICTION AND REMOVAL IS PROPER**

29. Based on the foregoing facts and allegations, this Court has original jurisdiction over this action pursuant to 28 U.S.C. § 1332(d) because:

a. This is a civil action which is a class action within the meaning of § 1332(d)(1)(B);

b. The action involves a putative class of at least 100 persons as required by § 1332(d)(5)(B);

c. At least one member of the putative class is a citizen of a state different from that of any defendant as required by § 1332(d)(2)(A); and

d. The amount in controversy exceeds $5 million, exclusive of interest and costs, as required by § 1332(d)(2).

Accordingly, this action is properly removable under 28 U.S.C. §§ 1441, 1446, and 1453.

30. The United States District Court for Central District of California, Western Division, is the federal judicial district in which the Los Angeles County Superior Court sits. This action was originally filed in the Los Angeles County Superior Court, rendering venue in this federal judicial district and division proper.[3] *See* 28 U.S.C. §§ 84(c)(2), 1441(a).

31. In accordance with 28 U.S.C. § 1446(a), true and correct copies of all process, pleadings, and orders served upon JUUL Labs are attached as Exhibits A–E to the Declaration of Austin V. Schwing filed concurrently herewith. These filings constitute the complete record of all records and proceedings in the state court that have been served upon JUUL Labs.

32. Upon filing the Notice of Removal, JUUL Labs will furnish written notice to Plaintiffs' counsel, and will file and serve a copy of this Notice with the Clerk of the Los Angeles County Superior Court, pursuant to 28 U.S.C. § 1446(d).

33. WHEREFORE, JUUL Labs hereby removes to the Court the above action pending against it in Los Angeles Superior Court.

[3] Defendant reserves the right to seek to transfer this action from the Central District of California to another United States District Court.

Dated: November 14, 2018

GIBSON, DUNN & CRUTCHER LLP

BY: */s/ Austin V. Schwing*
Austin V. Schwing

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UNITED STATES DISTRICT COURT

CENTRAL DISTRICT OF CALIFORNIA

WESTERN DIVISION

TIMOTHY MALANEY; and BRENDAN GORMAN,

Plaintiffs,

v.

JUUL LABS, INC.; and PAX LABS, INC.,

Defendants.

CASE NO. 2:18-CV-09605

**DECLARATION OF AUSTIN V. SCHWING IN SUPPORT OF NOTICE OF REMOVAL**

(Los Angeles County Superior Court Case No. 18STCV02948)

Action Filed: October 26, 2018
Trial Date: None Set

**DECLARATION OF AUSTIN V. SCHWING**

I, Austin V. Schwing, declare as follows:

1. I am an attorney admitted to practice law before all courts of the State of California and in the United States District Court for the Central District of California. I am a partner in the law firm of Gibson, Dunn & Crutcher LLP, and I am one of the attorneys primarily responsible for the representation of Defendant JUUL Labs, Inc. in this matter. I offer this declaration in support of JUUL Labs' Notice of Removal of this action from the California Superior Court, County of Los Angeles, to the United States District Court for the Central District of California. Unless otherwise stated, the following facts are within my personal knowledge and, if called and sworn as a witness, I could and would testify competently thereto.

2. Attached hereto as **Exhibit A** is a true and correct copy of the Class Action Complaint in *Malaney et al. v. JUUL Labs, Inc. et al.*, Case No. 18STCV02948, filed on October 26, 2018 in the Superior Court of California, County of Los Angeles.

3. Attached hereto as **Exhibit B** is a true and correct copy of the Civil Case Cover Sheet filed by Plaintiffs on October 26, 2018 in the Superior Court of California, County of Los Angeles.

4. Attached hereto as **Exhibit C** is a true and correct copy of the Summons filed by the Clerk on October 26, 2018 in the Superior Court of California, County of Los Angeles.

5. Attached hereto as **Exhibit D** is a true and correct copy of the Notice of Case Assignment – Unlimited Civil Case filed by the Clerk on October 26, 2018 in the Superior Court of California, County of Los Angeles.

6. Attached hereto as **Exhibit E** is a true and correct copy of the Minute Order filed by the Clerk on November 13, 2018 in the Superior Court of California, County of Los Angeles.

7. Plaintiffs served JUUL Labs, through JUUL Labs' agent for service of process with the Summons and Complaint on November 6, 2018.

8. In accordance with 28 U.S.C. § 1446(a), Exhibits A through E include "all process, pleadings and orders served upon" the Defendants in this action. Defendants have not been served with any other filings in this action.

I declare under penalty of perjury under the laws of the United States that the foregoing is true and correct.

Executed on this 14th day of November, 2018 in San Francisco, California.

*/s/ Austin V. Schwing*
Austin V. Schwing

Attorney for Defendant JUUL Labs, Inc.

# EXHIBIT A

Dept. # 14 Assigned Freeman

CAC 9 —

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**SUPERIOR COURT OF THE STATE OF CALIFORNIA**

**COUNTY OF LOS ANGELES**

Timothy Malaney; and Brendan Gorman,

Plaintiffs,

v.

JUUL LABS, INC; and PAX LABS, INC

Defendants

Case No. 18STCV02948

**CLASS-ACTION COMPLAINT FOR DAMAGES & MEDICAL MONITORING**

**JURY TRIAL DEMANDED**

BY FAX

## PARTIES

1. Plaintiff Timothy Malaney is a resident of California, residing at 2832 Menlo Avenue in Los Angeles, California in the County of Los Angeles.

2. Plaintiff Brendan Gorman is a resident of Alabama, residing at 2308 Maury Place in Hoover, Alabama in Jefferson County.

3. Defendant PAX Labs, Inc. ("PAX") is a Delaware corporation. Its principal place of business is in San Francisco, California. PAX's Registered Agent is Incorporating Services, LTD., 660 Alabama St FL 2, San Francisco, CA 94110.

4. Defendant JUUL Labs, Inc. ("JUUL") is a Delaware corporation. Its principal place of business is in San Francisco, California. JUUL was originally within PAX but became a separate entity in 2017. A substantial portion of the events set forth below occurred when JUUL was within PAX. JUUL's Registered Agent is InCorp Services, Inc., 5716 Corsa Ave, Ste. 110, Westlake Village, CA 91362-7354.

## JURISDICTION

5. The Court has jurisdiction over this action pursuant to California Constitution, Article 6, § 10 and Code of California Civil Procedure § 382.

## VENUE

6. Venue is proper pursuant to Code of California Civil Procedure § 395.5.

## ALLEGATIONS

7. This case arises from JUUL's failure to properly assess and warn about the harm that its products cause to the human lungs and body. JUUL failed to evaluate and warn about the dangers of its products, and it falsely markets and falsely advertises its e-cigarette system as a safe alternative to traditional cigarettes. JUUL evaluated and knew or should have known the potential dangers of its products, but failed to adequately ascertain and warn about those dangers. This action seeks certification of a class for medical monitoring and personal-injury damages. The Plaintiffs seek that relief for themselves individually and for the class.

8. JUUL is a pioneer in Electronic Nicotine Delivery Systems ("ENDS") and related technologies. ENDS typically are touted as a safe alternative to traditional combustible cigarettes. JUUL

introduced its ENDS-branded, innovative commercial product to the United States market in 2015. JUUL products are available via retail locations in 150 countries and the JUUL online store.

9. The JUUL system is comprised of two components: (i) a vaporizer device and (ii) disposable pods that are prefilled with a proprietary mixture of vaporizer carriers, nicotine salt extracts, and flavoring (together, "e-liquid"). When a user inserts a pod into the device and inhales using the mouthpiece, the device rapidly heats the e-liquid, aerosolizing it to allow the user to inhale a puff of the vaporized e-liquid. The labels for both the JUUL e-cigarette and pods contain California Proposition 65 warnings that the product contains a substance known to cause cancer. Yet the labels contain no warnings about the potential dangers of using JUUL products, including long-term effects of vaping and inhaling nicotine salts and flavored chemicals on the pulmonary, neurological, and cardiovascular systems. JUUL Labs, Inc., owns and operates juullabs.com and juulvapor.com where it markets, advertises, and sells e-cigarettes and pods.

10. JUUL markets and advertises its e-cigarettes and pods deliberately to attract minors and young adults, including those who have never even been regular tobacco smokers. The JUUL system delivers more potent doses of nicotine than traditional cigarettes. JUUL thus exposes these nonregular-tobacco users to a highly-addictive product under the guise of a safe alternative. The flavored product coupled with the patented nicotine formation creates a perfect storm for addiction among high school, college students, and adults. The JUUL e-cigarettes and pods are available for purchase online. There is also a subscription service on JUUL's website. The JUUL system has been widely adopted and attained tremendous commercial success, currently holding over 70% of the e-cigarette market share. JUUL e-cigarettes are sleek, discrete, and easy to hide. The system looks like a USB flash drive and can even be charged using the USB port of a computer. On its face, JUUL does not appear to be a tobacco-related product. Pods come in flavors that appeal to high-school and college students, including mango, fruit medley, crème brûlée, cool mint, and cool cucumber. Flavors play a key role in the use of tobacco products in teens and young adults. Numerous physician and health organizations have urged the FDA to act on this epidemic, citing the FDA's Population Assessment of Tobacco and Health (PATH) Study found that "85 percent of current e-cigarette users aged 12-17 had used flavored product in the past month and 81.5 percent of those young users cited flavors as the reason for their use of the product". Exhibit A. JUUL e-

cigarettes deliver nicotine more quickly, more effectively, and at higher doses than other e-cigarettes, increasing the users' risk of addiction. Each pod of e-liquid contains as much nicotine as a pack of cigarettes (i.e., about 200 puffs).

11. E-cigarettes were largely unregulated until May 10, 2016, when the Department of Health and Human Services, Food and Drug Administration, passed 21 CFR Parts 1100, 1140, and 1143: "Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act; Restrictions on the Sale and Distribution of Tobacco Products and Required Warning Statement for Tobacco Products". The FDA has allowed e-cigarettes that were already on the market as of August 8, 2016, to stay on the market until at least 2022 without filing applications or undergoing public health review by the FDA. The sale and market growth of JUUL e-cigarettes and pods has thus occurred without any regulatory oversight into the health risks of the vaporization of nicotine salts. On April 24, 2018, however, the FDA requested that JUUL submit to the FDA "documents relating to market practices and research on marketing, effect of product design, public health impact, and adverse experiences and complaints related to JUUL products". Exhibit B.

12. The simple yet almost unfathomable reality is that, until recently, very little was known about the detrimental health effects that JUUL e-cigarettes and pods cause to the lungs and bodies of its users, which includes teens, young adults, and older adults. In accordance with section 904(b) of the FD&C Act, the FDA requested that JUUL "submit all documents relating to marketing practices and research activities and research findings, conducted, supported, or possessed by you or your agents relating to a specific set of topics…research may include, but is not limited to, focus groups, surveys, experimental clinical studies, toxicological and biochemical assays, *in vivo* and *in vitro* assays including animal testing, laboratory formulation and processing testing, taste panels, and assessments of the effectiveness of product marketing practices". Modern science has thus been playing catch-up with the effects of e-cigarettes on humans. Since the science has developed, we have found that JUUL is a wolf-in-sheep's-clothing, delivering as much or more nicotine and harmful chemicals as bigger, more conspicuous e-cigarettes. What has been marketed and sold as a fun, harmless, and trendy pastime is anything but that. This year the American vaporizer market will grow to five and a half billion dollars, an increase of twenty-five per cent from 2017. 70% of that market belongs to JUUL.

13. Nicotine is both a stimulant and relaxant. Biochemically, it works by binding to receptors in multiple regions of the brain. It raises dopamine levels and can mimic key neurotransmitters that affect focus and arousal. The nicotine delivery for JUUL is enhanced by adding benzoic acid to nicotine salts, which occur naturally in tobacco, allowing for rapid nicotine delivery in vapor form that is quickly absorbed into the lungs and brain. When inhaled, the flavored vapor is pleasing to the palate and the nicotine produces a rush to the brain.

14. Dr. Johnathan Winickoff, the former chair if the American Academy of Pediatrics Tobacco Consortium, in March 2018 said that "JUUL is already a massive public-health disaster-and without dramatic action it's going to get much, much worse." Dr. Winickoff, who is also a pediatrician at Massachusetts General Hospital and Professor at Harvard Medical School also noted that: "[i]f you were to design your ideal nicotine-delivery device to addict a large numbers of United States kids, you'd invent JUUL". Of the emerging e-cigs, JUULs have all the necessary elements to take over a substantial portion of the market share. Its batteries can be recharged in an hour, it is flavored, it can often be used without detection, and it contains somewhere around twice the concentration of nicotine as other vape products. For those aged 18 to 24, 40 percent were not smokers before using the device. Exhibit C. On September 1, 2018, the Israeli Government instituted a ban on the sale of JUUL e-cigarettes in Israel, citing that JUUL poses "a grave danger to public health".

15. JUUL's e-liquid contains five ingredients: glycerol, propylene glycol, nicotine, benzoic acid, and food-grade flavoring. Glycerol is a sweet liquid that has been used in antifreeze and toothpaste. Propylene glycol is used in asthma nebulizers. Benzoic acid is a common food preservative. Vaporing these liquids at elevated temperatures may result in the generation of known pulmonary toxicants, including acrolein, acetaldehyde, and formaldehyde. Some of the chemicals in the flavoring have known adverse respiratory effects. Marketed as a transitional product to help adult smokers stop smoking cigarettes, many physicians question whether the higher doses of nicotine delivered in a JUUL-vape draw just makes the user want more. Nicotine affects young peoples' cognitive development, making them more susceptible to other addictions later in life. A Lancet study in March 2007 ranked nicotine as more addictive than alcohol or barbiturates. The National Academies recently published *Public Health*

*Consequences of E-Cigarettes*, which shows incontrovertible evidence that using e-cigarettes creates dependence.

16. JUUL is patent-protected and has an expanding customer base. It has seen exponential growth that has far exceeded expectations. The company's patented JUULSalts approach to nicotine delivery is due to compounds called nicotine salts, which develop in heat-dried tobacco leaves. According to JUUL's website, freebase nicotine is mixed with benzoic acid to make the e-liquid, which has a chemical reaction that produces the nicotine salts. JUUL U.S. Patent No. 9,215,895 covers its process to produce nicotine salts. The patented process allows 20% more nicotine to enter the blood stream than a Pall Mall cigarette, rendering the risk of addiction and abuse higher for JUUL users versus those who use traditional cigarettes.

17. JUUL heavily promotes its products via social media platforms. It presents the product as a trendy, fresh, and safer alternative to cigarettes, minimizing the health risks and addictiveness of "juuling". This marketing mirrors in many ways how the tobacco industry promoted cigarettes as being cool while suppressing the long-term adverse health complications.

18. It took time and resources for healthcare researchers and clinicians to research the effects of vaping on the lungs and human body. The evidence now shown by numerous clinical and scientific studies is not favorable for JUUL. Stanton Glanz, Professor of Medicine with the University of California, San Francisco Center for Tobacco Control, Research and Education, said "it's important to understand that e-cigs have an entirely different toxicological profile" than cigarettes. Glanz notes that "[t]he assumption has been that at least e-cigarettes aren't worse. But this suggests that they have something in them that isn't even in standard cigarettes that's worth being worried about".

19. Recent studies examining the effects of e-cigarettes on the lungs show some of the dangers of vaping. A study form the Marsico Lung Institute and the Department of Pathology at the University of North Carolina-Chapel Hill shows that vaping from e-cigarettes causes a unique innate immune response in the lung, involving increased neutrophilic activation and altered mucin secretion. The authors wrote "taken together, our results indicate that the effects of e-cigarettes are both overlapping with and distinct from what is observed in otherwise healthy cigarette smokers. In conclusion, our results challenge the concept that e-cigarettes are a healthier alternative to cigarettes and reverse smoking-induced adverse

health effects." Reidel et al, *E-Cigarette Use Causes a Unique Innate Immune Response in the Lung, Involving Increased Neutrophilic Activation and Altered Mucin Secretion*, AM J RESPIR CRIT CARE MED (2018 Feb 15;197(4):492-501). Another study by Dr. Casey G. Sommerfield MD with the Children's Hospital of Pittsburgh of UPMC reported the first case of hypersensitivity pneumonitis and acute respiratory distress syndrome as a risk of e-cigarette use in an adolescent. Exhibit D, Sommerfield et al, *Hypersensitivity Pneumonitis and Acute Respiratory Distress Syndrome From E-Cigarette Use* PEDIATRICS 2018 Jun;141(6). Dr. Sommerfield's case report involves an 18-year-old woman who presented with severe inflammatory disease of the lung called hypersensitivity pneumonitis. In an acute setting, hypersensitivity pneumonitis may be secondary to chemical exposure, which is found in e-cigarette vapor. The case report thus shows a life-threatening risk of e-cigarette use in an adolescent patient. Another study from the Comprehensive Cancer Center at the Ohio State University found that "the induction of inflammation by e-cigs may differentially impact lung cancer and COPD risks" and that "the role of nicotine also needs to be considered, as it had both pro-and-anti-inflammatory potential, making it unclear how nicotine content may mediate the effects of the other aerosol constituents". Exhibit E, Shields et al, *A Review of Pulmonary Toxicity of Electronic Cigarettes in the Context of Smoking: A focus on Inflammation*, CANCER EPIDEMIOL BIOMARKERS PREV 2017 Aug;26(8):1175-1191. Studies conducted at Tulane University show that "e-cigarette aerosol is capable of inducing reactive oxygen species, DNA damage, and cell death in human umbilical vein endothelial cells" and that "*in vivo* studies of e-cigarette aerosol's effect on the cardiovascular system have shown broad spectrum of potentially negative effects." Exhibit F, Anderson et al, *E-Cigarette Aerosol Exposure Induces Reactive Oxygen Species, DNA Damage, and Cell Death in Vascular Endothelial Cells*, TOXICOLOGICAL SCIENCES 154(2), 2016 332-340. Researchers at the West Virginia University School of Medicine published an animal study showing that the cardiovascular effects of long-term vaping may be as dire as smoking cigarettes. Results indicate that chronic exposure to e-cigarette vapor stiffened the aorta 2.5 times more than the regular aging process did in a vapor or smoke-free environment. In comparison, cigarette smoke caused a 2.8-fold increase. Exhibit G, Olfert et al, *Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice*, J APPLIED PHYSIOL, 2018 Mar 1;124(3):573-582

20. The regular use of e-cigarettes, including JUUL, presents a clear and present danger of acute and chronic injury to the pulmonary and cardiovascular systems of nonsmokers and adults who were not consistent traditional cigarette smokers. Science News reports that about 1.9 million American adults who have never consistently smoked traditional cigarettes use e-cigarettes. About 60% of these users were between the ages of 18 and 24. These numbers were based on the analysis of data from 2016 and are much higher in 2018. We know that there are carcinogens in the vapor created by JUUL e-cigarettes and scientists are concerned about the addictive risk of nicotine and multiple chemicals that are contained in the vapor and how nicotine may serve as a catalyst to increase the risk of cancer. There is concern about the addictive properties of the JUUL e-cigarette in both nicotine addiction and behavioral aspects of juuling.

21. JUUL has been incredibly successful through its marketing and branding, inducing youngsters, college students, and high-school students to start vaping. It has done so with wholly inadequate warnings about the potential health hazards of using the JUUL e-cigarettes and pods. Medical evidence shows significant health issues relating to the transmission of glycerol, propylene glycol, nicotine, benzoic acid, and food-grade flavoring in the vapor itself. The cytotoxic properties of these vaporized chemicals causes cellular damage to pulmonary and vascular cells that is acute and may lead to hypersensitivity pneumonitis and restrictive airway disease. There is growing scientific concern among public-health officials that vaping may cause a much higher rate of chronic obstructive pulmonary disease (COPD) in young adults and that vaping may evolve into a national health epidemic because it has become a means to nicotine addiction, rather than an end. Vaping among young people is surpassing all other forms of tobacco use.

22. There is a subset of adults who use JUUL e-cigarettes and who will develop significant acute pulmonary inflammation, leading to pneumonitis and pneumonia that will require medical intervention, including hospitalization and potentially mechanical ventilation. There is another subset of JUUL users who will sustain varying degrees of pulmonary injury that, over time, may cause shortness of breath, dyspnea, restrictive airway disease, and COPD. Modern pulmonary medicine allows physicians to assess and measure the level of pulmonary injury regarding both restrictive airway disease and inflammatory changes through advanced non-invasive pulmonary function tests and bronchoscopy. Past

and current JUUL users who have exposed and continue to expose their pulmonary and cardiovascular systems to vapor will need medical monitoring to assess how badly the vaping has affected them. Restrictive airway disease is often permanent and JUUL users will need to know how continued use may permanently impair their health and restrict their mobility. The evidence also shows that never smokers who use JUUL will have increased risks of cancer from the carcinogenic compounds found in the vapor.

23. Timothy Malaney, a college student at USC, occasionally and intermittently began smoking traditional cigarettes about two years ago. About one year ago, he began using JUUL to stop his casual traditional cigarette smoking and transition to using e-cigarettes. Malaney chose the JUUL e-cigarette specifically because of its small easily concealable and convenient size; the fruity and minty flavors available; the fact that JUUL vapor does not create a lingering odor in indoor spaces, in vehicles, or on clothes; the lack of ashes and cigarette butts to clean up; and the nicotine buzz that JUUL provides over competing products. He uses JUUL every day, usually the mint or mango pods.

24. Brendan Gorman, born September 2, 1996, occasionally and intermittently began smoking traditional cigarettes at or about age 19. Gorman only used traditional cigarettes in social settings, purchasing about 6-7 packs per month and oftentimes not smoking for days to weeks at a time between social gatherings. Around January 2017, Mr. Gorman began using the JUUL electronic cigarette to completely stop his occasional and intermittent use of traditional cigarettes. Mr. Gorman chose the JUUL electronic cigarette specifically because of its small easily concealable and convenient size; the fruity and minty flavors available; the fact that JUUL vapor does not create a lingering odor in indoor spaces, in vehicles, or on clothes; the lack of ashes and cigarette butts to clean up; and the nicotine buzz that JUUL provides over competing products. Mr. Gorman's intended to use JUUL to reduce his nicotine dependence and cease all nicotine containing products. But Mr. Gorman has been unable to cease use. In fact, Mr. Gorman, who was never an everyday traditional cigarette smoker now uses JUUL products every day, often taking his first puff as soon as he wakes up and using at least one JUUL pod per day (the equivalent of 1 pack of traditional cigarettes). Mr. Gorman has made numerous attempt to "kick the habit", but he has unfortunately been unable to do so because of the nicotine withdrawals, which is something he never contended with regarding traditional cigarettes.

## CLASS ALLEGATIONS

25. Plaintiffs bring this action against Defendants on behalf of themselves and all others similarly situated, as a class action pursuant to California Code of Civil Procedure Section 382.

The proposed class is defined as follows:

a. All persons who were never smokers, or who were not regular traditional tobacco smokers, who purchased or used or consumed in the United States JUUL e-cigarettes or pods and suffered personal injury to their pulmonary, cardiovascular, neurological, or behavioral health. This class also includes appropriate medical monitoring for assessment of both acute and chronic physical medical illnesses relating to the use of JUUL e-cigarettes or pods.

b. Plaintiffs reserve the right to alter, change, narrow, or broaden the class definition based on specific evidence developed during discovery and propose appropriate sub-classes based on ascertained risks and necessary medical monitoring.

26. This action has been filed with the purpose of maintaining it as a class against the Defendants, pursuant to the provisions of § 382. The need for medical monitoring applies to all members of the class, including many young adults and teenagers.

27. The Plaintiffs anticipate that the class will be in the hundreds of thousands to potentially millions. At this time, the number of members in the class is so numerous that the disposition of the claims for medical monitoring in a class action rather than individual actions will benefit the parties and the courts.

28. The common areas in the Class Member's claims include: (a) Defendants marketed and sold their JUUL E-cigarettes and pods as less likely to cause addiction than traditional cigarettes; (b) Defendants had not done adequate medical research on the deleterious medical effects of the vaporization of chemicals and compounds used in their proprietary products, or, alternatively, Defendants conducted adequate medical research but failed to warn about the dangers learned; (c) Defendants failed to warn about the direct cellular and tissue injury to both the lungs and vascular endothelial tissues of JUUL users, including causing both acute and chronic pulmonary injury, leading to long-term restrictive airway disease, COPD, and increased risk of heart disease. Class Members' claims regarding personal injury are based on the biological effects of JUUL on human tissues—the mechanism of injury is the same, but the

degree of injury will vary. Class Members also share the same need for medical monitoring to assess the level of injury sustained from using JUUL products and to ensure proper medical treatment intervention occurs to treat those injuries.

29. The common questions of law and fact are:

a. Whether Defendants adequately warned the Class Members of the deleterious direct health effects to the human body from the vapor inhaled while using a JUUL e-cigarette or pod;

b. Whether Defendants did enough research as to the health effects to the human body from the vapor inhaled while using a JUUL e-cigarette or pod before they began selling JUUL products;

c. Whether Defendants were aware of the levels of nicotine absorption into the human body from the use of JUUL e-cigarettes and pods and what, if anything, the Defendants did to reduce the amount of nicotine absorbed;

d. Whether the Defendants did any research on the health effects to the human body from the vaporization of multiple chemicals (glycerol, propylene glycol, nicotine, benzoic acid, and food-grade flavoring) and how these chemicals affect each other and any additive effects from their comingling;

e. Whether the Defendant engaged in conduct knowingly, recklessly, or negligently to keep high nicotine absorption levels in their JUUL vapor to induce higher use of their product among Class Members;

f. Whether the Defendants knew or should have known that JUUL products are being sold to and used by minors;

g. Whether Defendants should have taken reasonable steps to prevent youth access to JUUL products;

h. Whether it was foreseeable to Defendants that knockoff or copycat JUUL-like products would be made, sold to JUUL users, and used in lieu of or in combination with genuine JUUL products;

i. The amount of unjust enrichment enjoyed by the Defendants because of their conduct;

j. Whether Class Members are entitled to injunctive relief, including lowering the amount of nicotine delivered by the JUUL system and reducing the amount of nicotine in the JUUL pod, and other equitable relief;

k. Whether Class Members are entitled to medical monitoring;

l. Whether Class Members are entitled to personal-injury damages for the development of personal injury, including inflammatory lung disease, COPD, restrictive airway disease, hypersensitivity pneumonitis, vascular disease including myocardial infarction, nicotine addiction, and other diseases and injuries affecting pulmonary, cardiovascular, neurological, or behavioral health, including cancer;

## COUNT ONE

## NEGLIGENCE

30. Plaintiff realleges all preceding paragraphs.

31. Defendants as designers, manufacturers, retailers, wholesalers, suppliers, and distributors of JUUL e-cigarettes and pods were negligent in carrying out the manufacturing, retailing, design, wholesaling, testing, advertising, promotion, and distribution of the products. Defendants' negligence proximately caused the defects inherent in the JUUL e-cigarettes and pods. Each plaintiff has suffered personal injury because of the inherent defects and each plaintiff suffers from the continuing likelihood of medical problems as described herein and the attendant emotional stress that is constantly present.

32. As a further proximate cause of Defendants' negligence, each plaintiff must employ clinicians to examine, treat, and care for them, and will incur medical, hospital, pharmaceutical, and incidental and consequential expenses. They will continue to incur such medical, hospital, pharmaceutical, and incidental and consequential expenses in the future.

## COUNT TWO

## STRICT LIABILITY

33. Plaintiff realleges all preceding paragraphs.

34. Defendants as the designers, manufacturers, retailers, wholesalers, suppliers, and distributors of the JUUL e-cigarettes and pods are strictly liable to the Class for manufacturing, designing, retailing, distributing, wholesaling, supplying, and placing on the market and in the flow of commerce a defective product knowing that the product would be used by the public and particularly by the recipients without adequate research, inspection, testing, and warning. The JUUL e-cigarettes and pods were not fit for their intended purpose and/or the risks inherent in the design of the JUUL e-cigarettes and pods outweighed the benefits and/or the JUUL e-cigarettes and pods were more dangerous than Class Members anticipated. All these defects proximately caused the personal injuries and damages to each member of the class as alleged herein.

35. The JUUL e-cigarettes and pods were defective, unsafe, and unreasonably dangerous for use and caused and will continue to cause serious bodily injury when used for such purposes.

36. As a further proximate cause of Defendants' actions, each plaintiff must employ clinicians to examine, treat, and care for them, and will incur medical, hospital, pharmaceutical, and incidental and consequential expenses. They will continue to incur such medical, hospital, pharmaceutical, and incidental and consequential expenses in the future.

## COUNT THREE

## FAILURE TO WARN

37. Plaintiff realleges all preceding paragraphs.

38. At the time the JUUL e-cigarettes and pods were supplied to the recipient class, the product was defective as a result of Defendants' failure to adequately test for safety, and to give adequate warnings or instructions regarding the possibility of adverse health consequences, including to the lungs, brain, and cardiovascular systems, the development of medical problems associated with the use as described herein, including cancer, and other dangers that might be associated with use. Defendants also failed to inform and warn plaintiff and the Class that they had failed to perform adequate testing of the JUUL e-cigarettes and pods to ensure safety, including long-term testing of the product, testing for injury to the lungs, brain, and cardiovascular systems, and other related medical conditions, including COPD and cancer.

39. Defendants failed to adequately test JUUL e-cigarettes and pods before marketing it to consumers such as plaintiff and the Class Members; failed to disclose to plaintiff and the recipient class

members that such testing had not been done and which testing would have disclosed the magnitude of the potential risks associated with the use of the JUUL e-cigarettes and pods. In the alternative, Defendants conducted adequate testing but failed to warn about the dangers of using JUUL e-cigarettes and pods.

40. Defendants' failure to warn was willful and malicious in that the conduct was carried on with a conscious disregard for the safety and the rights of the Class. As a proximate result of Defendants' failure to warn regarding the dangers of the JUUL e-cigarettes and pods, each plaintiff must employ clinicians to examine, treat, and care for them, and will incur medical, hospital, pharmaceutical, and incidental and consequential expenses. They will continue to incur such medical, hospital, pharmaceutical, and incidental and consequential expenses in the future. In addition, Defendants' conduct proximately caused each class member to live under the continued likelihood of medical injury and the increased risk of developing medical problems associated with the use of JUUL e-cigarettes and pods as described herein, and the attendant emotional stress that is constantly present.

## COUNT FOUR

## NEGLIGENT MISREPRESENTATION

41. Plaintiff realleges all preceding paragraphs.

42. During the period of time that defendants designed, manufactured, distributed, advertised, promoted, supplied, and marketed the JUUL e-cigarettes and pods, Defendants falsely and negligently represented to the class, the consumers of the product, and the FDA that the JUUL e-cigarettes and pods were safe for use, safer than traditional cigarettes, not harmful like traditional cigarettes, and were fit for their intended purposes; that JUUL e-cigarettes and pods were not dangerous and did not impose any health risks; and that the products would function without defect.

43. The representations made by defendants were false. The true facts that defendants concealed, falsified, or misrepresented to the public, the FDA, and to medical providers was that the JUUL e-cigarettes and pods the JUUL e-cigarettes and pods were safe for use, safer than traditional cigarettes, not harmful like traditional cigarettes, and were fit for their intended purposes, even though we know that use of the JUUL e-cigarettes and pods may cause severe medical problems as described herein.

44. When defendants made these representations, they knew or should have known that the representations were false and that they were made with no reasonable ground for believing them to be

true. The representations were made by defendants with intent to deceive users of the JUUL e-cigarettes and pods, the FDA, and the public, with intent to induce them to use JUUL e-cigarettes and pods.

45. At the time these representations were made, defendants concealed from plaintiff, the FDA, and the class, their lack of adequate testing and research and their lack of information about the safety of the JUUL e-cigarettes and pods.

46. Plaintiff and Class Members, at the time these representations were made by Defendants and at the time Plaintiff purchased and used JUUL e-cigarettes and pods, were ignorant of the falsity of Defendants' representations and believed that the JUUL e-cigarettes and pods were safe and fit for their intended use.

47. In reliance on Defendants' representations, Plaintiff and Class Members were induced to and did purchase and use JUUL e-cigarettes and pods and suffered injury as described herein. Had Plaintiff and Class Members known of the true facts, then they would not have taken such actions.

48. Plaintiff and Class Members reliance on Defendants' representations was justified because they reasonably relied upon Defendants' representations concerning the product, having no independent expertise of their own to evaluate the product or the representations to be anything other than what defendants stated.

49. As a proximate cause of Defendants' actions, each Plaintiff and Class Member must employ clinicians to examine, treat, and care for them, and will incur medical, hospital, pharmaceutical, and incidental and consequential expenses. They will continue to incur such medical, hospital, pharmaceutical, and incidental and consequential expenses in the future.

## COUNT FIVE

## FRAUDULENT MISREPRESENTATION

50. Plaintiff realleges all preceding paragraphs.

51. During the period of time that Defendants designed, manufactured, distributed, advertised, promoted, supplied and marketed the JUUL e-cigarettes and pods, they knowingly and purposely represented to the Plaintiff, Class Members, the FDA, and users of JUUL e-cigarettes and pods JUUL e-cigarettes and pods that the products were fit for their intended purposes, would function without defect, and were appropriate for use.

52. Defendants knew that these representations were false when made. The true facts that Defendants concealed were that the JUUL e-cigarettes and pods are dangerous and may cause long-term adverse health consequences, including injury to the lungs, brain, and cardiovascular systems.

53. When Defendants made these representations, they knew that the representations were false. The representations were made by defendants with intent to deceive the public, including teenagers and young adults, users of JUUL e-cigarettes and pods, and the FDA, with intent to induce them to purchase and use JUUL e-cigarettes and pods.

54. The teenagers, young adults, and users of JUUL e-cigarettes and pods, at the time these representations were made by Defendants and at the time the JUUL e-cigarettes and pods were purchased and used, were ignorant of the falsity of Defendants' representations and believed that the JUUL e-cigarettes and pods were safe for use, safer than traditional cigarettes, not harmful like traditional cigarettes, and were fit for their intended purposes, and that JUUL e-cigarettes and pods were not dangerous and did not impose any health risks, and would function without defect.

55. Plaintiff and Class Members reliance on Defendants' representations was justified because Plaintiff and Class Members reasonably relied upon Defendants' representations concerning the product, having no independent expertise of their own to evaluate the product or the representations to be anything other than what defendants stated.

56. As a proximate cause of Defendants' actions, each Plaintiff and Class Member has suffered personal injury and must employ clinicians to examine, treat, and care for them, and will incur medical, hospital, pharmaceutical, and incidental and consequential expenses. They will continue to incur such medical, hospital, pharmaceutical, and incidental and consequential expenses in the future.

## COUNT SIX

## BREACH OF IMPLIED WARRANTY

57. Plaintiff realleges all preceding paragraphs.

58. Defendants designed, manufactured, distributed, packaged, compounded, merchandised, advertised, promoted, supplied and sold JUUL e-cigarettes and pods and, before the JUUL e-cigarettes and pods were purchased and used, Defendants impliedly warranted to each named plaintiff, the class

members, and the FDA that the JUUL e-cigarettes and pods were of merchantable quality and safe for the use for which they were intended.

59. Each named plaintiff and class member relied on the skill, judgment, and representations of Defendants in purchasing and using the JUUL e-cigarettes and pods.

60. The JUUL e-cigarettes and pods were unsafe for their intended use and were not of merchantable quality as warranted by Defendants in that they had dangerous propensities when put to their intended use and would cause severe injury to the user.

61. The JUUL e-cigarettes and pods designed, manufactured, distributed, packaged, compounded, merchandised, advertised, promoted, supplied, and sold by defendants proximately and directly caused plaintiff and class members to sustain damages as set forth herein.

## COUNT SEVEN

## BREACH OF EXPRESS WARRANTY

62. Plaintiff realleges all preceding paragraphs.

63. Defendants designed, manufactured, distributed, packaged, compounded, merchandised, advertised, promoted, supplied and sold JUUL e-cigarettes and pods and, before the JUUL e-cigarettes and pods were purchased and used, Defendants expressly warranted to each named plaintiff and class member that the JUUL e-cigarettes and pods were of merchantable quality and safe for the use for which they were intended.

64. At the time of making said express warranties, defendants had knowledge of the purpose for which the JUUL e-cigarettes and pods were to be used and warranted them to be, in all respects, fit, safe, and effective and proper for such purposes.

65. Each named plaintiff and class member relied on the skill, judgment and express warranties and representations of Defendants in having the purchasing and using the JUUL e-cigarettes and pods.

66. These warranties were false and untrue at the time they were made. Defendants knew that the JUUL e-cigarettes and pods were unsafe and unsuited for the use for which they were intended, and that they could cause attendant medical problems as described herein. Further, the JUUL e-cigarettes and pods were unsafe for their intended use and were not of merchantable quality as warranted by defendants

in that they had dangerous propensities when put to their intended use and would cause severe injury to the user.

67. The JUUL e-cigarettes and pods designed, manufactured, distributed, packaged, compounded, merchandised, advertised, promoted, supplied and/or sold by defendants, proximately and directly caused plaintiff and class members to sustain damages as set forth herein.

## COUNT EIGHT

## INTENTIONAL INFLICTION OF EMOTIONAL DISTRESS

68. Plaintiff realleges all preceding paragraphs.

69. Defendants placed into the stream of commerce defective JUUL e-cigarettes and pods knowing that the JUUL e-cigarettes and pods were not fit for their intended purposes.

70. Defendants also knew that users of the JUUL e-cigarettes and pods would suffer emotional distress upon learning that the JUUL e-cigarettes and pods are defective and could cause attendant medical problems as described herein.

71. Defendants' conduct in manufacturing, retailing, distributing, advertising, promoting, wholesaling, and placing on the market and into the flow of commerce a known defective product constitutes the intentional infliction of emotional distress upon each plaintiff and class member and has proximately caused emotional distress for each plaintiff and class member.

## COUNT NINE

## NEGLIGENT INFLICTION OF EMOTIONAL DISTRESS

72. Plaintiff realleges all preceding paragraphs.

73. Defendants placed into the stream of commerce defective JUUL e-cigarettes and pods knowing that the JUUL e-cigarettes and pods were not fit for their intended purposes.

74. Defendants also knew that users of the JUUL e-cigarettes and pods would suffer emotional distress upon learning that the JUUL e-cigarettes and pods are defective and could cause attendant medical problems as described herein.

75. Defendants' conduct in manufacturing, distributing, wholesaling, advertising, promoting, retailing, and placing on the market and into the flow of commerce for human use a known defective

product constitutes the negligent infliction of emotional distress upon each plaintiff and class member and has proximately caused emotional distress in each plaintiff and class member.

## COUNT TEN

## EQUITABLE RELIEF: MEDICAL-MONITORING PROGRAM

76. Plaintiff realleges all preceding paragraphs.

77. As a direct and proximate result of Defendants' acts, each plaintiff and class member faces an increased susceptibility to injuries and this irreparable threat to their health can only be mitigated by the creation of a medical-monitoring fund to provide for a medical-monitoring program, including notifying purchasers and users of the defects and the potential medical harm; funding of a program monitor and measure injury, including to the lungs, brain, and cardiovascular systems; funding a study of the long-term effects of using the products; funding research into possible cures of the detrimental effects of using JUUL e-cigarettes and pods; gathering and forwarding to treating physicians information relating to the diagnosis and treatment of injuries that may result from using the product; aiding in the early diagnosis and treatment of resulting injuries; and providing funding for diagnosis and preventable medical treatment.

78. The users of JUUL e-cigarettes and pods have no adequate remedy at law in that monetary damages alone cannot entirely compensate them for the insidious and continuing nature of the harm to them, and only a monitoring program that notifies the users and aids in correcting the problems can prevent the greater harms that may not occur immediately and that may be preventable if proper research is conducted and the health risks are diagnosed and treated before they occur or become worse.

79. The users of JUUL e-cigarettes and pods have suffered irreparable harm as alleged herein and, in the absence of equitable relief, they will suffer further irreparable harm, including development of restrictive airway disease, hypersensitivity pneumonitis, pneumonia, decreased immune response to both bacterial and viral infections, nicotine addiction, behavioral addiction, cardiovascular endothelial cell injury leading to myocardial infarction, death and severe and debilitating injuries. Without a medical-monitoring program, Plaintiff and Class Members might not receive prompt medical care that could prolong their productive lives by earlier diagnosis of restrictive airway diseases, increase prospects for improvement, and minimize disability.

## COUNT ELEVEN

## PUNITIVE DAMAGES

80. Plaintiff realleges all preceding paragraphs.

81. Defendants' acts were willful and malicious in that their conduct was carried on with a conscious disregard for the safety and rights of the users of JUUL e-cigarettes and pods. Defendants' unconscionable conduct thereby warrants an assessment of exemplary and punitive damages against each defendant in an amount appropriate to punish the defendant and set an example of it. For these reasons, Plaintiff and Class Members, on behalf of themselves and all other members of their respective classes, ask for judgment against defendants as follows:

i. For a trial by jury on all issues;

ii. For general damages in an amount to be proven at time of trial, including prejudgment interest;

iii. For special damages in an amount to be proven at the time of trial;

iv. For exemplary and punitive damages in an amount to be proven at the time of trial, and sufficient to punish defendants or to deter them and others from repeating the injurious conduct alleged herein;

v. For restitution and disgorgement of profits;

vi. For the establishment and funding of a medical-monitoring program, at Defendants' expense, to notify users of the defects and potential harm; to study the effects of the defective product; to research appropriate medical intervention; and to gather and pool information relating to the diagnosis and treatment of injuries to provide funding for future research, diagnosis, medical advice, and treatment;

vii. For costs of suit and attorneys' fees; and

viii. All other relief that plaintiff and class members may be entitled to at equity or at law.

(signatures on next page)

Respectfully submitted,

Date: October 22, 2018

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EXHIBIT A

April 18, 2018

Dr. Scott Gottlieb
Commissioner
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Re: Need for immediate FDA action to protect young people from Juul electronic cigarettes

Dear Dr. Gottlieb:

The Campaign for Tobacco-Free Kids, Truth Initiative, American Academy of Pediatrics, American Cancer Society Cancer Action Network, American Heart Association and American Lung Association urge you to take immediate action to protect the nation's young people, and the public health, from the dramatic rise in teen usage of Juul electronic cigarettes.

According to widespread news reports, Juul electronic cigarettes have skyrocketed in popularity with teens across the United States. Educators and students report an alarming level of Juul use in middle and high schools, making this an urgent public health problem. Recent news coverage includes stories by The New York Times, Wall Street Journal and NBC Today Show.

Several factors have contributed to Juul's rising popularity with teens:

- Juul e-cigarettes are sleek, high tech and easy to hide. They look just like USB flash drives and can be charged in the USB port of a computer. They don't look anything like a traditional tobacco product. A Juul is also small enough to fit in a closed hand.

- Juul comes in sweet flavors that appeal to youth, including mango, fruit medley, crème brulee, cool mint and cool cucumber. The evidence is clear that flavors play a key role in youth use of tobacco products, including e-cigarettes. FDA's own Population Assessment of Tobacco and Health (PATH) study found that 85 percent of current e-cigarette users aged 12-17 had used a flavored product in the past month and 81.5 percent of those young users cited flavors as the reason for their use of the product.

- Juul appears to deliver nicotine more quickly, more effectively and at higher doses than other e-cigarettes, increasing users' risk of addiction. The manufacturer claims each Juul cartridge of nicotine liquid (called a "Juul pod") contains as much nicotine as a pack of cigarettes (about 200 puffs). The manufacturer also claims that Juul "delivers a nicotine experience truly akin to a cigarette, with two times the nicotine strength … of leading competitive products" (April 21, 2015, press release). However, research conducted by Truth Initiative and newly published in *Tobacco Control* found that 63 percent of Juul

users aged 15-24 did not know that the product always contains nicotine. This finding may in part be explained by the fact that the same research also found that a significant portion of those who recognized Juul (25 percent) reported that use of the product is called "juuling," indicating they may not realize it is an e-cigarette or tobacco product.

Juul sales have grown dramatically and now make up more than half the e-cigarette market. A 2018 report by the National Academies of Sciences, Engineering and Medicine, *Public Health Consequences of E-Cigarettes,* concluded that there is "substantial evidence" that e-cigarette use increases the risk of ever using combustible tobacco cigarettes among youth and young adults. Juul is putting kids at risk of nicotine addiction and threatens to undermine decades of progress in reducing youth tobacco use.

The alarming increase in youth use of Juul makes this an urgent public health problem that requires strong and immediate action by the Food and Drug Administration to protect kids. The FDA is responsible for regulating tobacco products, including e-cigarettes, and it is unacceptable that the FDA has yet to take action to address the skyrocketing youth use of Juul.

The FDA should take immediate steps to protect kids including, but not limited to, the following:

- The FDA should immediately order the removal of any Juul flavors, including the highly popular "mango" and "cool cucumber" flavors, which were introduced after August 8, 2016, without first seeking the required FDA authorization. Such flavors violate FDA's Deeming Rule that extended the agency's regulatory authority to additional tobacco products, including e-cigarettes, and prohibits the introduction of new or changed e-cigarettes after the August 8, 2016 effective date of the Rule, without prior FDA review and authorization. According to Juul's own social media posts, the "mango" and "cool cucumber" flavors were not introduced until 2017.

- As Juul's popularity has grown, new products that look and are alleged to perform like Juul have been introduced without first seeking FDA review. FDA should order the removal of these products unless and until they comply with the law by going through FDA review.

- The FDA should suspend internet sales of Juul until adequate rules are established to prevent those sales to kids by requiring effective age verification both at the time of sale and delivery. At the same time, FDA should dramatically step up its enforcement of the ban on underage sales of Juul by brick-and-mortar retailers.

- The FDA should reverse its unlawful 2017 decision that allows e-cigarettes that were already on the market as of August 8, 2016, to stay on the market until at least 2022 without filing applications and undergoing a public health review by the FDA. The rapid growth in Juul's popularity with kids underscores the public health importance of requiring manufacturers of these products to undergo agency review and to demonstrate that the sale of these products is appropriate for the protection of public health, including specifying the safeguards being implemented to protect kids. The FDA should be reviewing these products and taking action to protect kids now, not waiting until 2022.

- Merchandise with the Juul name and using Juul trademarks, including t-shirts, hoodies and Juul "wraps" or "skins," are being sold on the internet and have helped fuel the brand's popularity with kids. FDA rules prohibit cigarette brand names from being used on other products because of the impact on kids. FDA should apply the same rule to Juul.

The rapid growth in Juul use by high school students demonstrates that the FDA and Juul's manufacturer must do more to prevent the marketing and sale of the product to kids and ensure it is marketed and sold responsibly, consistent with the company's own stated mission of providing "an alternative to smoking" for adults. If Juul fails to take the steps necessary to curtail youth use before the start of the next school year in fall 2018, the FDA should take strong, additional enforcement action, up to and including suspension of Juul sales until it does so.

Thank you for your attention to this urgent threat to public health.

Campaign for Tobacco-Free Kids

Truth Initiative

American Academy of Pediatrics

American Cancer Society Cancer Action Network

American Heart Association

American Lung Association

EXHIBIT B

# B

U.S. FOOD & DRUG ADMINISTRATION

U.S. Food & Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
www.fda.gov

**April 24, 2018**

Submission Tracking Number (STN): RD0000476

JUUL Labs, Inc.
660 Alabama Street
2nd Floor
San Francisco, CA 94110

Dear Mr. Ziad Rouag:

Under Section 904(b) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), FDA is requesting that JUUL Labs, Inc. (JUUL) submit documents relating to marketing practices and research on marketing, effects of product design, public health impact, and adverse experiences and complaints related to JUUL products. This request applies to research relating to all such tobacco products and their components or parts, including those products for research, investigational use, developmental studies, test marketing, and/or commercial marketing.

FDA is requesting these documents based on growing concern about the popularity of JUUL products among youth. JUUL product use appears to be common in middle and high schools based on widespread media reporting describing a rapid growth of use among youth in general and on school property,[1] numerous complaints that have been received by CTP, small research studies that have

[1] *NY Times*, April, 2018: https://www.nytimes.com/2018/04/02/health/vaping-ecigarettes-addiction-teen.html; *Vogue*, April 2, 2018: https://www.vogue.com/article/vaping-health-risks-e-cigarettes-teenagers-addiction-toxic-metal-heat-lungs-heart-attack-juul; *Chicago Tribune*, February 2018: http://www.chicagotribune.com/news/ct-met-juul-ecigarettes-at-schools-20180209-story.html; *Buzzfeed*, February 2018: https://www.buzzfeed.com/carolinekee/juul-ecigarette-vape-health-effects?utm_term=.thP0N67x7p#.pkN2Ee0q0l; *Business Insider*, March 2018: http://www.businessinsider.com/juul-e-cig-vaping-health-effects-2018-3; *Sioux Falls (SD) Argus Leader*, April 2018: https://www.argusleader.com/story/news/2018/04/17/concerns-grow-more-kids-caught-vaping-juuling-s-d-schools/523447002/; North New Jersey, January 2018: https://www.northjersey.com/story/news/passaic/wanaque/2018/01/16/students-vaping-epidemic-schools/1006178001/; *US News*, March 2018: https://health.usnews.com/wellness/health-buzz/articles/2018-03-15/kids-are-trying-juul-e-cigarettes-and-experts-are-concerned; *Pittsburgh Post-Gazette*, December 2017: http://www.post-gazette.com/local/region/2017/12/12/JUUL-vaporizer-nicotine-flash-drive-small-concealable-e-cigarette/stories/201712120151; *MSN*, April 2018: https://www.msn.com/en-us/health/watch/%E2%80%98juuling%E2%80%99-is-%E2%80%98not-safe%E2%80%99-medical-expert-warns-on-megyn-kelly-today/vp-AAvnnc1; *NPR*, December 2017: https://www.npr.org/sections/health-shots/2017/12/04/568273801/teenagers-embrace-juul-saying-its-discreet-enough-to-vape-in-class

raised concerns,[2,3] and social media evidence of youth use.[4,5] Widespread reports of youth use of JUUL products are of great public health concern and no child or teenager should ever use any tobacco product. Nicotine affects the developing brain[6] and youth may not understand the nicotine or other characteristics of JUUL.[3,7] JUUL products may have features that make them more appealing to kids and easier to use, thus causing increased initiation and/or use among youth. Similar to other electronic nicotine delivery system (ENDS) products, JUUL product use during adolescence may lead to cigarette smoking or use of other tobacco products in the future.[8] Their appeal may be related to different aspects of the product, including the product design, promotion, or distribution, and CTP seeks information to further understand the appeal and use.

**I. Submission Content**

**A. Submission of Documents Pursuant to a Section 904(b) Request**

In accordance with section 904(b) of the FD&C Act, FDA requests that you submit all documents (including underlying scientific and financial information, as specified below) relating to marketing practices and research activities and research findings, conducted, supported, or possessed by you or your agents relating to a specified set of topics, as set forth below. The request includes but is not limited to documents relating to research findings and activities, if any, that you possess as the result of acquiring or merging with, or obtaining the services or products of another company. For purposes of this request, "research" may include, but is not limited to focus groups, surveys, experimental clinical studies, toxicological and biochemical assays, *in vivo* and *in vitro* assays including animal testing, laboratory formulation and processing testing, taste panels, and assessments of the effectiveness of product marketing practices. The request applies to research relating to any and all ENDS products including the components or parts of such products, including but not limited to products for research, investigational use, developmental studies, test marketing, and/or commercial marketing.

For products not manufactured in the United States, the request applies to the extent you have imported such products into the United States. An importer of a tobacco product not manufactured in the United States is required to supply the information required of the manufacturer of that product.

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[2] Jackson A, Kong G, Camenga D et al. (March 2018). *High School Adolescents Use Several Types of E-cigarette Devices.* Poster session presented at Society for Research on Nicotine and Tobacco, Baltimore, MD.

[3] Willlet JG, Bennett M, Hair, EC, et al. *Recognition, use and perceptions of JUUL among youth and young adults.* Tobacco Control. Epub ahead of print: April 17, 2018. doi: 10.1136/tobaccocontrol-2018-054273.

[4] Kavuluru R, Han S, Hahn EJ. *On the popularity of the USB flash drive-shaped electronic cigarette Juul.* Tobacco Control Epub ahead of print: April 17, 2018. doi:10.1136/tobaccocontrol-2018-054259.

[5] Brett E, Hebert E, Stevens E, et al. (March 2018). *An Analysis of JUUL discussions on social Media: Using Reddit to understand patterns of use and perceptions of JUUL.* Poster session presented at Society for Research on Nicotine and Tobacco, Baltimore, MD.

[6] U.S. Department of Health and Human Services. *Preventing Tobacco Use Among Youth and Young Adults: A Report of the Surgeon General.* Atlanta, GA: U.S. Department of Health and Human. Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention.

[7] https://www.marketwatch.com/story/many-young-people-are-missing-something-important-about-popular-e-cigarette-juul-2018-04-18

[8] National Academies of Science, Engineering, and Medicine. 2018. *Public Health Consequences of E-cigarettes.* Washington, DC: The National Academies Press. https://doi.org/10.17226/24952.

***1. Topics***

Pursuant to section 904(b), FDA requests all documents (including underlying scientific and financial information, as specified below) relating to research activities and findings (including marketing research) and marketing practices, developed for JUUL subject to the limitations in I.A.2 of this letter, on *all* of the following topics:

1. Product marketing— documents (including underlying scientific or financial information) related to marketing research or marketing practices and the effectiveness of such practices. Potential relevant areas of research or marketing practices include:
    - Target consumer groups, including direct (e.g., smokers, ENDS users) and indirect (e.g., youth)
    - Consumer perception studies/market testing
    - Marketing themes and content, including depictions of young people and how ENDS are characterized
    - Means of advertisement and promotion, such as:
        - General marketing strategies (e.g., social media)
        - Price promotions, promotional games and contests
        - Retailer agreements/incentives or partnerships with media and publishing organizations
        - Any other consumer or business-to-business advertising or promotion strategies not listed above
        - Educational materials or products for schools to limit youth use
    - Means of product distribution as it might relate to youth exposure to marketing or youth access to JUUL products
    - Information about how youth are accessing JUUL and information about how the company plans to prevent youth from gaining access to JUUL

2. Product design— documents (including underlying scientific information) related to research on the health, toxicological, behavioral, and physiologic effects, including appeal or addictive potential for youth, as it relates to product design, including the following:
    - Product shape or form (e.g., similarity in appearance to USB stick)
    - Nicotine formulation, (e.g., nicotine salt formula) and nicotine concentration/content
    - Flavors
    - Product features such as: appearance, or lack thereof, of plume; safety features/prevention of misuse; USB port rechargeability

3. Public health impacts involving youth—documents (including underlying scientific information, such as survey information) related to research on the health, toxicological, behavioral, or physiologic effects of JUUL products on youth, including, but not limited to:
    - Awareness, susceptibility, intentions to use, and use patterns (e.g., frequency of use, dual use with other tobacco products; pharmacokinetics and topography)
    - Perceptions of risk, harm, and addictiveness compared to other ENDS products, other tobacco products, and in general
    - Appeal, liking, product satisfaction

- Health impacts of short-term and long-term use

4. Adverse experiences and complaints involving youth—documents (including underlying scientific information) related to research on health, toxicological, behavioral, or physiologic effects described in adverse experience reports or consumer complaints related to youth use associated with JUUL products, including:
   - Reports of youth use and uptake
   - Reports of addiction or withdrawal
   - Reports of acute hazards or risk of injury

***2. Limitations — types of documents and information***

With respect to the topics listed above, FDA requests *all* of the following documents and information:

- Study proposals, original implemented protocols (including all amendments), analysis plans, agreements, notebooks, data collection tools, including, but not limited to, forms and assessment scales for planned, ongoing, or completed studies, surveys, and other research, whether for external release or internal use
- Final data analyses and reports regarding studies, surveys, data compilations, or other research, whether for external or internal use (if there were no final analyses, interim data analyses should be submitted)
- Posters and/or presentations exhibited or to be exhibited at external meetings or conferences if the underlying data has not been presented in other documents and information within this request
- Manuscripts, articles, editorials, and letters that have been submitted for publication but not yet published (e.g., in review, accepted, rejected)
- Underlying data (e.g., in the form of spreadsheets, datasets, charts, tables, and diagrams) analyzed to produce any of the data analyses, reports, posters, manuscripts, or articles requested above

With respect to documents, FDA requests only the final version, or in the absence of a final version, the most recent draft of each document. Please do not submit (a) past iterations of a completed or more recent document, (b) document duplicates, or (c) near duplicates that only vary in minor ways (e.g., differences in addressee or changes in letterhead). FDA does not request published (publicly available) press releases, abstracts, editorials, letters, manuscripts, material safety data sheets (MSDS), and HHS correspondences; if you seek to voluntarily submit such information, we request a list of such publications be provided as a separate appendix only, in lieu of submitting such publications. Electronic mail should be in portable document format (.pdf) and responsive to the above topic areas. Transmittal email should not be included. Submitted documents should not be redacted.

Included within the request are supporting summary reports and the underlying data that support those reports. FDA asks that spreadsheets or datasets be submitted both in pdf and in a file type and structured format that allows for meaningful review and analysis of the data (e.g., Excel (.xls), comma separated values (.csv), or SAS transport (.xpt). Where relevant, data submissions should be accompanied by the name and version of software used to create the file, names and definitions of variables, and copies of programs and macros needed to generate

your analyses. Your submission should include any data analyses that stratify scientific results by one or more of the following: gender, race/ethnicity, age, health condition, or other similar factors.

As an option, information responsive to this 904(b) request that has been previously provided to FDA under section 904 the FD&C Act does not have to be re-submitted as long as the document is fully referenced in the metadata load file.

***3. Date for submission of documents***

All information for this request is to be received by FDA no later than June 19, 2018. **If you do not have any documents responsive to this request, inform FDA of this in writing by June 19, 2018.** If you anticipate difficulties with this document production, please contact FDA within 30 days of this letter so that we may assist you in resolving any technical difficulties you may have and facilitate compliance with the above time line.

***Failure to provide information requested by FDA in accordance with section 904(b) of the FD&C Act is a violation of the FD&C Act and subject to regulatory and enforcement action by FDA.***

**B. Submission of Additional Information**

To provide context and background for the 904(b) requests in section I.A of this letter, FDA also asks that you voluntarily submit a summary (one to five pages in length) for each of the topics in section I.A that includes the number and type of documents included, and a high-level overview of the content

**II. Submission Instructions**

Consistent with applicable statutes and regulations, the confidentiality of trade secret and confidential commercial information submitted to FDA pursuant to this request will be preserved.

Please see the enclosed document for guidance in preparing your submission to FDA.

Clearly identify the manufacturer's or importer's name and address, include the label "**FDA 04-2018 JUUL Request for RD0000476**", and submitted electronically via the CTP Portal[9] using eSubmitter[10].

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[9] http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/Manufacturing/ucm515047.htm. FDA's Electronic Submission Gateway (ESG) is still available as an alternative to the CTP Portal.

[10] http://www.fda.gov/ForIndustry/FDAeSubmitter

Alternatively, CD-ROM, DVD, or hard drive submissions may be mailed to:

Food and Drug Administration
Center for Tobacco Products
Document Control Center (DCC)
Building 71, Room G335
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

The CTP Portal and FDA Electronic Submission Gateway (ESG) are both generally available 24 hours a day, seven days a week. Submissions delivered to DCC by couriers or physical mail will be considered timely if received during delivery hours on or before the due date[11]; if the due date falls on a weekend or holiday the delivery must be received on the prior business day. We are unable to accept regulatory submissions by e-mail.

If you have questions regarding this document request, please contact Jaime Golwalla, Regulatory Health Project Manager, at 301-796-2878.

Sincerely,

Digitally signed by Matthew R. Holman -S
Date: 2018.04.24 08:15:33 -04'00'

Matthew R. Holman, Ph.D.
Director
Office of Science
Center for Tobacco Products

Enclosure

[11] http://www.fda.gov/TobaccoProducts/AboutCTP/ContactUs/default.htm

**Enclosure: Submission Information**

**A. General Instructions**

We request that you submit documents and related material on a CD-ROM, DVD, or hard drive. Documents should be in text-searchable PDF file(s) per FDA guidance on electronic submissions, the FDA eSubmitter User Manual, and the National Archives and Records Administration (NARA) Technical Guidelines for Digitizing Archival Materials for Electronic Access, for document preservation of content and format. The files should include a signed cover letter prominently identified as "**FDA 04-2018 JUUL Request for RD0000476**," and should also identify the software (name, version, and company) that you used to confirm the submission is free of viruses or other malware. The cover letter should include the number of documents you are submitting for each of the topics. The electronic media should be labeled with your company name, a contact phone number, "FDA 04-2018 JUUL Request for RD0000476," submission date, and series number (e.g., "disc 1 of 2").

In order for FDA to accept, access, review, and archive the documents, all documents are to be submitted in their native color and files, including compressed files and archives, cannot be password protected. File formats that should be avoided are proprietary, requiring specialized software to read, and active content that can contain macros or change the content upon opening the file. Ensure all documents are text-searchable and restriction settings under Document Properties are set to "allowed". If you submit PDF files, they should not contain any attached, embedded, or bundled files. If any documents are scanned, you should verify the accuracy of optical character recognition and legibility of the document. In addition, multi-page documents should be properly unitized, instead of several single-page files.

**B. Instructions for Information Submitted Under Section I.A**

To ensure accessibility of your documents and facilitate more fluent and efficient communication between you and FDA regarding your submissions, FDA recommends that you take the following steps:

- Uniquely number all pages of your submission, a process commonly referred to in the litigation context as Bates numbering
- Translate all foreign language documents into English
- Create and submit a glossary or explanation of any abbreviations, jargon, or internal names (e.g., code names)

To provide context and background for each document, FDA recommends inclusion of a load file containing the following metadata for each document:

- Manufacturer filing the document
- Filename
- Document date
- Document author(s)
- Document recipient(s)
- Document custodian
- Document title or identification number
- Beginning and ending Bates numbers
- Bates number ranges for other documents physically or digitally attached to the document

- OCR text (for scanned paper documents)
- Identification of each document as one of the following document types: Email, Briefing Slides, Publication, Memo, Report, Meeting minutes, Proposal, Study design, Other;
- Topic(s) (i.e., the topic or topics listed in Section I.A.1 of the attached letter to which the document relates)
- Product name(s) (e.g., brand or sub-brand, or a unique, consistent identifying name for any tobacco product in research or development)
- Product identification number
- Identify the presence of each document in the University of California San Francisco Truth Tobacco Industry Documents Library[12] (formerly Legacy Tobacco Documents Library) as one of the following: present with the Bates number (begin Bates number to end Bates number), not present, or unknown
- For information previously provided to FDA:
    - Date of previous FDA submission
    - Regulatory section under which the document was submitted
    - File name
    - File extension
    - Bates number (begin Bates number to end Bates number)
    - Relevant page numbers

FDA requests that load files containing metadata be submitted in a comma delimited ASCII text or spreadsheet format and be organized so that data fields will appear in the same order as they appear here (i.e., "Manufacturer filing the document" should be the first field, and "Relevant page numbers" should be the last field). Metadata load file delimiters should be as follows:

**Metadata Load File Delimiters**

| | |
|---|---|
| Field separator: | **Vertical Pipe (ASCII 124)** |
| Field encapsulate: | **Carat (ASCII 094)** |
| Return value in data: | **Tilde (ASCII 126)** |
| Multi-value field: | **Semi Colon (ASCII 059)** |
| Dates format: | **MM/DD/YYYY** |

Hard Returns should appear only at the end of each record.

If you scan paper documents for digital production, please use optical character recognition software (OCR) technology to render the images as functional text against the resulting PDF. Any extracted searchable text should be produced with the document as metadata.

The instructions in this enclosure are based on communications that FDA has received from industry and our evaluation of submissions received under the FD&C Act to date. If you have questions about how to prepare your submission, please contact us.

[12] If a responsive document is present in the University of California San Francisco Truth Tobacco Industry Documents library, that does not preclude it from this request.

EXHIBIT C

C

Centers for Disease Control and Prevention
MMWR
Morbidity and Mortality Weekly Report
Weekly / Vol. 66 / No. 23
June 16, 2017

# Tobacco Use Among Middle and High School Students — United States, 2011–2016

Ahmed Jamal, MBBS[1]; Andrea Gentzke, PhD[1]; S. Sean Hu, MD[1]; Karen A. Cullen, PhD[2]; Benjamin J. Apelberg, PhD[2]; David M. Homa, PhD[1]; Brian A. King, PhD[1]

Tobacco use is the leading cause of preventable disease and death in the United States; nearly all tobacco use begins during youth and young adulthood (*1*,*2*). Among youths, use of tobacco products in any form is unsafe (*1*,*3*). CDC and the Food and Drug Administration (FDA) analyzed data from the 2011–2016 National Youth Tobacco Surveys (NYTS) to determine recent patterns of current (past 30-day) use of seven tobacco product types among U.S. middle (grades 6–8) and high (grades 9–12) school students. In 2016, 20.2% of surveyed high school students and 7.2% of middle school students reported current tobacco product use. In 2016, among current tobacco product users, 47.2% of high school students and 42.4% of middle school students used ≥2 tobacco products, and electronic cigarettes (e-cigarettes) were the most commonly used tobacco product among high (11.3%) and middle (4.3%) school students. Current use of any tobacco product did not change significantly during 2011–2016 among high or middle school students, although combustible tobacco product use declined. However, during 2015–2016, among high school students, decreases were observed in current use of any tobacco product, any combustible product, ≥2 tobacco products, e-cigarettes, and hookahs. Among middle school students, current use of e-cigarettes decreased. Comprehensive and sustained strategies can help prevent and reduce the use of all forms of tobacco products among U.S. youths (*1*–*3*).

NYTS is a cross-sectional, voluntary, school-based, self-administered, pencil-and-paper questionnaire administered to U.S. middle and high school students. A three-stage cluster sampling procedure was used to generate a nationally representative sample of U.S. students attending public and private schools in grades 6–12. This report uses data from six NYTS waves (2011–2016). Sample sizes and response rates for 2011, 2012, 2013, 2014, 2015, and 2016 were 18,866 (72.7%), 24,658 (73.6%), 18,406 (67.8%), 22,007 (73.3%), 17,711 (63.4%), and 20,675 (71.6%), respectively.

INSIDE



**Continuing Education** examination available at https://www.cdc.gov/mmwr/cme/conted_info.html#weekly.

**U.S. Department of Health and Human Services**
Centers for Disease Control and Prevention

Participants were asked about current use of cigarettes, cigars, smokeless tobacco,* e-cigarettes,† hookahs (water pipes used to smoke tobacco),§ pipe tobacco,¶ and bidis (small imported cigarettes wrapped in a leaf). Current use for each product was defined as use on ≥1 day during the past 30 days. "Any tobacco product use" was defined as current use of one or more tobacco products, and "≥2 tobacco product use" was defined as current use of two or more tobacco products.** "Any combustible tobacco product use" was defined as current use of cigarettes, cigars, hookahs, pipe tobacco, and/or bidis.

Data were weighted to account for the complex survey design and adjusted for nonresponse; national prevalence estimates, 95% confidence intervals, and population estimates were computed and rounded down to the nearest 10,000. Current use estimates for 2016 are presented for any tobacco product, any combustible tobacco product, ≥2 tobacco products, and each tobacco product individually, by selected demographics for each school type (high school and middle school). Results were assessed for the presence of linear and quadratic trends during 2011–2016, adjusting for race/ethnicity, sex, and school

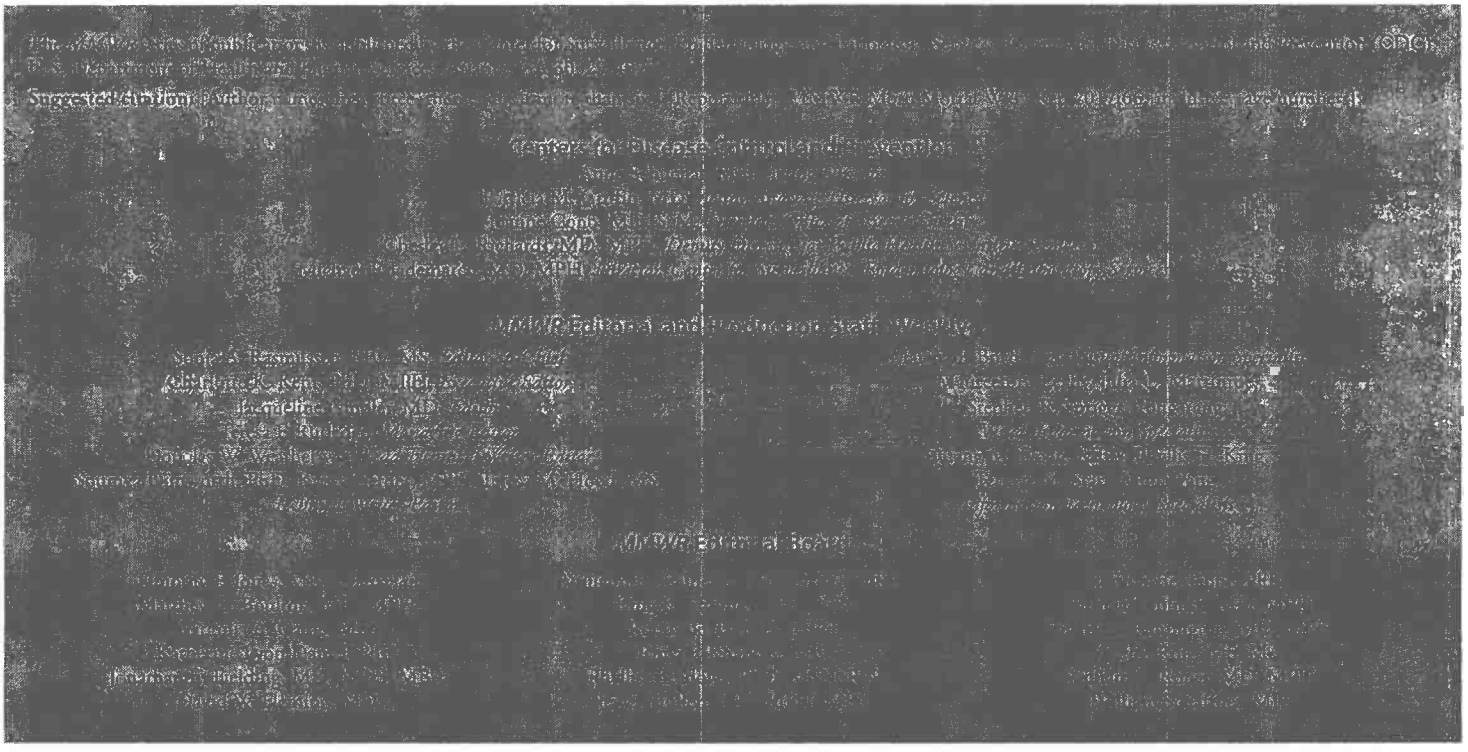
* Beginning in 2015, the definition of smokeless tobacco included chewing tobacco/snuff/dip, snus, and dissolvable tobacco because of limited sample sizes for individual products (snus, dissolvable). In figures 1 and 2, this definition was applied across all years (2011–2016) for comparability purposes. The definition of smokeless tobacco in previously published reports (NYTS 2014 and earlier) included only chewing tobacco/snuff/dip, whereas snus and dissolvable tobacco were reported as separate products.

† In 2015 and 2016, current use of e-cigarettes was assessed by the question "During the past 30 days, on how many days did you use electronic cigarettes or e-cigarettes?" E-cigarette questions were preceded by an introductory paragraph. In 2016, this paragraph read: "The next thirteen questions are about electronic cigarettes or e-cigarettes. E-cigarettes are battery-powered devices that usually contain a nicotine-based liquid that is vaporized and inhaled. You may also know them as vape-pens, hookah-pens, e-hookahs, e-cigars, e-pipes, personal vaporizers or mods. Some brand examples are NJOY, Blu, Vuse, MarkTen, Logic, Vapin Plus, eGo, Halo." A similar introductory paragraph preceded e-cigarette questions in 2015. In 2014, current use of e-cigarettes was assessed by the question "During the past 30 days, on how many days did you use e-cigarettes such as Blu, 21st Century Smoke, or NJOY?"; and in 2011 to 2013, e-cigarette use was assessed by the question "In the past 30 days, which of the following products have you used on at least one day?," and the response option for e-cigarettes was "Electronic cigarettes or e-cigarettes such as Ruyan or NJOY."

§ In 2016, current use of hookahs was assessed by the question "In the past 30 days, on how many days did you smoke tobacco in a hookah or waterpipe? Hookah questions were preceded by an introductory statement: "The next eight questions are about smoking tobacco in a hookah, which is a type of waterpipe. Shisha (or hookah tobacco) is smoked in a hookah." From 2011–2015, current hookah use was assessed by the question "In the past 30 days, which of the following products have you used on at least one day?" Hookah was the fourth response option in 2015, the first response option in 2014, and was the fourth or fifth response option from 2011 to 2013.

¶ From 2014 to 2016, current use of tobacco pipes was assessed by the question "In the past 30 days, which of the following products have you used on at least one day?" and the response option for pipe tobacco was "Pipe filled with tobacco (not waterpipe)." Pipe tobacco was the second response option available in 2016, the fifth option in 2015, and the second option available in 2014. From 2011 to 2013, tobacco pipe use was assessed by the question "During the past 30 days, on how many days did you smoke tobacco in a pipe?"

** In 2015 and 2016, the definition of ≥2 tobacco product–use includes the updated definition of smokeless tobacco, thereby analyzing chewing tobacco/snuff/dip, snus, and dissolvable tobacco as a single tobacco product type compared with previously published NYTS reports, which analyzed chewing tobacco/snuff/dip, snus, and dissolvable tobacco as separate products.



grade.[††] T-tests were performed to examine differences between findings in 2015 and 2016. For all analyses, p-values <0.05 were considered statistically significant.

[††] A test for linear trend is significant if an overall statistically significant decrease or increase occurs during the study period. Data were also assessed for the presence of quadratic trends; a significant quadratic trend indicates that the rate of change accelerated or decelerated across the study period. Trends were only assessed when statistically stable data were available for all 6 years. A significant positive linear trend and nonsignificant quadratic trend signifies the presence of a linear increase; a significant negative linear trend and nonsignificant quadratic trends signifies the presence of a linear decrease; a significant positive linear trend and significant positive or negative quadratic trend signifies the presence of a nonlinear increase; a significant negative linear trend and significant positive or negative quadratic trend signifies the presence of a nonlinear decrease; a nonsignificant linear trend and significant positive or negative quadratic trend signifies the presence of a nonlinear change.

In 2016, 20.2% of high school students (estimated 3.05 million) reported current use of any tobacco product, including 9.6% (1.44 million; 47.2% of current tobacco product users) who reported current use of ≥2 tobacco products. Among high school students, e-cigarettes were the most commonly used tobacco product (11.3% of current users), followed by cigarettes (8.0%), cigars (7.7%), smokeless tobacco (5.8%), hookahs (4.8%), pipe tobacco (1.4%), and bidis (0.5%) (Table). Males reported higher use of any tobacco product, ≥2 tobacco products, cigars, smokeless tobacco, and pipe tobacco than did females. E-cigarettes were the most commonly used tobacco product among non-Hispanic white (13.7%) and Hispanic

**TABLE. Estimated percentage of middle and high school students who used tobacco products in the past 30 days, by product,* school level, sex, and race/ethnicity — National Youth Tobacco Survey, United States, 2016**

| | Sex % (95% CI) | | Race/Ethnicity % (95% CI) | | | | Total | |
|---|---|---|---|---|---|---|---|---|
| Tobacco product | Female | Male | White, non-Hispanic | Black, non-Hispanic | Hispanic | Other, non-Hispanic | % (95% CI) | Estimated no. of users[†] |
| **High school students** | | | | | | | | |
| Electronic cigarettes | 9.5 (7.8–11.5) | 13.1 (11.4–14.9) | 13.7 (11.9–15.7) | 6.2 (4.8–7.9) | 10.3 (8.2–12.8) | 5.4 (3.6–8.0) | 11.3 (9.9–12.9) | 1,680,000 |
| Cigarettes | 6.9 (5.4–8.8) | 9.1 (7.6–11.0) | 9.9 (8.2–11.8) | 3.9 (2.9–5.3) | 6.4 (4.9–8.4) | 4.8 (3.1–7.6) | 8.0 (6.7–9.6) | 1,180,000 |
| Cigars | 5.6 (4.3–7.2) | 9.0 (8.6–11.2) | 7.9 (6.5–9.6) | 9.5 (7.8–11.5) | 7.2 (5.7–9.1) | 3.7 (2.4–5.7) | 7.7 (6.6–8.9) | 1,130,000 |
| Smokeless tobacco | 3.3 (2.4–4.4) | 8.3 (6.8–10.1) | 7.4 (6.0–9.1) | 2.1 (1.5–3.1) | 4.4 (3.4–5.7) | 3.8 (2.1–6.8) | 5.8 (4.8–7.0) | 860,000 |
| Hookah | 5.1 (4.1–6.3) | 4.5 (3.8–5.4) | 4.5 (3.7–5.4) | 4.1 (3.2–5.3) | 6.4 (4.8–8.3) | 3.4 (2.1–5.5) | 4.8 (4.1–5.7) | 700,000 |
| Pipe tobacco | 0.9 (0.7–1.2) | 1.8 (1.5–2.4) | 1.4 (1.1–1.8) | 1.2 (0.7–2.0) | 1.2 (0.9–1.8) | —[§] | 1.4 (1.1–1.7) | 190,000 |
| Bidis | 0.3 (0.2–0.6) | 0.7 (0.5–0.9) | 0.4 (0.2–0.7) | — | 0.6 (0.4–1.1) | — | 0.5 (0.3–0.7) | 70,000 |
| **Any tobacco product[¶]** | **17.0 (14.9–19.3)** | **23.5 (21.3–25.8)** | **23.0 (20.7–25.6)** | **16.4 (14.1–18.9)** | **18.3 (15.8–21.0)** | **11.3 (8.7–14.5)** | **20.2 (18.4–22.3)** | **3,050,000** |
| ≥2 tobacco products** | 7.8 (6.3–9.7) | 11.4 (9.9–13.0) | 11.3 (9.6–13.2) | 6.1 (5.2–7.3) | 8.9 (7.1–11.2) | 5.0 (3.2–7.7) | 9.6 (8.3–11.1) | 1,440,000 |
| Any combustible tobacco product[††] | 12.4 (10.7–14.4) | 15.3 (13.7–17.1) | 15.1 (13.1–17.3) | 12.9 (11.0–15.1) | 12.9 (11.1–14.9) | 8.1 (5.9–11.1) | 13.8 (12.3–15.5) | 2,080,000 |
| **Middle school students** | | | | | | | | |
| Electronic cigarettes | 3.4 (2.7–4.3) | 5.1 (4.2–6.1) | 3.7 (3.0–4.7) | 4.0 (2.6–6.0) | 5.6 (4.3–7.4) | — | 4.3 (3.7–4.9) | 500,000 |
| Cigarettes | 1.8 (1.3–2.5) | 2.5 (1.8–3.4) | 1.9 (1.4–2.6) | — | 2.5 (1.8–3.5) | — | 2.2 (1.7–2.7) | 250,000 |
| Cigars | 1.7 (1.1–2.4) | 2.7 (1.9–3.9) | 1.4 (0.9–2.2) | 4.5 (2.8–7.1) | 2.8 (1.9–4.2) | — | 2.2 (1.7–2.9) | 260,000 |
| Smokeless tobacco | 1.5 (0.9–2.4) | 3.0 (2.2–4.0) | 2.1 (1.5–3.0) | — | 3.0 (2.1–3.4) | — | 2.2 (1.6–3.1) | 260,000 |
| Hookah | 1.9 (1.5–2.5) | 2.1 (1.5–2.9) | 0.9 (0.6–1.4) | 2.8 (1.8–4.4) | 3.7 (3.0–4.7) | — | 2.0 (1.6–2.5) | 230,000 |
| Pipe tobacco | 0.6 (0.3–1.0) | 0.8 (0.5–1.3) | — | — | 1.7 (1.1–2.6) | — | 0.7 (0.5–1.0) | 70,000 |
| Bidis | — | 0.4 (0.2–0.7) | — | — | 0.6 (0.4–1.1) | — | 0.3 (0.2–0.5) | 30,000 |
| **Any tobacco product[¶]** | **5.9 (4.9–7.3)** | **8.3 (6.8–9.9)** | **5.9 (4.7–7.3)** | **7.5 (5.5–10.1)** | **9.5 (7.5–11.8)** | — | **7.2 (6.1–8.4)** | **850,000** |
| ≥2 tobacco products** | 2.5 (1.8–3.4) | 3.6 (2.7–4.7) | 2.3 (1.7–3.0) | 3.0 (2.0–4.3) | 4.5 (3.3–6.1) | — | 3.1 (2.5–3.8) | 360,000 |
| Any combustible tobacco product[††] | 3.9 (3.0–5.0) | 4.6 (3.4–6.2) | 2.9 (2.2–3.7) | 5.8 (4.0–8.3) | 6.1 (4.7–7.9) | — | 4.3 (3.5–5.2) | 510,000 |

**Abbreviation:** CI = confidence interval.

* Past 30-day use of electronic cigarettes was determined by asking, "During the past 30 days, on how many days did you use electronic cigarettes or e-cigarettes?" Past 30-day use of cigarettes was determined by asking, "During the past 30 days, on how many days did you smoke cigarettes?" Past 30-day use of cigars was determined by asking, "During the past 30 days, on how many days did you smoke cigars, cigarillos, or little cigars?" Past 30-day use of hookahs was determined by asking, "During the past 30 days, on how many days did you smoke tobacco in a hookah or waterpipe?" Smokeless tobacco was defined as use of chewing tobacco, snuff, dip, snus, and/or dissolvable tobacco products. Past 30-day use of smokeless tobacco was determined by asking the following question regarding chewing tobacco, snuff, and dip: "During the past 30 days, on how many days did you use chewing tobacco, snuff, or dip?," and the following question for use of snus and dissolvable tobacco products: "In the past 30 days, which of the following products did you use on at least one day: snus, dissolvable tobacco products?" Responses from these questions were combined to derive overall smokeless tobacco use. Past 30-day use of pipe tobacco and bidis were determined by asking, "In the past 30 days, which of the following products have you used on at least one day: pipe filled with tobacco (not waterpipe), bidis (small brown cigarettes wrapped in a leaf)?"

† Estimated total number of users is rounded down to the nearest 10,000 persons.

§ Data are statistically unreliable because samples size was <50 or relative standard error was >0.3.

¶ Any tobacco product use is defined as use of any tobacco product (electronic cigarettes, cigarettes, cigars, smokeless tobacco, hookahs, pipe tobacco, and/or bidis) on at least one day in the past 30 days.

** ≥2 tobacco product use is defined as use of two or more tobacco products (electronic cigarettes, cigarettes, cigars, smokeless tobacco, hookahs, pipe tobacco, and/or bidis) on at least one day in the past 30 days.

†† Any combustible tobacco use defined as use of cigarettes, cigars, hookahs, pipe tobacco, and/or bidis on at least one day in the past 30 days.

(10.3%) high school students, whereas cigars were the most commonly used tobacco product among non-Hispanic black high school students (9.5%).

Among middle school students, 7.2% (0.85 million) reported current use of any tobacco product, and 3.1% (0.36 million; 42.4% of current tobacco users) reported current use of ≥2 tobacco products (Table). Among middle school students, e-cigarettes were the most commonly used tobacco product (4.3%), followed by cigarettes (2.2%), cigars (2.2%), smokeless tobacco (2.2%), hookahs (2.0%), pipe tobacco (0.7%), and bidis (0.3%). Among males, current use of any tobacco product was 8.3%, and among females, was 5.9%. Hispanics reported higher use of any tobacco product, use of ≥2 tobacco products, and use of hookahs than did non-Hispanic whites (Table).

Among all high school students, current use of any tobacco product did not change significantly from 2011 (24.2%) to 2016 (20.2%); however, a nonlinear decrease occurred in current use of any combustible tobacco product (21.8% to 13.8%), and ≥2 tobacco products (12.0% to 9.6%) during this time (Figure 1). By product type, nonlinear increases occurred for current use of e-cigarettes (1.5% to 11.3%) and hookahs (4.1% to 4.8%) (p for trend <0.05); however, a linear decrease occurred in current use of cigarettes (15.8% to 8.0%), cigars (11.6% to 7.7%), and smokeless tobacco (7.9% to 5.8%), and a nonlinear decrease occurred in current use of pipe tobacco (4.0% to 1.4%) and bidis (2.0% to 0.5%) (p<0.05 for trend) (Figure 1). During 2011–2016, among middle school students, a linear decrease occurred in current use of any combustible tobacco products (6.4% to 4.3%), cigarettes (4.3% to 2.2%), cigars (3.5% to 2.2%), and pipe tobacco (2.2% to 0.7%) (p for trend <0.05), whereas no significant linear or quadratic trends were observed for current use of any tobacco product or ≥2 tobacco products (Figure 2). A nonlinear increase occurred in current use of e-cigarettes (0.6% to 4.3%), and a linear increase occurred for current use of hookahs (1.0% to 2.0%) (p for trend <0.05).

During 2015–2016, among high school students, decreases occurred in the use of any tobacco product (25.3% to 20.2%), any combustible tobacco product (17.2% to 13.8%), ≥2 tobacco products (13.0% to 9.6%), e-cigarettes (16.0% to 11.3%), and hookahs (7.2% to 4.8%) (p<0.05). Among middle school students, e-cigarette use decreased from 5.3% in 2015 to 4.3% in 2016 (p<0.05). Among middle and high school students, use of other tobacco products, including cigarettes, cigars, smokeless tobacco, pipe, and bidis, did not change significantly during 2015–2016.

## Discussion

During 2015–2016, the use of any tobacco product, any combustible tobacco product, ≥2 tobacco products, e-cigarettes, and hookahs declined among high school students, and e-cigarette use declined among middle school students. This is in contrast to prior recent years, when declines in the reported use of cigarettes and cigars occurred alongside increases in the use of other tobacco products, including e-cigarettes and hookahs, resulting in no change in the use of any tobacco product during 2011–2016. In 2016, an estimated 3.9 million U.S. middle and high school students currently used any tobacco product, with 1.8 million reporting current use of ≥2 tobacco products. Among youths, symptoms of nicotine dependence are increased in multiple tobacco product–users compared with single product–users (*4*).

Tobacco prevention and control strategies at the national, state, and local levels likely have contributed to the reduction in use of certain tobacco products, including e-cigarettes, among youths in recent years (*2*). Efforts to address youths' use of tobacco products include youth access restrictions, smoke-free policies that include e-cigarettes, and media campaigns warning about the risks of youth tobacco product use. For example, since February 2014, FDA's first national tobacco public education campaign, The Real Cost, has broadcasted tobacco education advertising designed for youths aged 12–17 years; the campaign was associated with an estimated 348,398 U.S. youths who did not initiate cigarette smoking during

**Summary**

**What is already known about this topic?**

Tobacco use is the leading cause of preventable disease and death in the United States, and nearly all tobacco use begins during youth and young adulthood. Among youths, use of tobacco products in any form is unsafe.

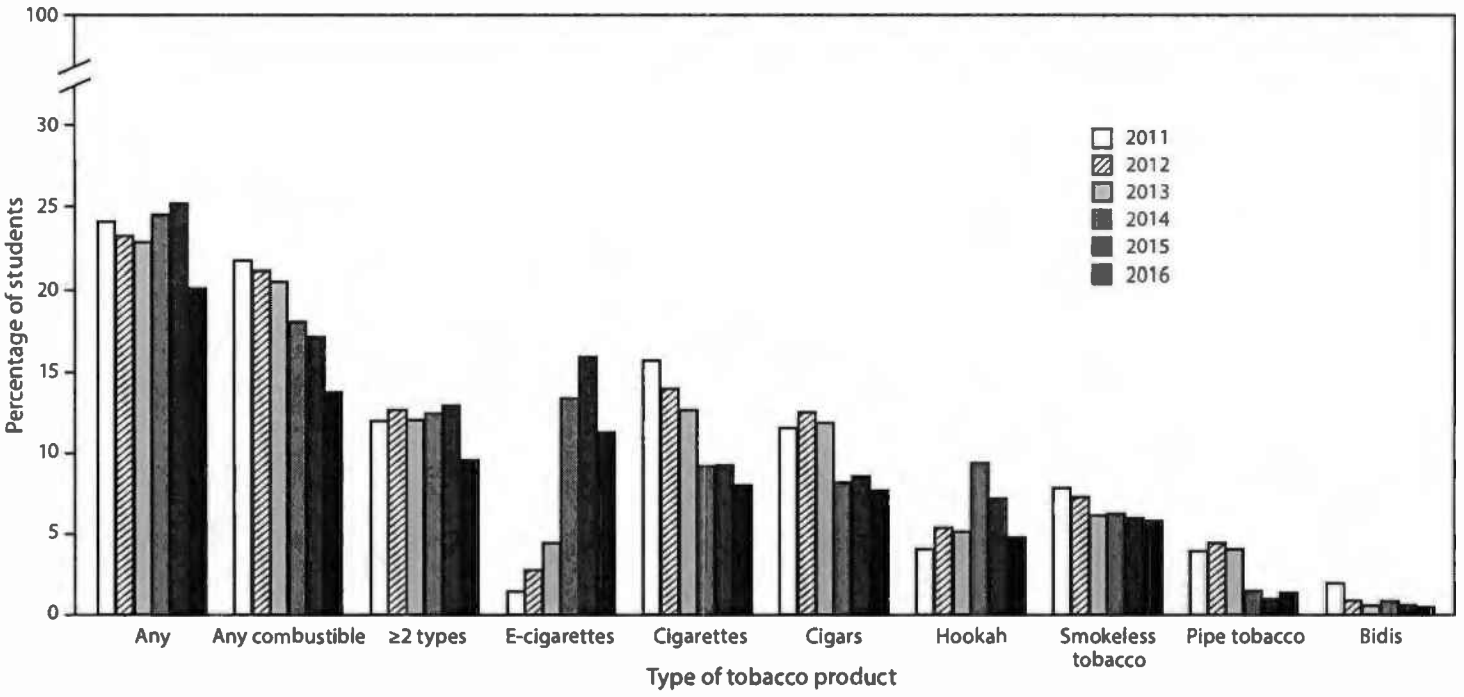
**What is added by this report?**

In 2016, one in five high school students and one in 14 middle school students reported current use of a tobacco product on ≥1 of the past 30 days (3.9 million tobacco users). Moreover, 47.2% of high school students and 42.4% of middle school students who used a tobacco product in the past 30 days used ≥2 tobacco products. During 2015–2016, current use of electronic cigarettes (e-cigarettes) decreased among middle school students, and decreases in current use of any tobacco product, any combustible tobacco product, ≥2 tobacco products, e-cigarettes, and hookahs occurred among high school students. However, decreases in cigarette and cigar use during 2011–2016 were offset by increases in hookah and e-cigarette use, resulting in no significant change in any tobacco use. In 2016, e-cigarettes remained the most commonly used tobacco product among high (11.3%) and middle (4.3%) school students.

**What are the implications for public health practice?**

Sustained efforts to implement proven tobacco control strategies focusing on all types of tobacco products are critical to reduce tobacco product use among U.S. youths.

**FIGURE 1. Estimated percentage of high school students who currently use any tobacco products,* any combustible tobacco products,† ≥2 tobacco products,§ and selected tobacco products — National Youth Tobacco Survey, United States, 2011–2016¶,**,††**



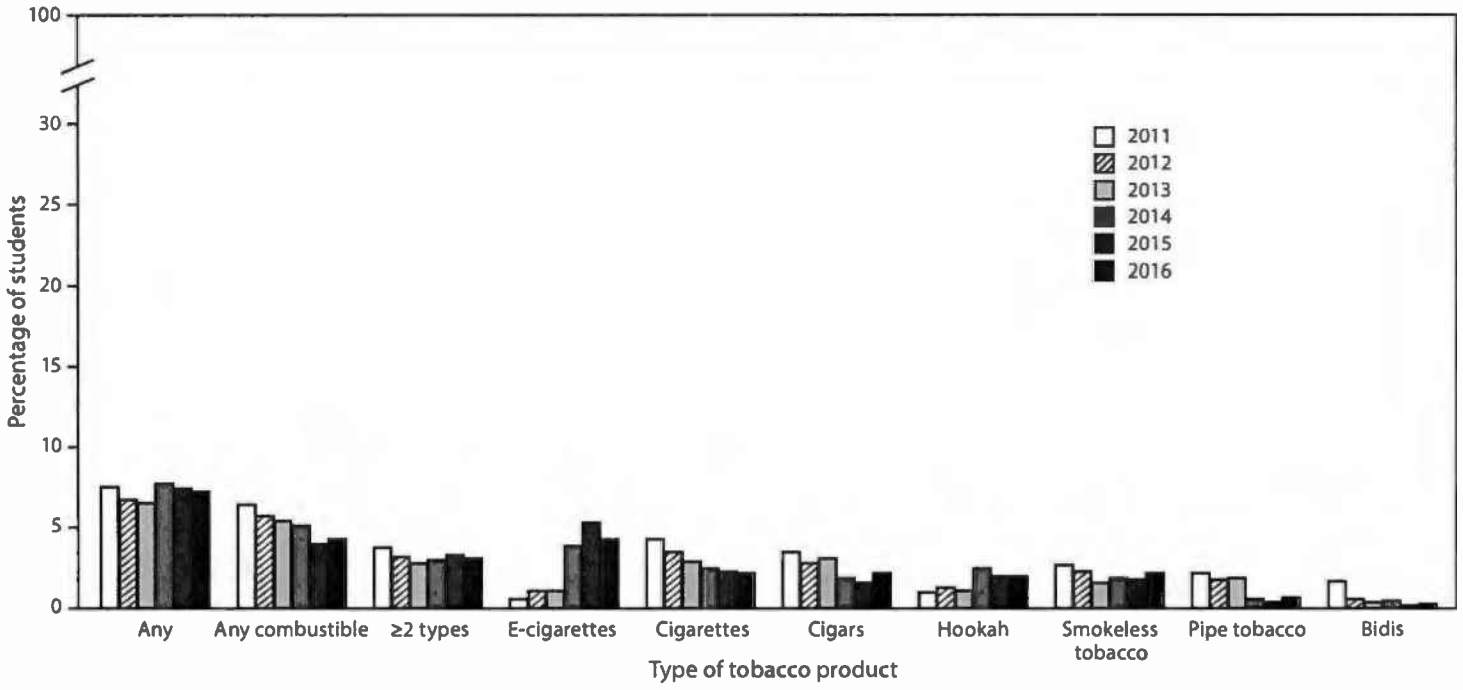

* Any tobacco product use is defined as past 30-day use of electronic cigarettes, cigarettes, cigars, hookahs, smokeless tobacco, pipe tobacco and/or bidis.
† Any combustible tobacco use is defined as use of cigarettes, cigars, hookahs, pipe tobacco, and/or bidis on at least one day in the past 30 days.
§ ≥2 tobacco product use is defined as past 30-day use of two or more of the following tobacco products: electronic cigarettes, cigarettes, cigars, hookahs, smokeless tobacco, pipe tobacco, and/or bidis.
¶ From 2015 to 2016, a significant decrease in use of any tobacco product, any combustible tobacco product, ≥2 tobacco products, electronic cigarettes, and hookahs was observed ($p<0.05$).
** During 2011–2016, use of electronic cigarettes and hookahs exhibited a nonlinear increase ($p<0.05$). Use of cigarettes, cigars, and smokeless tobacco exhibited a linear decrease ($p<0.05$). Any combustible tobacco use, pipe tobacco, and bidis exhibited a nonlinear decrease ($p<0.05$). There was a nonlinear change during this time in the use of ≥2 types of tobacco products ($p<0.05$). No significant trend in current use of any tobacco product was observed during 2011–2016.
†† Beginning in 2015, the definition of smokeless tobacco included chewing tobacco/snuff/dip, snus, and dissolvable tobacco because of limited sample sizes for individual products; this definition was applied across 2011–2016 for comparability purposes. In previous reports (National Youth Tobacco Survey 2014 and earlier) smokeless tobacco included only chewing tobacco/snuff/dip; snus and dissolvable tobacco were reported as separate products

February 2014–March 2016 (*5*). Continued implementation of these strategies can help prevent and further reduce the use of all forms of tobacco product among U.S. youths (*1–3*).

The findings in this report are subject to at least three limitations. First, NYTS only recruited students from public and private schools; therefore, the findings might not be generalizable to youths who are being home-schooled, have dropped out of school, or are in detention centers. Second, data were self-reported; thus, the findings are subject to recall and response bias. Finally, changes in the wording and placement of survey questions about certain products (e.g., e-cigarettes, hookahs, and pipe tobacco) during 2011–2016 might have had an impact on reported use. Despite these limitations, overall trends are generally similar to those found in other nationally representative surveys (*6,7*).

Sustained efforts to implement proven tobacco control policies and strategies are critical to preventing youth use of all tobacco products. Effective August 8, 2016, FDA finalized its deeming rule, which gave FDA jurisdiction over products made or derived from tobacco, including e-cigarettes, cigars, pipe tobacco, and hookah tobacco (*8*). Regulation of the manufacturing, distribution, and marketing of tobacco products by FDA, coupled with full implementation of comprehensive tobacco control and prevention strategies at CDC-recommended funding levels (*9*), could reduce youth tobacco product initiation and use (*1,2,9*). Strategies to reduce youth tobacco product use include increasing the price of tobacco products, protecting people from secondhand exposure to combustible tobacco smoke and e-cigarette aerosol, implementing advertising and promotion restrictions and national public education media campaigns, and raising the minimum age of purchase for tobacco products to 21 years (*9,10*). Continued monitoring of all forms of youth tobacco product use is critical to determine whether current patterns in use persist over time.

**FIGURE 2. Estimated percentage of middle school students who currently use any tobacco products,* any combustible tobacco product,† ≥2 tobacco products,§ and selected tobacco products — National Youth Tobacco Survey, 2011–2016¶,**,††**




* Any tobacco product use is defined as past 30-day use of electronic cigarettes, cigarettes, cigars, hookahs, smokeless tobacco, pipe tobacco and/or bidis.
† Any combustible tobacco use is defined as use of cigarettes, cigars, hookahs, pipe tobacco, and/or bidis on at least one day in the past 30 days.
§ ≥2 tobacco product use is defined as past 30-day use of two or more of the following tobacco products: electronic cigarettes, cigarettes, cigars, hookahs, smokeless tobacco, pipe tobacco, and/or bidis.
¶ From 2015 to 2016, a significant decrease in use of electronic cigarettes was observed ($p<0.05$).
** During 2011–2016, electronic cigarette use exhibited a nonlinear increase ($p<0.05$). Hookah use exhibited a linear increase ($p<0.05$). Use of any combustible tobacco, cigarettes, cigars, and pipe tobacco exhibited a linear decrease ($p<0.05$). Bidi use exhibited a nonlinear decrease ($p<0.05$). Smokeless tobacco use exhibited a nonlinear change over this time period ($p<0.05$). No change in current use of any product or ≥2 types of products was observed during 2011–2016.
†† Beginning in 2015, the definition of smokeless tobacco included chewing tobacco/snuff/dip, snus, and dissolvable tobacco because of limited sample sizes for individual products; this definition was applied across 2011–2016 for comparability purposes. In previous reports (National Youth Tobacco Survey 2014 and earlier) smokeless tobacco included only chewing tobacco/snuff/dip; snus and dissolvable tobacco were reported as separate products.

## Conflict of Interest

No conflicts of interest were reported.

[1]Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, CDC; [2]Center for Tobacco Products, Food and Drug Administration.

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EXHIBIT D

# D

# Hypersensitivity Pneumonitis and Acute Respiratory Distress Syndrome From E-Cigarette Use

Casey G Sommerfeld, MD, Daniel J Weiner, MD, Andrew Nowalk, MD, PhD, Allyson Larkin, MD

Electronic cigarette (e-cigarette) use, or "vaping," is gaining widespread popularity as an alternative to conventional cigarettes among adolescents. Little is known of the health risks of e-cigarette use, especially in children and adolescents. We present a Case Report of a previously healthy 18-year-old woman who presented with dyspnea, cough, and pleuritic chest pain after e-cigarette use. She developed respiratory failure with hypoxia and was intubated, and ultimately met diagnostic criteria for acute respiratory distress syndrome. Chest tubes were placed to drain worsening pleural effusions. Computed tomography of the chest revealed dependent opacities in both lung bases, superimposed smooth interlobular septal thickening, and pleural effusions. Bronchoalveolar lavage revealed cellular debris and reactive mononuclear cells, and cell counts were remarkable for elevated mononuclear cells and eosinophilia. After the results of a workup for an infectious etiology came back negative, the patient was diagnosed with hypersensitivity pneumonitis and intravenous methylprednisolone therapy was initiated. After this the patient rapidly improved, was weaned off vasopressor support, and was extubated. This is the first reported case of hypersensitivity pneumonitis and acute respiratory distress syndrome as a risk of e-cigarette use in an adolescent, and it should prompt pediatricians to discuss the potential harms of vaping with their patients. Hypersensitivity pneumonitis, lipid pneumonia, and eosinophilic pneumonia should be included in the differential diagnosis of patients who exhibit respiratory symptoms after the use of an e-cigarette.

Tobacco use remains a significant public health issue in pediatric patients. The use of electronic cigarettes (e-cigarettes), or "vaping," is gaining widespread popularity as an alternative to conventional cigarettes. Recent data from the National Youth Tobacco survey revealed a threefold increase in e-cigarette use between the years 2011 and 2013 in adolescents without a previous history of smoking.[1]

Currently there are limited data on the health risks of e-cigarettes in pediatric patients because in previous reports researchers have documented respiratory consequences mostly in the adult population. The youngest patient so far described is a 20-year-old man diagnosed with acute eosinophilic pneumonitis immediately after smoking an e-cigarette. Although he presented with respiratory symptoms, his oxygen saturations remained at 100% on room air and he did not require respiratory support during his admission.[2] In another report of a 60-year-old man with a presumptive diagnosis of acute hypersensitivity pneumonitis, this disorder is connected to the

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Dr Sommerfeld cared for the patient as a resident on the Infectious Disease service, drafted the initial manuscript, and critically reviewed the manuscript; Dr Nowalk was the Infectious Disease attending who was consulted on this patient, and he assisted in the interpretation of data, contributed his expertise toward the patient's care, and critically reviewed and revised the manuscript for important intellectual content; Dr Weiner was the Pulmonology attending who was consulted on this patient, and he assisted in the interpretation of data, contributed his expertise toward the patient's care, and critically reviewed and revised the manuscript for important intellectual content; Dr Larkin was the Allergy and Immunology attending who was consulted on this patient, and she assisted in the interpretation of data, contributed her expertise toward the patient's care, and critically reviewed and revised the manuscript for important intellectual content; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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use of e-cigarettes. This patient required oxygen supplementation for hypoxemia but, again, no further respiratory support.[3] In 2 further reports, researchers describe lipid pneumonia secondary to e-cigarette use, and 1 patient required intubation for acute respiratory distress syndrome.[4,5] There are no case reports in the literature in which researchers describe respiratory failure secondary to hypersensitivity pneumonitis as a consequence of e-cigarette use in the pediatric population.

## CASE REPORT

An 18-year-old woman presented to the emergency department with a chief complaint of 2 days of progressive dyspnea, cough, and pleuritic chest pain. She was afebrile during this time and without any upper respiratory symptoms. Her past medical history was significant for mild intermittent exertional asthma, with only rare use of inhaled albuterol. Recently the patient had a reaction (hives and lip swelling) to a Brazil nut that resolved with diphenhydramine. She had not been evaluated for nut allergies, but she had tolerated other nuts without reaction.

The patient lived in a rural town and had no recent bird or farm animal exposure. She had no recent travel, reverse travel, or close contact with incarcerated individuals. The patient recently started to use e-cigarettes over the last 2 to 3 weeks and had been using them 1 to 2 days before the onset of symptoms. She was employed as a hostess in a local restaurant.

On presentation, the patient's vital signs were as follows: temperature of 36.8°C, heart rate of 130 beats per minute, respiratory rate of 32 breaths per minute, and oxygen saturation of 84% on room air. Her cardiac examination did not reveal any rubs, gallops, or murmurs. Her lung examination was notable for use of accessory muscles and diminished but clear breath sounds bilaterally at the bases. There was no hepatosplenomegaly or digital clubbing.

An initial complete blood cell count revealed an elevated white blood cell count of 35.9 ($\times 10^3$/mL), with 93% neutrophils, 4% bands, 1% lymphocytes, and 2% monocytes. Her hemoglobin level was 13.5 gm/dL, with a platelet count of 309 000/mL. Erythrocyte sedimentation rate was normal, with an elevated C-reactive protein level of 17.4 mg/L. Electrolytes and transaminases were normal. Urinalysis and urine drug screen results were both negative. A chest radiograph revealed patchy bilateral pulmonary infiltrates. Computed tomography (CT) angiography of the chest was negative for pulmonary emboli but did reveal dependent opacities in both lung bases, superimposed smooth interlobular septal thickening in the dependent areas of the lungs, and bilateral, small-to-moderate pleural effusions. Brain natriuretic peptide and cortisol levels were both normal. An echocardiogram revealed normal left ventricle systolic function with no valvular dysfunction.

The patient was admitted to the PICU and started on broad-spectrum antibiotics. Her respiratory distress rapidly worsened, and she was intubated for respiratory failure. She met diagnostic criteria for acute respiratory distress syndrome, requiring a >90% fraction of inspired oxygen with a $Pao_2$ of ~70 mm Hg. She was ventilated with a peak inspiratory pressure of up to 36 cm $H_2O$ and positive end-expiratory pressure of 12 cm $H_2O$. Norepinephrine therapy was initiated for poor perfusion, and bilateral chest tubes were placed for worsening pleural effusions. Bronchoscopy revealed normal mucosa of the trachea and mainstem bronchi, with clear frothy secretions. The results of a respiratory viral panel were negative. Bronchoalveolar lavage (BAL) revealed cellular debris and reactive mononuclear cells. BAL cell counts were notable for a 900 red blood cell count and a 340 white blood cell count (differential of 26% neutrophils, 13% lymphocytes, 14% monocytes, 25% mononuclear cells, and 22% eosinophils). The results of BAL testing for *Mycoplasma* polymerase chain reaction, *Legionella* direct fluorescent antibody, and aerobic and fungal cultures were all negative. BAL cytology revealed no *Pneumocystis* but abundant lipid-laden macrophages on an Oil Red O stain. The patient was started on 40 mg of intravenous methylprednisolone twice daily. After steroid initiation, she was quickly weaned from vasopressor support and was extubated 5 days after initial presentation. She was eventually discharged from the hospital on a prednisone taper with a diagnosis of hypersensitivity pneumonitis, likely secondary to e-cigarette exposure.

## DISCUSSION

Hypersensitivity pneumonitis is an inflammatory disease of the lung parenchyma that is the result of an immune response to inhaled antigens. Typically, hypersensitivity pneumonitis is associated with antigens from microbial agents, such as moldy hay or grains (farmer's lung), or with animal proteins in avian droppings (bird fancier's lung). In the acute setting, hypersensitivity pneumonitis can be secondary to chemical exposure, some of which can be found in e-cigarettes.[6]

Hypersensitivity pneumonitis can be categorized by the duration of illness as an acute, subacute, or chronic process. The typical manifestations of acute or subacute hypersensitivity pneumonitis can mimic a viral illness, with symptoms including fever, cough, dyspnea, myalgias, and arthralgias. In an acute presentation,

symptoms will often begin hours after antigen exposure. In a subacute or chronic presentation, the symptoms tend to be prolonged and less severe. With repetitive antigen exposure, patients may develop a progressive chronic respiratory disease secondary to pulmonary fibrosis.

The diagnosis of hypersensitivity pneumonitis is made by a combination of laboratory studies, imaging, BAL, and histologic findings. If possible, serum immunoglobulin G antibodies to specific antigens should be obtained. A positive serology result is suggestive but not diagnostic of hypersensitivity pneumonitis, and the absence of specific antibodies (especially in acute presentations) does not rule out this condition. Chest CT in the acute and subacute setting may reveal nodular, ground glass, or airspace opacities. There may be small nodules present, which represent granulomas.[7] The use of pulmonary function tests can be used to support the diagnosis of hypersensitivity pneumonitis, typically revealing a reduced diffusing capacity of the lung for carbon monoxide. BAL fluid is helpful in diagnosis, in which the leukocyte differential may reveal lymphocytosis. Neutrophil predominance can also be seen in either the acute phase with recent exposures or with more advanced disease. Increased eosinophil numbers can also be seen in BAL samples.[8]

BAL eosinophilia is also present in acute eosinophilic pneumonia, which may present similarly to hypersensitivity pneumonitis. Typical symptoms include fever, nonproductive cough, dyspnea, myalgias, and malaise.[9] A majority of patients do not have peripheral blood eosinophilia at the time of presentation. A complete blood cell count differential reveals a neutrophilic leukocytosis early in the course, followed by an eosinophil predominance with disease progression.[10] Chest CT will reveal patchy ground glass opacities, usually located along bronchovascular bundles.[11] The diagnosis of acute eosinophilic pneumonia can be made on the basis of clinical features, CT findings, and a BAL sample with >25% eosinophilia.

The treatment of both hypersensitivity pneumonitis and acute eosinophilic pneumonia is centered on avoidance of inciting agents. In more severely ill patients, intravenous corticosteroids have been shown to accelerate lung recovery.[9,12] Because this is an inflammatory response, antibiotics are not useful unless bacterial superinfection is suspected.

## CONCLUSIONS

With this case, we highlight hypersensitivity pneumonitis as a life-threatening health risk of e-cigarette use in an adolescent patient. Although little is known of e-cigarette health risks, especially in children and adolescents, their use in the pediatric population is growing rapidly. This should prompt pediatricians to discuss the potential harms of e-cigarette use with their patients. Hypersensitivity pneumonitis, lipid pneumonia, and eosinophilic pneumonia should be included in the differential diagnosis of patients who exhibit respiratory symptoms after the use of e-cigarettes.

**ABBREVIATIONS**

BAL: bronchoalveolar lavage
CT: computed tomography
e-cigarette: electronic cigarette

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# Hypersensitivity Pneumonitis and Acute Respiratory Distress Syndrome From E-Cigarette Use

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# A Review of Pulmonary Toxicity of Electronic Cigarettes In The Context of Smoking: A Focus On Inflammation

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## Abstract

The use of electronic cigarettes (e-cigs) is increasing rapidly, but their effects on lung toxicity are largely unknown. Smoking is a well-established cause of lung cancer and respiratory disease, in part through inflammation. It is plausible that e-cig use might affect similar inflammatory pathways. E-cigs are used by some smokers as an aid for quitting or smoking reduction, and by never smokers (e.g., adolescents and young adults). The relative effects for impacting disease risk may differ for these groups. Cell culture and experimental animal data indicate that e-cigs have the potential for inducing inflammation, albeit much less than smoking. Human studies show that e-cig use in smokers is associated with substantial reductions in blood or urinary biomarkers of tobacco toxicants when completely switching and somewhat for dual use. However, the extent to which these biomarkers are surrogates for potential lung toxicity remains unclear. The FDA now has regulatory authority over e-cigs and can regulate product and e-liquid design features such as nicotine content and delivery, voltage, e-liquid formulations, and flavors. All of these factors may impact pulmonary toxicity. This review summarizes current data on pulmonary inflammation related to both smoking and e-cig use, with a focus on human lung biomarkers.

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**Keywords**

inflammation; flavors; nicotine; biomarkers; gene expression; metabolomics

## INTRODUCTION

The category of electronic cigarettes (e-cigs) includes a wide variety of products that result in aerosolizing (vaporizing) nicotine and/or flavors for inhalation, along with a carrier (1). Some e-cigs look like cigarettes that have LED lights opposite the mouthpiece (known as a "cig-alike"), some have e-liquid cartridges or refillable tanks, and others are hookah-like. All of these products are battery powered with electronic heating elements that aerosolize carrier liquids that usually contain nicotine. The carriers are vegetable glycerol (VG) and/or propylene glycol (PG). The use of e-cigs and similar products is rapidly rising, with sales totaling more than $3.7 billion per year. All of the major tobacco manufacturers are marketing these products (2). The rates of e-cig use among youth are now higher than cigarette use, although the estimate of use may vary depending on the method of survey (3–5). Nonetheless, many youth with no history of cigarette use are using e-cigs. In 2015, the prevalence of never-smokers using e-cigs was as high as 19% among youths, and about 10% for adults. About 5% of college students who have never smoked are using e-cigs (6). Fifty percent of adult smokers in the US have tried e-cigs, and 23% currently use both cigarettes and e-cigs (termed dual users) (5, 7–9). For adults and youth who use multiple tobacco products, the most common combination is cigarettes and e-cigs (5). The reasons for adult e-cig use vary and include hoping to quit smoking, health concerns, and convenience (10). Contributing to the popularity of e-cigs is the availability of many e-liquid flavors, which are attractive to a variety of smokers and non-smokers. However, there is concern that the availability of flavors may promote uptake of other tobacco products among non-smokers and possibly hinder cessation among smokers (11).

There has been significant controversy in the public health community regarding the risks and benefits of e-cigs, resulting in confusion among health care practitioners and the general population (1, 12–20). Despite the paucity of human data, there is a growing perception among lay adults that e-cigs are as risky as cigarettes (21–23). Most professional organizations have been cautious in their assessment of what is known regarding benefits and risks of e-cigs (24–27), reflecting the lack of data regarding e-cigs' toxicity, particularly relative to that of cigarette smoke. Adding to the difficulty of providing evidence based policy recommendations is the considerable diversity of products in terms of devices, flavors, and solvents. Thus, there is considerable need for studies on e-cig use, behavior, and toxicity (14, 22, 24).

In 2016, the Food and Drug Administration (FDA) Center for Tobacco Products finalized a "deeming" regulation extending its tobacco-related regulatory authority to e-cigs that contain nicotine derived from tobacco, and its current research priorities include the study of e-cig toxicity (1). However, some have voiced concern that increased regulation too soon would hinder an emerging market with the promise for a positive health impact, and also impair long-term observational research needed to assess the risks of e-cigs use at the

population level (28). At this time, much of the evidence regarding effects of e-cigs comes from cell culture and animal studies. Biomarkers from the lung, e.g., sputum, exhaled air, and samples collected by bronchoscopy (inserting a scope through the mouth or nose into the lung for bronchial alveolar lavage [BAL], bronchial brushings and biopsies) provide direct evidence for assessing lung toxicity in humans. Although the study of biomarkers in the sputum and exhaled air are useful because they are non-invasive, they also provide more conflicting data and their relevance to lung toxicity is not well understood (29). In contrast, bronchoscopy specimens measure physiological changes directly from lung samples and not subject to factors such as sputum production or gases exhaled that circulated through the body.

When making policy, the FDA based its decisions on likely population-level public health impact of its decisions. Thus, when available, regulatory judgments about e-cigs should be informed by human toxicity data, which ideally considers the heterogeneity in the population, e.g., smoking history (current smokers using e-cigs to quit, former smokers at risk for future cancers and smoking relapse, and never-smokers including adolescents or young adults), age, gender, and rural vs. urban. It also needs to consider patterns of use, including whether e-cigs are being used concurrently with cigarettes or other tobacco products. The FDA has not clarified what evaluation frameworks and risk assessment methods it will use, there are available frameworks to consider that include a robust research agenda for human studies (30).

In this review, we summarize the available bronchoscopy evidence regarding lung inflammation associated with smoking and e-cig used. We focus on inflammation because this pathway is plausibly affected by e-cigs and is important in the etiology of lung cancer and chronic obstructive pulmonary disease (COPD). While there is an extensive literature for the relationship of inflammation to lung cancer and respiratory disease developed from the laboratory (31–36), this review will focus on human studies of cigarette smokers and e-cig users. The data reviewed focus on methods for considering a validated biomarker for inflammation that reflects differences between smokers and non-smokers, shows a dose-response relationship with smoking, identifies changes in levels after quitting towards that of a non-smoker, and has the sensitivity to show differences when switching to a less harmful product (37).

## Smoking, Inflammation, and the Human Lung

Cigarette smoking is the major cause of lung cancer and COPD, accounting for about 90% of all cases (38–40). The smoke contains numerous toxicants that promote inflammatory responses that contribute to the risk for these diseases (31, 32, 34, 40–42). Inflammation is considered a hallmark of cancer (43) and COPD (31, 32). The pro-inflammatory effects on the lung are observable in healthy smokers before the onset of disease (36). Cigarette smoke activates alveolar macrophages and airway epithelial cells to release proinflammatory cytokines, resulting in the recruitment of infiltrating inflammatory cells from the blood to the lung. At the same time, normal protective mechanisms for adequate tissue repair by fibroblasts are hindered by cigarette smoke: pro-inflammatory pathways are upregulated and anti-inflammatory ones are down-regulated. Key inflammatory cytokines (e.g., TNF-α,

interleukins [IL], and interferons) and cytotoxic mediators such as reactive oxygen species, metalloproteinases and soluble mediators of cell death are induced by smoking with chronic inflammation promoting unregulated cell proliferation, cell invasion, and angiogenesis and genomic instability (34, 44). Smoking drives KRAS oncogenesis (frequently mutated in lung cancer) via inflammation induced by the activation of NF-κB and STAT3, and stimulating lung cell survival (31, 45–47). In experimental animals, chemopreventive agents that inhibit inflammation reduce lung tumorigenesis (48). In humans, there is some evidence that non-steroidal anti-inflammatory agents reduce lung cancer risk, although not consistently (34, 49–52). COPD is a known risk factor for lung cancer, indicating some shared mechanisms that include an effect on inflammation, although each may have pathways that are not shared (53–59).

There are numerous biomarkers that have been used for sampling the lung for inflammation. These will be reviewed below. Each have the potential for assessing inflammatory responses from e-cigs.

**Inflammatory cell infiltrates**—There are numerous studies indicating that induced sputum has higher inflammatory cell content (e.g., neutrophils) in smokers compared to non-smokers (29, 34, 60); counts tend to be increased with increased smoking exposure. Sputum neutrophils decreased after 6 weeks of smoking cessation (61, 62) in two studies; in a small sputum study there was not a change 4 weeks after quitting (63). Macrophages decrease as early as 1 week following smoking cessation (64). Based on bronchoscopy data, total cell counts, macrophages, lymphocytes, neutrophils, eosinophils and basophils, are much higher in smokers compared to non-smokers (65–75). For example, in a study with 132 smokers and 295 never-smokers who underwent bronchoscopy, the smokers had increased numbers of inflammatory cells in BAL samples, most noticeably for macrophages with lesser effects on neutrophils and lymphocytes in a dose dependent manner associated with smoking status (76). Results are similar for studies of bronchial biopsies; e.g., 45 asymptomatic smokers compared to never-smokers had statistically higher numbers of neutrophils, eosinophils, mast cells, and macrophages, with means differing 2–4 fold (70). Important evidence comes from smoking cessation studies. In a study of 28 smokers who underwent bronchoscopy, 12 months after quitting they had reduced numbers of inflammatory cells compared to those who continued smoking (77). Reducing cigarettes per day by more than 50% was also associated with decreased BAL macrophages and neutrophils at 2 months (78).

**Inflammatory cytokines**—Lung cytokines also are affected by smoking (e.g., IL6, IL8, IL10, and IL33); these cytokines have been shown to be associated with risk of lung cancer and other lung diseases (65, 72, 79–86). In sputum, an exposure-response gradient with increased numbers of packs per day has been reported (60, 87). For example, in a bronchial biopsy study of 45 asymptomatic smokers and never-smokers, smokers had 2- to 4-fold higher IL8 compared to never smokers (70). In another study that used bronchial biopsies and immunohistochemistry in 47 subjects, IL6 was associated with smoking (85). Inflammatory cytokines, such as IL8, are higher in patients with emphysema (79). While in one cross-sectional study, there was no difference between smokers and non-smokers in IL6 and IL8 (88), a smoking cessation study reported statistically significant reductions at 12

months for IL8 (65). The reliability of repeated measures for BAL cytokines has been demonstrated, but it also should be noted that blood cytokines are not a good surrogate for lung cytokines (75).

**mRNA expression—**Differences in mRNA expression for smokers versus non-smokers have been well described. These differences, including those related to inflammation, are used for the early detection of lung cancer (89–96). Expression profiles in the lung for genes that are up- and down-regulated have been described and shown to cluster with smoking status (90). In comparisons of 16 smokers and 17 non-smokers, genes coding for inflammatory cytokines and innate immunity, and response to oxidants and xenobiotics were differentially expressed (91). Dose-response mRNA expression changes to urine cotinine have been identified in 121 subjects who were smoking the equivalent of only a few cigarettes per day (95). In this large cross-sectional study, pathway analysis implicated genes involved in the metabolism of xenobiotics, eicosanoid metabolism, and oxidative stress responses.

**MicroRNAs (miRNAs)—**MiRNAs are short non-coding single-stranded RNA transcripts that negatively regulate mRNA expression at the post-transcriptional level. There are many studies linking smoking and COPD via changes in miRNA expression and inflammation pathways, for example miR-146a altered by smoking (97–101). *In vitro* studies using cigarette smoke condensate (CSC) on human bronchial epithelial cell lines show up-regulation of miR-101 and miR-144, which target the cystic fibrosis transmembrane conductance regulator found to mitigate airway cell inflammation, and also are found to be up-regulated in COPD (102, 103). Other changes *in vitro* include a decrease in miR-200c, related to NF-κB-mediated inflammation and thought to increase epithelial to mesenchymal transition (EMT) associated with tissue remodeling and cigarette smoking in COPD (104–107). Experimental animal models for cigarette smoke exposure have identified altered expression of several miRNAs including, miR-146a, miR-92a-2*, miR-147, miR-21 miR-20 and miR-181. Both miR21 and miR-181a are involved in chronic systemic inflammation (108) and have been reported to be affected by smoking in humans (109). Cross-sectional studies assessing the sputum of smokers and non-smokers identified let-7c as over-expressed and inversely correlated with tumor necrosis factor receptor type II, implicated in COPD and inflammation pathogenesis and a predicted target gene of let-7c) was inversely correlated with the sputum levels of let-7c (29, 110, 111), and alveolar macrophages alter expression of miR-210, miR-150, miR-146b-3p, and miR-452 (112). The latter miRNA targets matrix metalloproteinase-12, which is increased in the sputum of patients with COPD and contributes the development of emphysema (113, 114). In a recent study of 19 subjects in a 3-month smoking cessation trial, 34 miRNAs in bronchial brushings were differentially expressed between the smokers and baseline non-smokers, and 22 of these decreased with smoking cessation (115). The major function of both the up- and down-regulated miRNAs was inflammation, with several targets associated with NF-κB pathway. There are other examples of miRNAs related to cigarette smoke and inflammation considered to be involved in COPD, such as effects in smooth muscle, fibroblasts, macrophages and neutrophils, and specific miRNA changes in bronchial epithelia of smokers versus non-smokers (97, 116).

**Untargeted metabolomic profiles**—Metabolomics is an emerging technology that is being used to identify new biomarkers of tobacco smoke exposure (117–125), and for studying COPD (126–128). The assay can be used to identify thousands of small molecules (<1500 Daltons) reflective of exogenous exposures and cellular responses to those exposures. Metabolomics is now being widely applied to evaluate disease and disease causation (129–132). In the case of smoking, metabolomic screening can reveal changes induced by cigarette smoke constituents as well as those due to endogenous cellular responses to cigarette smoke. In an animal model, BAL metabolomics have mapped with emphysema progression, identifying a lung specific L-carnitine as a central metabolite (133). In our studies, we have 1) demonstrated the feasibility for assessing smoking-related biomarkers in blood and urine (134); 2) identified novel biomarkers related to smoking (e.g., glycophospholipids and pathways related to inhibition of cAMP), including some that differ by gender and race (117); and, 3) identified the presence of menthol metabolites (117). We are not aware of metabolomics studies in the lung for smoking-related changes, but metabolomics have shown changes in smokers' sputum (135), and have been used in a bronchoscopy study for air pollution (136).

**Nitric oxide**—Fractional exhaled nitric oxide (FeNO) is a validated marker of lower airway inflammation that is simple to assess, non-invasive and reproducible (137, 138). It is used for the diagnosis and treatment of asthma in children (139–143). Nitric oxide (NO) is synthesized in the lung by NO synthase (NOS) and the oxidation of L-arginine to L-citrulline. The inducible NOS (iNOS) is transcriptionally regulated by pro-inflammatory cytokines in epithelial cells and macrophages in the airways (144). FeNO has been shown to be decreased by almost 50% in smokers in several cross-sectional studies (145–148), possibly related to the large amount of NO in cigarette smoke (146). The reduction in FeNO also is thought to be related to nitric oxide synthase inhibition due to cigarette smoke carbon monoxide and/or oxygen free radicals (146, 149). Reduced FeNO has been reported to be significantly associated with increased neutrophilic inflammation (150).

## E-Cig Toxicity

While there are numerous recent reviews for the risks and benefits of e-cigs, there are substantial research gaps in our knowledge of the effects of e-cigs on inflammation (20, 22). There is some evidence that they do affect inflammation as indicated below. However, there are only a few studies that provide data related to lung inflammation; most human studies assess cigarette smoke exposure biomarkers. This section reviews recent studies that support the hypothesis that e-cigs might affect inflammation in the human lung.

**E-cig aerosol constituents**—E-liquids, in addition to nicotine, are composed mostly of PG, VG, and flavors. When used in foods and skin products, these carriers and flavors are "generally regarded as safe" by the FDA (151, 152). However, it is unknown what happens to the lung when these constituents are heated and inhaled. E-cig heated PG can be converted to propylene oxide (1, 153), which is an irritant and an International Agency for Research on Cancer group 2b carcinogen (possibly carcinogenic to humans) (154). Heated VG and PG can be converted to acrolein, acetaldehyde, and formaldehyde, which also are known strong irritants that affect inflammation (155–157). In addition, the e-cig aerosols

include many chemical constituents in e-cig flavors, including glycidol, acetol, and diacetyl (158) as well as tobacco specific nitrosamines (TSNAs), aromatic hydrocarbons, acetone, and volatile organic compounds (VOC) (e.g., benzaldehyde, propionaldehyde, crotonaldehyde) (1, 22, 157, 159–176). A recent study using mass spectroscopy identified over 115 VOCs in e-cig aerosol, many that were not present in the unheated liquids (160), while another identified trace quantities of benzene, methyl ethyl ketone, toluene, xylene, styrene, and acetic acid (177). However, their presence is substantially reduced compared to cigarette smoke.

The amount of aerosol and constituent levels in e-cig aerosols can greatly increase under different heating conditions that occur when using higher voltages of the device. For example, increasing temperature overall increases the overall amount of aerosol of flavor-free liquids, as well as total aldehydes, formaldehyde, acetaldehyde and acrolein, and the release of inflammatory cytokines, as much as 10-fold with higher voltages (157, 158, 178–182).

**Laboratory Studies—**There has been some toxicology testing for e-cig liquids and aerosols, but these are limited and the relationship to human disease risk is unclear (12, 183, 184). Existing studies suggest that the toxicological responses are qualitatively similar to smoking, e.g., exposing cell lines and cultures to the aerosols induces a pro-inflammatory effect (185, 186), disruption to epithelia barriers (187), oxidative stress (188), cytotoxicity (189), neutrophil inflammatory response (190) and DNA damage (191, 192). However, the magnitude of effect is low compared to cigarette smoke and aerosols were not found to be mutagenic (193). Normal human bronchial epithelial (NHBE) cells exposed to e-cig aerosols, with or without nicotine, increase IL-6 and IL-8 cytokine levels (194). Another study reported a change in the gene expression pattern of NHBE cells with silenced p53 and activated KRAS when exposed to e-cig aerosol (153). Separately, e-cig liquid was assessed in NHBE cells in parallel with a knock-out mouse model; there were increased rates of infection, inflammatory markers and altered gene expression (195). Metals present in e-cig aerosol are capable of causing cell injury and inflammatory cytokine induction, e.g., in human lung fibroblasts (196). There have been some studies of gene expression in cultured human bronchial epithelial cells showing changes in profiles that are much less than smoking but clearly distinctive (197). The pathways that have been implicated in these studies include phospholipid and fatty acid triacylglycerol metabolism, with enrichment of cell cycle associated functions (e.g., cell cycle checkpoint regulation, control of mitosis) and immune system function.

*In vitro* studies using human bronchial epithelial cells demonstrate that increasing voltage decreases cell viability and increases the release of inflammatory cytokines (IL-1β, IL-6, IL-10,CXCL1, CXCL2 and CXCL10) (178). Experimental animal studies have also shown that there are some toxic effects in the lungs of e-cig aerosols, which includes pro-inflammatory responses (12, 184, 198). While *in vivo* studies indicate that aerosolized PG or VG alone only have slight toxic effects in the lung (199–202), more recent data using e-cig devices are identifying various effects on inflammatory and other responses. For example, mice exposed to e-cig aerosols with or without nicotine showed increased lung macrophages, neutrophils and lymphocytes (194). Separately, mice exposed to e-cig aerosol

intratracheally had an increased rate of inflammatory infiltrate and cytokines, and IgE production (203). Other studies report lung oxidant reactivity and reactive oxygen species increasing inflammatory cytokines (i.e., increasing IL-8), changes in lung fibroblasts thought to be part of COPD pathogenesis, and altered redox balance (204). There also is evidence that e-cig aerosols may promote oxidative damage, mitochondrial reactive oxygen species, a dose-dependent loss of lung epithelial barrier function and increased inflammation-related intracellular ceramides and myosin light chain phosphorylation (198). A recent animal study showed measurable effects on inflammation and lung injury for both cigarette smoke and e-cigs, but much less for the latter (186).

**Human Studies**—Important information about potential toxic exposures from e-cigs can be learned from human biomarker studies. There are several studies that indicate that e-cig users have substantially less toxicant exposure than cigarettes, depending on either complete quitting or the amount of smoking reduction, both for clinical symptoms and by reducing exposure to cigarette smoke exposure biomarkers. The studies are either cross-sectional studies or clinical trials that assess complete switching or dual use, but these studies are all small. The most informative studies are the ones that are published most recently, because they provide data for the most advanced generation e-cigs. All of the published studies that we are aware of use peripheral biomarkers (e.g., urine and blood) or exhaled air, and not those collected directly from the lung. They also represent only short term exposures, lacking direct data for the long term consequences, if any, of e-cig use.

In humans, e-cig acute health effects are minimal and short-lived (27, 205–212). The most common adverse effects reported across studies were nausea, headache, cough, and mouth/throat irritation, which were similar or less compared to nicotine patches. Although adolescents using e-cigs reported an overall increased rate of chronic bronchitis symptoms (213), smokers with COPD who switched to e-cigs had a reduction in symptoms and an improved quality of life (214, 215).

In studies of smokers completely switching to e-cigs, there are substantial reductions in such exposures. In a 2016 trial of 419 smokers randomized to an e-cig or continued smoking over 12 weeks, Cravo et al. (209), reported that assignment to e-cigs was associated with statistically significant decreases in urinary metabolites of acrolein (3-HPMA), benzene (S-PMA) and NNAL (a pulmonary carcinogen) compared to controls. Another important measure in that study was urinary PG, which almost doubled after one month of e-cig use, indicating that this could be a biomarker for exposure generally to e-cigs. In another recent study of 20 smokers switched for only two weeks, authors reported reductions for a large panel of biomarkers, including a 50% reduction in acrolein metabolites (carbon monoxide [CO], NNAL and all measured VOCs and PAHs) (216). McRobbie et al. (217) reported that among 40 smokers switched to e-cigs use, there was a statistically significant decrease in acrolein exposure after 4 weeks. Pulvers and co-workers (2016) studied 40 smokers switched to e-cigs and reported substantial reductions (to non-smoking levels) for urinary NNAL, but only for 2 (benzene and acrylonitrile) of 8 VOCs (218). CO also was substantially reduced. O'Connel et al. (219, 220), reported on a five day trial of 105 subjects confined to a clinical facility; they found similar reductions in the urinary biomarkers and CO. Lastly, a one-year clinical trial reported significant reductions in exhaled CO (221).

Thus, compared to smoking, there appears to be a significant overall reduction in biomarkers for persons completely switching to e-cigs, but it is not known if these peripheral biomarkers reflect effects in the lung.

There are 3 studies for e-cig use that includes smokers who dually use e-cigs (217, 222, 223). A cross-sectional study was published by Shahab and coworkers (2017), where 5 groups of long-term smokers or former smokers were recruited for a total n of 181 subjects (222). These groups were long term e-cig users, long term NRT users, smokers, and smokers who dually used either e-cigs or NRT. All groups had similar total nicotine equivalents, indicating that the products chosen by the smokers or former smokers all were able to deliver the particular levels of nicotine needed by the smoker. However, the levels were numerically higher compared to smokers for the e-cig dual users (157%), not being statistically different perhaps due to the small numbers of subjects. TSNAs were substantially and statistically significantly lower for the NRT-only (12% of smokers) and the e-cig-only groups (3% of smokers), and they were also statistically lower for the smoker-NRT dual users (57%). However, the levels were not statistically lower for the smoker-e-cig dual users (81%), also perhaps due to the small numbers. It may also be due to lower cigarettes per day, and while not statistically different, the mean numbers were 13.9 for the smokers, 10.8 for the smoker-NRT dual users and 11.9 for the smoker-e-cig dual users. The dual users with NRT or e-cigs, compared to smokers had similar acrolein levels (107% and 91%, respectively), and the exclusive NRT and e-cig users had similar levels (35% and 33%, respectively). The similar acrolein levels for the exclusive NRT and e-cig users indicates that there was no measurable increase in levels from e-cig aerosols. Other volatile organics had similar results, where there were clear decreases for complete switching to NRT or e-cigs, but there were not for the dual users. Thus, although the data is cross-sectional in nature, the results are consistent with substantial reductions in smoke toxicants when exclusively switching to e-cigs, but a reduction in dual use is more modest and likely depends on the amount of smoking reduction that can be achieved. Somewhat consistent with this cross-sectional study, McRobbie and coworkers (2015) reported that dual users after 4 weeks had reductions in cotinine, CO and acrolein compared to smokers based on the reduction in numbers of cigarettes used per day (217). Using a novel study design, Jorenby and coworkers (2017) studied long term smokers and e-cig dual users (n=74) and smokers (n=74) (223). Both groups were asked to reduce their cigarettes per day by 75% over 2 weeks, allowed to resume their regular use and then asked to quit smoking for 3 days. The e-cig users were free to increase their e-cig use using whatever e-cig device they normally used, and were found to have increased their vaping by more than 4 times while reducing smoking or quitting. CO substantially decreased during reduction and quitting, although the levels for the two groups did not differ from each other.

Four switching studies showed a decrease FeNO (219, 221, 224, 225) (including a 1-year trial), while another found no difference (226), and another with methodological limitations (i.e., e-cigs and controls were tested on different days) reported an increase (227).

**Flavors**—Most e-cig users indicate that their first and usual e-cigs are flavored, with non-tobacco flavors used by a strong majority of college students (95%) and young adult (71%) e-cigs users, but a minority (44%) of adults (228). In most cases, non-tobacco flavors are

fruit and candy flavors, especially among never-smokers and former smokers who take up e-cigs, without any discernible patterns for type of fruit or candy flavor. A 2016 study showed that adults prefer menthol, mint, and fruit, followed by candy and chocolate (229). A recent review by Hoffman et al. (230), provided similar results, including preferences for cherry, candy, strawberry, orange, apple and cinnamon, with these higher preferences in adolescents than adults. The choice among youth and former smokers typically is a fruit or candy flavor, while among smokers it is a tobacco flavor (228).

There are data that some flavorings may induce lung inflammation. For example, diacetyl present in many e-cig liquids (found in caramel, butterscotch, watermelon, pina colada, and strawberry) has received widespread attention because it is a cause of bronchiolitis obliterans (popcorn lung) in the occupational setting (231, 232). Additional research has indicated that some flavors may be a source of aldehydes (233). For example, cherry flavored e-cig liquids yield increased amount of benzaldehyde, a key ingredient for many fruit flavors (176). There are a few *in vitro* and *in vivo* studies for the effects of flavors in the context of e-cig aerosols (in contrast to food uses where they are generally regarded as safe). Using a high through-put screening method based on cell death endpoints, 7 flavors used in e-cigs showed positive results, such as the chocolate flavoring 2,5-dimethylpyrazine (234). Using a different cell culture model for cytotoxicity that assesses vapors from e-liquids (volatility of the liquid, not the aerosols emitted from an e-cig), cinnamon-flavorings had the most cytotoxicity among 36 different e-liquids and confirmed among sources from multiple manufacturers; the constituents in the cinnamon-flavored liquids thought to be responsible for the cytotoxicity were cinnamaldehyde (CAD) and 2-methoxycinnamaldehyde (2MOCA) (235, 236). *In vivo, o*ne study reported no effect in rats, but they chose a mixture of flavors with constituents not known to cause cell damage or inflammation (237). Menthol is a flavor of concern for enhancing the abuse liability in cigarettes (238). Although there are some toxic effects of menthol, there are no data for the human lung (239). Menthol flavorings for e-liquids may also have diacetyl (231). A recent study has demonstrated that several flavorings induce expression of inflammatory cytokines in lung cell cultures, where acetoin and maltol are among the most potent (240).

**Nicotine**—Nicotine content can be regulated by the FDA and some considerations for this will be affected by the addictiveness (i.e., abuse liability) of the product, but toxicity considerations may also apply. Nicotine content varies widely among e-cigs, and users can formulate e-liquids with their own choice of nicotine concentration. It is well established that nicotine is highly bioactive in that it induces proliferation, inhibits apoptosis, promotes the epithelial to mesenchymal transition (EMT), and promotes angiogenesis (55, 241). All of these are important components of cancer and COPD development (55, 198). To date, nicotine is not considered a carcinogen for humans, as nicotine replacement therapy (NRT) and low-TSNA smokeless tobacco (snus) have not demonstrated increased risks of cancer (242). Regarding inflammation, nicotine is both pro- and anti-inflammatory, and therefore theoretically able to affect cancer and COPD pathogenesis in different ways (241, 243–248). In cell culture studies of human bronchial epithelial cells, while cigarette smoke condensate increases inflammatory cytokine production, nicotine alone does not, and pretreatment with nicotine reduced the condensate effects (244). In a study of wound healing in smokers,

compared to continued smoking and quitting with or without nicotine, it was observed that NRT reduced inflammation and macrophage infiltration, but not angiogenesis (243). In human nasal epithelial cells, in contrast to cigarette smoke and acrolein, nicotine induced inflammatory cytokine response (249). *In* vivo, nicotine was able to inhibit acute lung injury in mice through anti-inflammatory effects (248). The anti-inflammatory effect may be through the stimulation of nicotinic receptors present in lung and other cells, and there is data that nicotinic receptor agonists reduce acute lung injury (245, 250, 251). There are nicotinic receptors on macrophages that reduce pro-inflammatory cytokines while having no effect on anti-inflammatory cytokines (252). In contrast to data for nicotine reducing inflammation, other data, using different experimental models, indicate that nicotine may increase inflammatory response because of its toxic effects on the lung epithelium (187, 195). Pro-inflammatory effects have been observed in cell culture models of vascular smooth muscles and in atherogenesis, because nicotine can induce oxidative damage (253, 254). It also has been reported that nicotinic receptors both increase and decrease inflammation pathways in human lung and lung cells, depending on the experimental model and receptor subunits (but better lung function (250)) (255–258). Because of the potential anti-inflammatory effect of nicotine, NRT has been explored as a treatment for inflammatory disease, such as ulcerative colitis, but results have been inconclusive to date (247, 259).

## Summary and Research Gaps

Numerous studies demonstrate that cigarette smoking induces pulmonary inflammation in humans, as measured by cellular infiltrates, altered cytokines, and changes in gene expression. Importantly, these are biomarkers of effect, rather than biomarkers of exposure, and many can be considered as validated for assessing smoking and harm reduction. Inflammation is considered important for the development of both lung cancer and COPD. There is sufficient data about e-cig aerosols to also indicate a pro-inflammatory effect that warrants further investigation, given the toxicant and irritant constituents in e-cig aerosols. The bronchoscopic biomarkers discussed in this review represent direct evidence for the inflammatory effects in the human lung, the target organ for lung cancer and COPD. The studies also indicate that they are valid markers of tobacco smoke exposure because of the identified differences between smokers and non-smokers, the dose-response with smoking levels, and the reversal of effects with cessation and smoking reduction (37). Thus, assessing inflammation for e-cig toxicity is feasible. An important research gap for currently available studies are the lack of assessing long term chronic effects; all studies to date assess short term exposures and acute changes in health effects or biomarkers of recent exposures. Thus, studies of longer clinical trials and observational cohort studies with repeated measures are needed. Focusing on the lung provides some data for more chronic effects, but definitive data would need to come longer term observational studies and clinical trials.

E-cigs may have the potential for supporting smoking cessation, although current data is not yet sufficient to support specific recommendations for their use (24, 260, 261). Whether or not the efficacy of e-cigs becomes established for assisting smoking cessation, their safety profile also needs to be determined. An important consideration about safety is the context of the e-cig user. While e-cigs are likely less toxic than smoking given the lack of most combustible tobacco constituents and evidence by human biomarker studies, the amount of

reduced toxicity that may occur in the lung remains unknown both for a long-term user who quits smoking and for dual users. For dual users, the extent of harm reduction, if any, will likely depend on the amount of smoking reduction. At the other end of the spectrum, while the conceptual effects of e-cig aerosols promoting inflammation may be much less than smoking, it also is unknown if the use of e-cigs in never smokers with naïve lungs (e.g., adolescents who become nicotine dependent with e-cigs) would have a clinically significant impact on future disease risk.

Given the chemical complexity of the e-cig aerosol, and that cigarette smoking induces pulmonary inflammation, studies for e-cig lung effects in both smokers and never-smokers are needed. While cross-sectional studies provide relevant information, they are subject to bias and confounding, and do not demonstrate causal relationships. In contrast, clinical trials for both smokers and never-smokers can provide better evidence for the uptake of e-cigs and related exposures. The studies to date, however, only measure blood and urine biomarkers, where it is unknown if these biomarkers are suitable surrogates for lung inflammation and disease risk. This could only be determined for humans using biomarkers obtained from lung sampling, i.e., bronchoscopy.

While bronchoscopy is an invasive procedure, research bronchoscopies are commonly done for healthy smokers and non-smokers to understand the effects of smoking, and are considered sufficiently safe for the research of healthy subjects (65–73, 76, 77, 86, 89, 94, 95, 115, 262–269). The risk of the procedure increases with the number of lavaged segments. For persons with reactive airway disease there can be wheezing and bronchospasm. Non-invasive tests are available to assess pulmonary inflammation, such as induced sputum, but these studies also have complications (e. g., inducing bronchospasm) and the results are less consistent than bronchoscopy studies. FeNO, however, is a validated marker with utility to assess e-cig use and lung effects.

The induction of inflammation by e-cigs may differentially impact lung cancer and COPD risk, because e-cig aerosols do not have the complexity of carcinogen exposure found in cigarette smoke. While it is entirely speculative at this point, it may be that long-term e-cig use heightens one's risk for COPD; whether the inflammatory effect is sufficient to increase risk in never smokers, or in smokers with existing lung damage, is an open research question. It may be that the risk for an individual smoker who switches to e-cigs may decrease, but as overall use in the population increases, including use by never smokers and former smokers, population-level risks might increase. (270, 271). Risk assessment models are being developed to estimate these possible effects (272–274). The role of nicotine also needs to be considered, as it has both pro- and anti-inflammatory potential, making it unclear how nicotine content may mediate the effects of the other aerosol constituents.

A methodological challenge to studying e-cigs and their health effects are the almost countless brands on the market of differing design and performance. There has been a successive generation of manufactured devices that have generally improved on use and nicotine delivery. Thus, the generalizability of studies that assess one type of e-cig may not be reflective of the marketplace, and which device was used is an important consideration. Another challenge to the researcher when studying particular products is that the

manufacturer may alter the design or withdraw the product from the market, which may affect the research results. These issues however, are somewhat addressed by the recently developed National Institutes of Drug Abuse production of a standardized research electronic cigarette (SREC; https://www.drugabuse.gov/funding/supplemental-information-nida-e-cig) that can be used for both laboratory and human studies. While this advancement will provide sustainability and allow for comparing data from different research studies, the generalizability would still be a continued limitation.

The FDA now has the regulatory authority to regulate e-cig product design and e-liquid formulations. Subjects for further research and possible regulation include voltage, flavors, and nicotine content. Voltage and higher temperatures have been shown to increase the toxicity of e-cig aerosol content. Flavors are not all one type of chemical constituent, and different flavors may impact morbidity risk differently. And nicotine content may play a protective or adverse effect that can be additive or synergistic. As indicated above, there is an urgent and broad research agenda to identify the magnitude of effect for e-cig pulmonary toxicity, and how that magnitude impacts the risk for never-smokers and smokers.

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Table 1

:er Studies

| ı | Population | Criteria for each group of tobacco user (baseline) | Duration | Products tested | Markers assessed* | Results |
|---|---|---|---|---|---|---|
| he safety profile of an e-<br>t (EVP; 2.0% nicotine)<br>f conventional cigarettes<br>ing to use the EVP | Healthy subjects (n=408) in UK<br>EVP group: (n=306):<br>- Mean age: 34<br>- Mean BMI: 26<br>- 55% Males<br>CC group (n=102):<br>- Mean age: 35<br>- Mean BMI: 25<br>- 57% Males | EVP group (n=306):<br>- CPD<br>5-10 CPD: 36%<br>11-20 CPD: 56%<br>21-30 CPD: 8%<br>- FTND<br>Mild: 30%<br>Moderate: 57%<br>Severe: 13%<br>CC group (n=102):<br>- CPD<br>5-10 CPD: 31%<br>11-20 CPD: 62%<br>21-30 CPD: 7%<br>- FTND*<br>Mild: 29%<br>Moderate: 54%<br>Severe: 17% | 12 weeks | EVP prototype developed by Fontem Ventures B.V.<br>A rechargeable battery (voltage range of 3.0-4.2 V), an atomizer and a capsule (small cartridge) containing e-liquid<br>The base components of the e-liquids: PG (70-75% w w), glycerol (18-20% w/w) and water (5% w/w) | Urine biomarkers: NEQ, SPMA, 3HPMA and total NNAL, PG | % change in week 12 from baseline: EVP vs. CC<br>- NEQ: −25% vs. −6%<br>- 3HPMA: −29% vs. 6%<br>- SPMA: −35% vs. 1%<br>- Total NNAL: −31% vs. 3%<br>- PG: 119% vs. −3% |
| ffects of e-cigarettes (e-<br>tine delivery and<br>elected carcinogens and<br>ı longitudinal study<br>:ts observational study | Healthy subjects (n=20) in Poland<br>- Aged 18 or older<br>- 100% Caucasian<br>- 40% Males<br>- Mean age: 31 | - Current daily cigarette smokers (>5 CPD within the last 12 months)<br>- Years of smoked: 12 | 2 weeks | An e-cig (M201 Mild, Poland) with 20 tobacco-flavored cartridges per week containing 11 mg of nicotine in a mixture of PG* and Gly* (50:50) | Urine biomarkers: *NEQ*, NNAL,<br>Volatile organics: HEMA, MHBMA, HPMMA, 33HPMA, SPMA, AAMA, CNEMA, and 2HPMA<br>*Metabolites of PAHs* (free plus conjugated): 2-naphthol, 1-hydroxyfluorene, 2- | Baseline/Week 1/Week 2, P-value<br>- NEQ(μmol/g): 50/45/43, NS<br>- NNAL (ng/g): 165/60/69, <0.001<br>- HEMA(ng/g): 3120/864/1573, 0.001<br>- MHBMA(ng/g):: 1283/478/887, <.001<br>- HPMMA(μg/g): 1379/387/575, <.001<br>- 3HPMA(μg/g): 700/455/465, 0.001<br>- SPMA(ng/g): 674/193/481, <.001 |

| | Population | Criteria for each group of tobacco user (baseline) | Duration | Products tested | Markers assessed* | Results |
|---|---|---|---|---|---|---|
| | | | | | hydroxyfluorene, 3-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3+4-ydroxyphenanthrene; 1-hydroxypyrene | - AAMA(μg/g): 148 188 97, 0.005<br>- CNEMA(μg/g): 178/58/66, <.001<br>- 2HPMA(μg/g): 24/18/15, <.001<br>- 1-Hydroxyfluorene(ng/g): 864/492/833, <.001<br>- 3-,4-Hydroxyphenanthrenes(ng/g): 669 544/1262, NS<br>- 2-Hydroxyfluorene(ng/g): 463/315/495, 0.048<br>- 1-Hydroxypyrene(ng/g): 338/279/627, NS<br>- 3-Hydroxyfluorene(ng/g): 312/192/349, 0.001<br>- 2-Hydroxyphenanthrene(ng/g): 333 492/800, NS -1-Hydroxyphenanthrene(ng/g): 211 196 415, NS<br>- 2-Naphthol(μg/g): 13/8/14, NS |
| e exposure to nicotine in before and after e- | Adult smokers (n=40) in UK<br>E-cigs use only (n=16)<br>- Mean age: 45<br>- 63% White<br>- 50% Males<br>Dual users (n=17)<br>- Mean age: 48<br>- 53% White<br>- 52.9% Males | E-cigs use only<br>- Mean CPD: 16<br>- Mean FTCD: 3.9<br>Dual users<br>- Mean CPD: 21<br>- Mean FTCD: 4.7 | 4 weeks | A Green Smoke EC (labeled 2.4% nicotine) | Urine biomarkers: 3-HPMA and cotinine | % reduction in week 4 from baseline: E-cigs use only vs. Dual users<br>- Cotinine (ng/mg creatinine): 17% vs. 44%, P =0.010<br>- 3-HPMA (ng/mg creatinine): 79% vs. 60% P <0.001 |
| ne consumption and sure of cigarette ching to e-cigs; l study of smokers e-cig independent of tion | Adult US smokers (n=40) | Male (73%)<br>Mean age: 30.08 (SD = 8.82 White 50% Hispanic 25% | 4 weeks | e-Go C non-variable battery and refillable atomizers and choice of eight flavors in 12 or 24 mg nicotine dosage | Urine biomarkers: cotinine, NNAL, VOCs | Reductions (p value):<br>Cigs/day 50% (<0.001)<br>CO 37% (<0.001)<br>Cotinine 23% 0.(90)<br>NNAL 46% (<0.01)<br>PMA 17% (0.01) |

| 1 | Population | Criteria for each group of tobacco user (baseline) | Duration | Products tested | Markers assessed* | Results |
|---|---|---|---|---|---|---|
| | | | | | | HEMA 14%(0.85)<br>MMA increased 11% (0.27)<br>CNEMA 52% (<0.01)<br>3-HPMA 21% (0.16)<br>2-HPMA 12% (0.96)<br>AAMA 12% (0.67)<br>HPMMA 14% (0.99) |
| ompare changes in<br>arkers among different<br>groups from usual brand<br>entional tobacco<br>rettes to e-cigs and dual<br><br>gs use only<br><br>Group A1: Tobacco flavor rechargeable bluTM e-cigs<br><br>Group A2: Cherry flavor rechargeable bluTM e-cigs<br><br>Group A3: Cherry flavor disposable bluTM e-cigs<br><br>l Use<br><br>Group B1, B2, and B3: Usual brand combustible tobacco cigarette plus products from Group A1, A2, or A3, respectively. | Healthy adult male or female smokers (n=105) in USA<br><br>E-cigs use (n=15 for each) Group: A1/A2/A3<br><br>- Mean age: 37, 40, 33<br>- 87%, 60%, 93% White<br>- 60%, 80%, 40% Males<br><br>Dual use (n=15 for each) Group: B1/B2/B3<br><br>- Mean age: 36, 36, 39<br>- 87%, 73%, 87% White<br>- 60%, 80%, 53% Males | E-cigs use only Group: A1/A2/A3<br><br>- CPD: 18 17/15<br>- Years smoked: 19/20/15<br>- FTND score: 5.3/5.1 5.3<br><br>Dual use Group: B1/B2/B3<br><br>- CPD: 18 20 21<br>- Years smoked: 19/14/21<br>- FTND score: 5.5/5.7 5.2 | 5 days | BluTM e-cigs<br><br>All e-cigs contained 24 mg/mL (2.4%) nicotine, vegetable glycerol (~50% in cherry flavor and ~80% in tobacco flavor), PG (45% in cherry flavor and ~10% in tobacco flavor), distilled water, and flavorings. | Urine biomarkers: NEQ, NNN, NNAL, 1OHP, 3HPMA, SPMA, MHBMA, HMPMA, CEMA | E-cigs use only groups A1/A2/A3, Day −1 vs. Day 5 days<br><br>- NNAL (ng/24 h), 423/384 299 vs. 174/150 111<br>- 3HPMA (lg 24 h): 1522/1903 1354 vs. 214/263 247<br>- HMPMA (lg 24 h): 523/657/533 vs. 71/83/78<br>- CEMA (lg/24 h): 220/266/201 vs. 33/41/26<br>- 1OHP (ng 24 h): 317/ 302/261 vs. 94/86/91<br>- NNN (ng/24 h): 19/14 14 vs. 1/0.7/1<br>- MHBMA (lg/24 h): 5/6/5 vs. 0.3/ 0.3/0.3<br>- SPMA (lg 24 h): 6.3 8.1/6.3 vs. 0.3 0.3/0.4<br>- NEQ (mg/24 h): 17 18/15 vs. 11/13/11<br><br>Dual use groups B1 B2/B3, Day −1 vs. Day 5 days<br><br>- NNAL (ng/24 h), 431/422/343 vs. 329/321/269<br>- 3HPMA (lg 24 h): 1644/1475/1490 vs. 1046/1071/1155<br>- HMPMA (lg 24 h): 591/598/505 vs. 392/395 387<br>- CEMA (lg/24 h): 256/246/223 vs. 172/168 173 |

| | Population | Criteria for each group of tobacco user (baseline) | Duration | Products tested | Markers assessed* | Results |
|---|---|---|---|---|---|---|
| | | | | | | - 1OHP (ng/24 h): 364/295/304 vs. 235/206/224<br>- NNN (ng/24 h): 14/12 11 vs. 9/8/7<br>- MHBMA (lg/24 h): 5/3/5 vs. 4/3/4*<br>- SPMA (lg/24 h): 7/5/7 vs. 5/4/6<br>- NEQ (mg/24 h): 17/16/16 vs. 18*/16*16*<br>Note: All levels in Day 5 from three groups were statistically different compared to the levels in Day-1, * not significant. |
| ıvestigate long-term ıges in exhaled breath surements and iratory symptoms in kers invited to quit or ce their cigarette umption by switching to ;s<br>ıp A: 12 weeks of inal either 2.4 mg/ml or ng/ml nicotine)<br>ıp B: 6 weeks of inal either 2.4 mg/ml a further 6 weeks of goria 1.8 mg/ml nicotine ) nicotine<br>ıp C: 12 weeks of inal 0% without tine(sweet tobacco ıa) | Regular smokers not intending to quit (n=134) in Italy<br>Group A (N = 49)<br>- Mean age: 45<br>- 26 Males<br>Group B (N 49)<br>- Mean age: 42<br>- 28 Males<br>Group C (N = 40)<br>- Mean age: 40<br>- 25 Males | Group A<br>- Packs/year: 25<br>- CPD: 20<br>- FTND: 5.5<br>- eCO: 18<br>- FeNO: 5.8<br>Group B<br>- Packs/year: 24<br>- CPD: 18<br>- FTND: 5.6<br>- eCO: 21<br>- FeNO: 5.9<br>Group C<br>- Packs/year: 24<br>- CPD: 20<br>- FTND: 5.8<br>- eCO: 19<br>- FeNO. 6.4 | Baseline and at week 12, week 24 and week 52 | E-cig model '401' with a rechargeable three-piece design | | - There was no difference of baseline characteristics between failures, reduces, and quitters.<br>- A significant effect of quitting classification was found on FeNo and eCO at all time points (P < 0.0001)<br>- Among quitters, FeNO rose from 5.5 ppb to 17.7 ppb by week 52.<br>- Baseline eCO decreased from 17 ppm to 3 ppm by week 52.<br>- No significant changes in FeNO and eCO levels were observed in failures and reducers. |
| valuate, nicotine levels smoking reduction ess for cigarette smokers | Regular smokers or dual users in USA<br>Smokers | Smoker<br>- Years smoked: 25<br>- Mean FTCD: 4.9 | 26 days | - Disposable:34%<br>- Replaceable cartridge:16% | Urine biomarkers: Nicotine, CO | - Compared to smokers, dual users did not smoke significantly fewer cigarettes during either periods of ab libitum use or during |

| | Population | Criteria for each group of tobacco user (baseline) | Duration | Products tested | Markers assessed* | Results |
|---|---|---|---|---|---|---|
| dual users of cigarettes e-cigs<br><br>rettes only (n=74)<br><br>l users: cigarettes + e- (n=74)<br><br>ibitum period Days 1-8 16-21<br><br>reduction period: Days<br><br>'o cessation period: Days 5 | - Mean age: 43<br>- 42% Males<br>- 80% White<br>Dual users<br>- Mean age: 33<br>- 41% Males<br>- 91% White | Dual users<br>- Years smoked: 17<br>- Mean FTCD: 4.5 | | - Tank system:14%<br>- Unknown:37%<br>Nicotine concentration<br>0.1-0.3%: 4%<br>0.4-0.6%: 8%<br>0.7-1.2%: 7%<br>1.3-1.8%: 8%<br>1.9+%: 1%<br>Unspecified: 72% | | periods of smoking restriction, nor did they produce lower CO levels.<br><br>Dual users increased vapes/day from 1.3 and 1.9 during Ad Libitum use to 6.3 and 4.4 during 75% Reduction for women and men, respectively. |
| ssess an impact of using -cigarette for 5 min on ulmonary function tests FENO of healthy adult kers<br><br>erimental group were ucted to use the e-cigs 'b for 5 min as they ld usually smoke.<br><br>control group subjects : asked to use the e- rette with similar uency, but without the e- cartridge included | Regular healthy smokers (n=40) in Greece<br><br>Experimental group (n=30) and control group (n=10) | A minimum pack-year: 5 | 5 min | NOBACCO MLB-MED filter, 11 mg of nicotine, PG>60%, linalool <5%, nicotine <10%, tobacco essence <5%, and methyl vanillin <1%; no polyaromatic hydrocarbons were detected. | FeNO | FeNO, ppb<br>- Experimental group Pre usage: 13 vs. Post usage: 11, P=0.005<br>- Control group Pre usage:9 vs. Post usage: 9, NS |
| he effects of ad libitum time free e-cigs or/and a 5 min in healthy adult 10) and non-smokers | Healthy subjects (n=20) in Italy<br><br>Smokers (n=30)<br>- Mean age: 42<br>- 40% Males<br>Non-smokers (n=10)<br>- Mean age: 36<br>- 30% Males | Smokers<br>- Packs/year: 19 | 5 min. | ELIPS C Series | FeNO and FeCO | FeNO<br>Smokers: no difference, NS<br>Non-smokers: no difference, NS<br>CO<br>Smokers: Decreased FeCO after e-cig use, P <0.001<br>Non-smokers: Decreased FeCO after e-cig use, P =0.048 |
| neasure indoor air ity and FeNO levels of : consumers | 9 healthy e-cig users in Germany<br>- 100% Males | All subjects were occasional smokers with a cigarette consumption of <10 cigarettes per week (no e-cigarettes) | 2 hours | Liquids (with and without nicotine, all with tobacco flavor) and rechargeable e- | CO and FeNO | - FeNO increased in 7 of 9 individuals after vaping a nicotinic e-cigs at P =0.030, but the effect was not significant when nicotine-free liquids were used. |

| | Population | Criteria for each group of tobacco user (baseline) | Duration | Products tested | Markers assessed* | Results |
|---|---|---|---|---|---|---|
| x vaping sessions, nine nteers consumed e-cigs and without nicotine | - Mean age: 25 | | | cigs from Red Kiwi, Seevetal, Germany<br>nicotine: 18mg/ml | | - eCO levels were not significantly influenced by e-cig consumption. |

al excretion of nitric oxide, FTND: Fagerstrom Test for Nicotine Dependence, Gly: glycerine, NEQ: nicotine equivalents. PG: propylene glycol tobacco specific nitrosamines: NNAL =, volatile organic compounds: SPMA, 3HPMA :NEMA, and 2HPMA

EXHIBIT F

# F

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# E-Cigarette Aerosol Exposure Induces Reactive Oxygen Species, DNA Damage, and Cell Death in Vascular Endothelial Cells

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## ABSTRACT

Cigarette smoking remains one of the leading causes of preventable death worldwide. Vascular cell death and dysfunction is a central or exacerbating component in the majority of cigarette smoking related pathologies. The recent development of the electronic nicotine delivery systems known as e-cigarettes provides an alternative to conventional cigarette smoking; however, the potential vascular health risks of e-cigarette use remain unclear. This study evaluates the effects of e-cigarette aerosol extract (EAE) and conventional cigarette smoke extract (CSE) on human umbilical vein endothelial cells (HUVECs). A laboratory apparatus was designed to produce extracts from e-cigarettes and conventional cigarettes according to established protocols for cigarette smoking. EAE or conventional CSE was applied to human vascular endothelial cells for 4–72 h, dependent on the assay. Treated cells were assayed for reactive oxygen species, DNA damage, cell viability, and markers of programmed cell death pathways. Additionally, the anti-oxidants α-tocopherol and n-acetyl-l-cysteine were used to attempt to rescue e-cigarette induced cell death. Our results indicate that e-cigarette aerosol is capable of inducing reactive oxygen species, causing DNA damage, and significantly reducing cell viability in a concentration dependent fashion. Immunofluorescent and flow cytometry analysis indicate that both the apoptosis and programmed necrosis pathways are triggered by e-cigarette aerosol treatment. Additionally, anti-oxidant treatment provides a partial rescue of the induced cell death, indicating that reactive oxygen species play a causal role in e-cigarette induced cytotoxicity.

Key words: anti-oxidants; cardiovascular system; cytotoxicity; e-cigarettes; endothelial cells; oxidative stress.

Over the past 50 years, the scientific and medical communities have amassed a wealth of data on the harmful effects of tobacco cigarettes. Cigarette smoking damages every major organ system and remains one of the leading causes of preventable death (U.S. Department of Health and Human Services, 2014). The World Health Organization estimates that cigarette smoking leads to around 6 million deaths per year worldwide and projects that number will increase to 8 million by the year 2030. Yet, approximately one-fifth of the world's population persists in cigarette smoking (WHO, 2015).

Recently, the development of the electronic nicotine delivery systems known as e-cigarettes has provided an alternative to conventional cigarettes. In a 2015 study, researchers noted that e-cigarettes have already become the most popular alternative to conventional cigarettes and are routinely used by about 1 in 5 American tobacco product users. It is of particular interest that 1 in 10 current e-cigarette users have no history of smoking tobacco cigarettes (Weaver *et al.*, 2015). Another recent study indicates that e-cigarettes have become the most commonly used tobacco product among middle and high schoolers with 3.9% of middle schoolers and 13.4% of high schoolers responding that they currently use e-cigarettes (Arrazola *et al.*, 2015). These statistics are troubling as there is currently no consensus on the health consequences of e-cigarette usage.

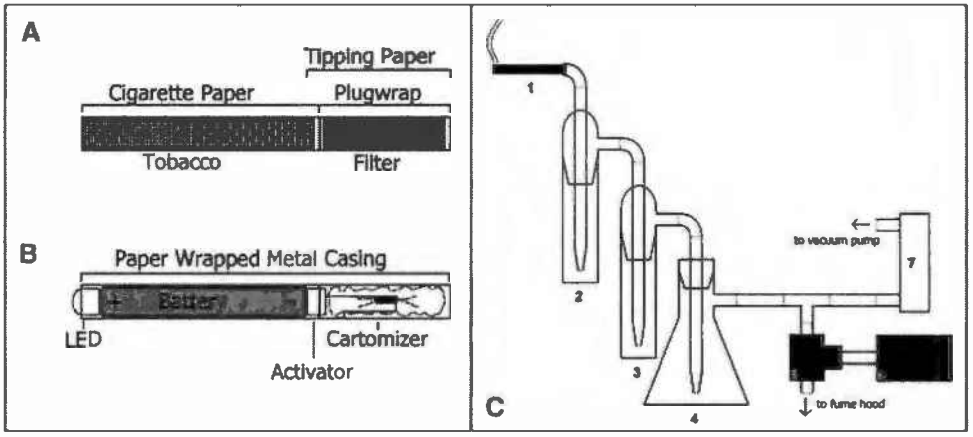


FIG. 1. Schematics of conventional cigarettes, electronic cigarettes, and smoke/aerosol extraction apparatus. A, Conventional tobacco cigarette; B, electronic cigarette; C, smoke/aerosol extraction apparatus: 1) cigarette, 2) primary extract impinger, 3) overflow impinger, 4) vacuum trap, 5) solenoid valve, 6) microcontroller, 7) flow regulator.

As e-cigarettes appear to contain fewer known toxic and carcinogenic compounds than conventional cigarettes, e-cigarette use has been advocated as a harm reduction strategy for cigarette smokers (Cahn and Siegel, 2011; Goniewicz *et al.*, 2014). However, current literature reviews on the question of e-cigarette safety are inconclusive and cite significant methodological problems and conflicts of interest in many studies (Callahan-Lyon, 2014; Pisinger and Døssing, 2014; West and Brown, 2014). Moreover, the rapid development of new models of e-cigarette and new flavors of e-cigarette liquid provides a challenge to meaningful research and regulation (Zhu *et al.*, 2014).

Many of the pathologies associated with conventional cigarette smoking possess a cardiovascular component (U.S. Department of Health and Human Services, 2014), and the core of these pathologies is death and dysfunction at the level of vascular cells (Messner and Bernhard, 2014; Morris *et al.*, 2015; Sobus and Warren, 2014). The mechanism of cigarette smoke induced cardiovascular cytotoxicity/genotoxicity has been well characterized: cigarette smoke increases the oxidative burden on the cell, dysregulates cellular metabolism, alters nitrogen oxide levels, and results in the production of toxic compounds such as peroxynitrate and peroxynitrite (Pryor and Stone, 1993; Irani, 2000; Smith and Fischer, 2001).

Specific studies of the cardiovascular effects of e-cigarettes tend to conclude that they are less hazardous than conventional cigarettes when used by healthy individuals; however, reviewers point out that these studies are few in number and often short term with no follow-ups (Benowitz and Burbank, 2016; Lippi *et al.*, 2013; Morris *et al.*, 2015; Nelluri *et al.*, 2016). It has been recently demonstrated that e-cigarette aerosol can induce cell death and oxidative stress both *in vitro* across multiple cell lines (Farsalinos *et al.*, 2013; Lerner *et al.*, 2015; Putzhammer *et al.*, 2016; Romagna *et al.*, 2013; Schweitzer *et al.*, 2015; Teasdale *et al.*, 2016) and *in vivo* in the serum of smokers and e-cigarette users (Carnevale *et al.*, 2016; Hom *et al.*, 2016; Schweitzer *et al.*, 2015).

The purpose of this study was to better characterize the effects of e-cigarette aerosol on human vascular endothelial cells. We developed a laboratory apparatus capable of creating extract from conventional cigarette smoke and e-cigarette aerosol. The aerosol from a panel of 4 brands of tobacco flavored e-cigarettes was compared with smoke from conventional tobacco cigarettes in its ability to induce reactive oxygen species formation, DNA damage, and cell death in human umbilical vein endothelial cells (HUVECs). To look more closely at the specific mechanisms of e-cigarette aerosol induced cell death, we investigate the presence of key proteins and cellular hallmarks of the apoptotic and necrotic pathways of programmed cell death. To determine the role of oxidative stress in e-cigarette induced cell death, we attempted to prevent e-cigarette induced cell death with anti-oxidant treatment. This work contributes to the growing body of literature on the potential vascular harm of e-cigarette aerosol and substantially advances our understanding of the mechanisms of e-cigarette aerosol induced cell death.

## MATERIALS AND METHODS

### Sample Selection

In this study, we chose to examine high revenue generating, tobacco flavored, cigarette-like e-cigarettes. Manufacturers were chosen from a list of companies that make up >1% of the multi-outlet market share (which consists of sales from grocery and food stores, drug stores, club stores, big box stores, dollar stores, mass merchandisers, and military commissaries) (Maier, 2015). The final panel consisted of tobacco flavored e-cigarettes of the brands Blu (Imperial Tobacco), Vuse (R.J. Reynolds), Green Smoke (Altria), and NJoy (NJoy). For comparison to conventional tobacco cigarettes, we used 3R4F research reference cigarettes from the University of Kentucky Center for Tobacco Reference Products. Figures 1A and B shows comparative schematics of a conventional tobacco cigarette (Figure 1A) and the model of e-cigarette used in this study (Figure 1B). All samples of each brand were acquired at the same time and stored in the dark at room temperature in airtight plastic bags. 3R4F reference cigarettes were stored in airtight plastic bags at 4 °C and pre-equilibrated in a humidor at room temperature at 60 ± 3% humidity for at least 30 min before use.

### Cell Culture

HUVECs (American Type Culture Collection) were maintained at 37 °C, 5% $CO_2$ in EGM-2 cell culture medium (Lonza). Cells were kept sub-confluent and used at passages 4-7. Cells were split for experiments using 0.25% Trypsin/EDTA (Gibco) and all samples were allowed to acclimate at least 12 h prior to treatment.

### Cigarette Smoke and E-Cigarette Aerosol Extraction and Preparation

Cigarette smoke extract (CSE) and e-cigarette aerosol extract (EAE) were extracted via a laboratory apparatus (Figure 1C) designed to function within WHO standard operating procedures for intense tobacco smoking: WHO TobLabNet SOP1 (2 s, 55 ml puffs, 2×/min) (WHO, 2012). Either conventional tobacco cigarettes or electronic cigarettes were placed in a plastic tube and PM-992 Parafilm (Bemis) was used to create an airtight seal (Figure 1C1). Puffs of 55 ml volume were taken with the smoke or aerosol being pulled through 2 midget impingers (Figs. 1C2 and 3). The first impinger contained EGM-2 and the second impinger caught any overflow from the first. Downstream of the impingers, an Erlenmeyer flask (Figure 1C4) containing desiccant was used as a moisture trap to protect the vacuum pump. Puffing was performed via opening and closing a 12 volt solenoid valve (US Solid) controlled by a Basic Stamp 2 Microcontroller (Parallax) (Figs. 1C5 and 6). The volume of the puff was controlled with an airflow regulator (Figure 1C7). To prevent operator exposure to smoke or aerosol, all extractions were performed in a chemical safety hood.

Pre and post extraction, e-cigarette cartridges were weighed on an analytical balance (Fisher). The weight of the consumed e-liquid was used to determine the weight of consumed nicotine. For 3R4F research reference cigarettes the amount of nicotine consumed by smoking one cigarette was considered to be 0.7 mg (Roemer *et al.*, 2014). The contents of the first and second impinger were collected and the volume of EGM-2 was adjusted so that the final concentrations of consumed nicotine were equivalent across all samples. Prior to being introduced to cell culture, all extracts were filtered through a 0.22 μm syringe filter (Millipore). Extracts were either used immediately or aliquoted into single use tubes and kept at −80 °C for up to 1 week.

### Neutral Red Uptake Cell Viability Assay

Endothelial cell viability assays were performed according to the protocol of Repetto *et al.* (2008). Treatment was performed by applying either EAE or CSE (in EGM-2 endothelial cell culture medium) at a range of 31.25–500 μM consumed nicotine across 7 wells of a multi-well plate. The cells were allowed to incubate with extract for either 24 or 72 h. After treatment, cells were rinsed with Tris buffered saline (TBS) and incubated with 0.4 mg/ml Neutral Red (Sigma-Aldrich) in cell culture medium for 2 h at 37 °C. Following incubation, cells were rinsed in TBS to remove excess dye and the remaining dye was solubilized with 50% ethanol/1% acetic acid. The relative quantity of neutral red was measured by reading absorbance at 540 nm (baseline 630 nm).

### TUNEL Assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed with a TMR-Red *in situ* Cell Death Detection Kit (Roche) per manufacturer's protocols. Treatment was carried out over 24 h at either 25 or 500 μM consumed nicotine. Positive control cells were treated with 5 U/ml DNase I (Thermo) for 10 min. Following treatment, cells were fixed in 10% formalin, permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate solution, and incubated with TUNEL reagent for 2 h at 37 °C in the dark. Samples were mounted with Vector mounting medium containing DAPI and imaged via fluorescence microscopy.

### Antibody Staining

Antibody staining was performed via conventional methods. Cells were incubated for 24 h with either EAE or CSE before being fixed in 10% formalin. Cells were washed in TBS, permeabilized with TBS with 0.1% Tween-20 (Sigma-Aldrich), and incubated overnight at 4 °C with primary antibodies (rabbit anti-human cleaved caspase-3 [Clvd Casp-3], Cell Signaling, 1:250; rabbit anti-human MLKL phospho s358, Abcam, 1:250). The next day, cells were washed with TBS and incubated for 1 h at room temperature in the dark with secondary antibody (Alexa-594 conjugated donkey anti-rabbit IgG, Invitrogen, 1:500). Samples were mounted with DAPI and imaged via fluorescence microscopy.

### ROS Detection Assay

Cells were analyzed for reactive oxygen species generation using a ROS-ID Total ROS Detection Kit (Enzo Life Sciences) per manufacturer's protocol. Cells were treated with ROS detection reagent at the same time as 500 μM consumed nicotine EAE or CSE and incubated for 4 h at 37 °C in the dark. Following incubation, ROS level was imaged via fluorescence microscopy and quantified as level of fluorescence per cell.

### Anti-Oxidant Rescue Experiments

Cells were exposed simultaneously to 500 μM consumed nicotine EAE and either 50 μM α-tocopherol (α-Toc) (Sigma-Aldrich) or 5 mM n-acetyl-l-cysteine (NAC) (Enzo Life Sciences) for either 24 or 72 h. Following treatment, the cells were collected and analyzed for extent and type of cell death using an Apoptosis & Necrosis Quantitation Kit Plus (Biotium) per manufacturer's protocol. Cells were spun down, washed in TBS, spun down again, washed in annexin V (Ax V)-binding buffer, and then incubated with Ax V/ethidium-III homodimer staining solution at room temperature in the dark. Following incubation, samples were run on a Guava EasyCyte Mini flow cytometer (Millipore).

### Statistical Analysis

All statistical analyses were performed in R. Significant differences among groups were analyzed via ANOVA followed by Tukey honest significant difference testing. A $P$ value $\leq .05$ was considered statistically significant.

## RESULTS

### E-Cigarette Aerosol Displays Reduced and Delayed Cytotoxicity as Compared with Cigarette Smoke

In order to determine the cytotoxic effects of e-cigarette aerosol as compared with conventional cigarette smoke, we made extracts from each brand of e-cigarette in our panel as well as 3R4F research reference cigarettes. As nicotine drives smoking behavior (Picciotto and Kenny, 2013), we adjusted the concentration of the extracts so that the same amount of nicotine was consumed by our smoking/vaping apparatus per final volume of cell culture medium. Figure 2 shows the percentage of cell viability after exposure to either EAE or CSE across a range of concentrations from 31.25 to 500 μM consumed nicotine. At 24 h, CSE displayed acute cytotoxicity across all concentrations while EAE displayed a limited cytotoxicity (up to 13% cell death) across the highest 2 concentrations (Figure 2A). When the experiment was extended out to 72 h, CSE still displayed acute cytotoxicity

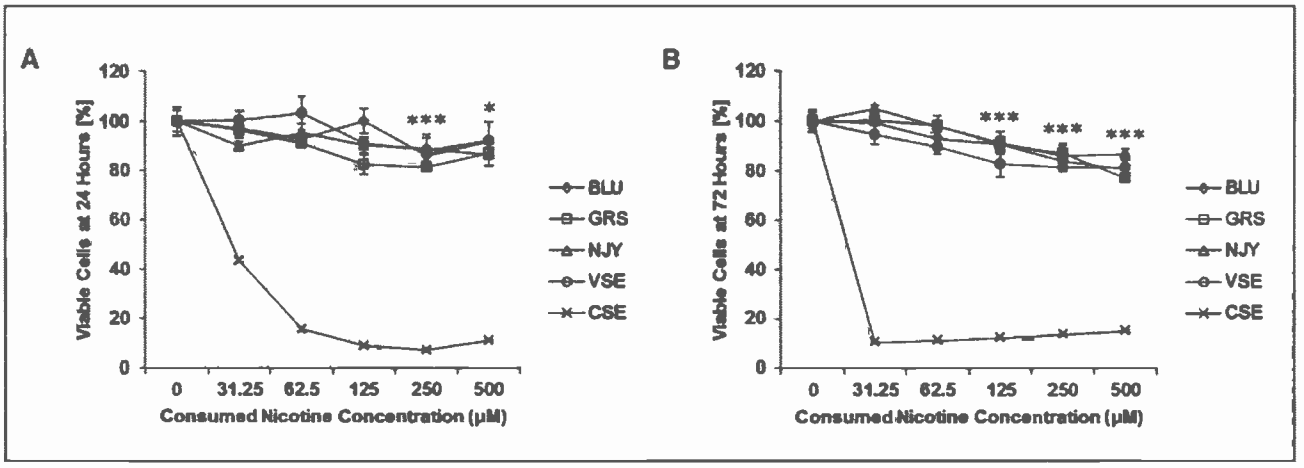


FIG. 2. Cell viability after 24 or 72 h of CSE or EAE exposure. Graphs show the number of viable endothelial cells, as measured by NRU assay, after A, 24, or B, 72 h of exposure to extract from one of 4 brands of e-cigarette aerosol or research reference cigarette smoke. BLU: Blu "Classic Tobacco" 2.4% nicotine by weight (NBW); GRS: Green Smoke "Red Label Tobacco" 2.4% NBW; NJY: NJoy "Bold Tobacco" 4.5% NBW; VSE, Vuse 4.8% NBW; CSE: 3R4F Research Reference Cigarettes. Points represent the mean ± SEM of 6 samples. No significant differences were observed between e-cigarette brands. All brands of EAE at all concentrations were highly significantly different from CSE. Significant differences between no treatment and all brands of EAE are indicated by $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

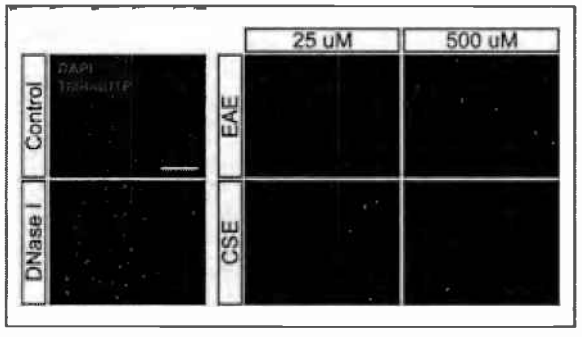


FIG. 3. TUNEL staining after 24 h of CSE or EAE exposure. Endothelial cells were treated for 24 h with either 25 or 500 µM of either CSE or EAE. Positive control cells were treated with 5 U/ml DNase I for 10 minutes. Cells were fixed, permeabilized, and incubated for 1 hour with TUNEL reaction mixture, which labels DNA breaks by the incorporation of red fluorophore conjugated nucleotides (TMR-dUTP) (red). Note that while both EAE and CSE induce TUNEL positivity at 500 µM, CSE displays a much lower cell number than EAE (consistent with Figure 2). Cells were counterstained with the DAPI (blue) prior to imaging. Scale bar = 200 µm.

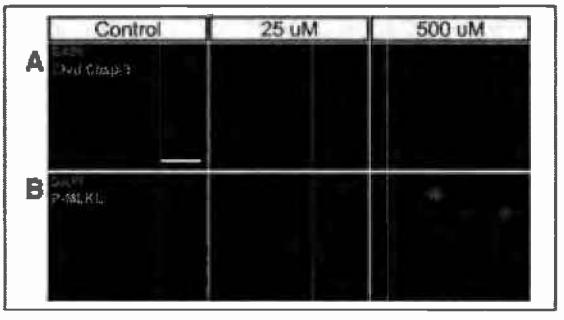


FIG. 4. Immunostaining of Clvd Casp-3 and phosphorylated MLKL after 24 h EAE exposure. Endothelial cells treated for 24 h with either 25 or 500 µM of EAE. Cells were fixed, permeabilized, and immunostained overnight with primary antibodies against either Clvd Casp-3 or phosphorylated MLKL (Phospho-MLKL). Following primary staining, cells were stained with Alexa-594 conjugated secondary antibodies (red) and counterstained with DAPI (blue) prior to imaging. Pictures Scale bar = 50 µm.

and a greater cytotoxic effect (up to 22% cell death) was evident at higher concentrations of EAE (Figure 2B). EAE was less cytotoxic than CSE at all treatment concentrations in both experiments. Neither experiment showed any significant variation within or among brands of e-cigarette.

## Both E-Cigarette Aerosol and Cigarette Smoke Cause DNA Damage

As e-cigarette aerosol caused significant cell death at higher concentrations; we performed a TUNEL assay to determine whether DNA damage, a hallmark of programmed cell death, could be detected. HUVECs were treated with either EAE from one of the e-cigarette in our panel or CSE. Figure 3 shows representative pictures of the results after 24 h of treatment with the extracts. CSE caused DNA damage at low (25 µM) concentrations while EAE did not; whereas both EAE and CSE caused DNA damage at high (500 µM) concentrations.

## E-Cigarette Aerosol Triggers Both Apoptotic and Necrotic Cell Death

Historically the TUNEL assay has been used as an indicator of apoptotic cell death. However, it has recently been shown that TUNEL positivity can occur as a result of either apoptosis or programmed necrosis (Hanus *et al.*, 2015). To determine which cell death pathway is triggered by EAE, we used antibodies against either active caspase-3 (an apoptosis marker) or phosphorylated MLKL (a programmed necrosis marker) (He *et al.*, 2016). Figure 4 shows representative pictures of the results after 24 h of treatment with EAE. A low concentration of EAE (25 µM) did not activate either pathway; however, a high dose (500 µM) induced activation of both the apoptotic pathway mediated by caspase-3 (Figure 4A) and the necrotic pathway mediated by MLKL (Figure 4B).

## Both E-Cigarette Aerosol and Cigarette Smoke Generate ROS

The cytotoxic effects of cigarette smoke are closely associated with its ability to induce oxidative stress through ROS (Morris *et al.*, 2015). To determine whether EAE could induce oxidative

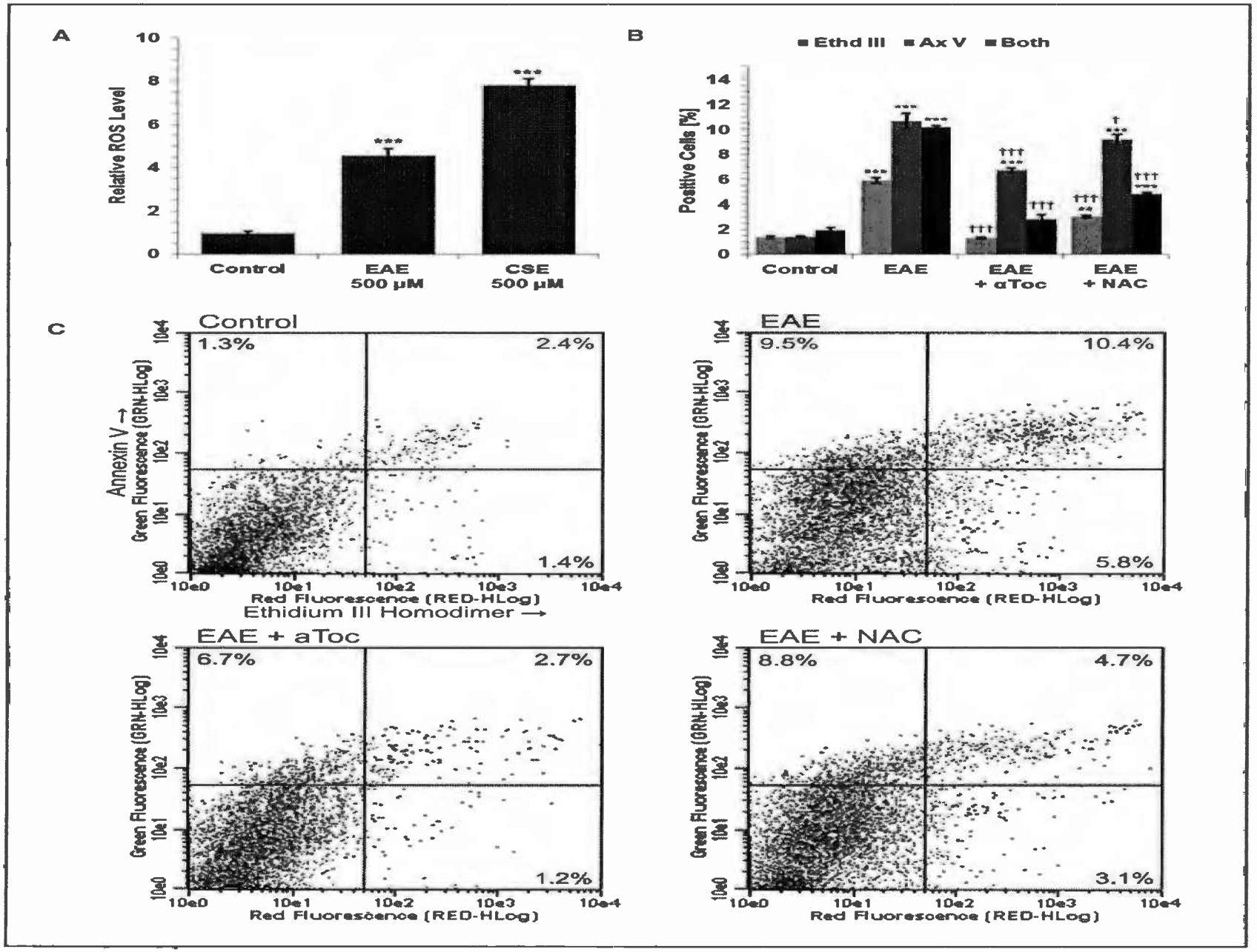


FIG. 5. ROS generation and flow cytometry analysis of apoptosis and necrosis after EAE exposure. A, Endothelial cells exposed to EAE for as little as 4 h display significant levels of ROS. Simultaneously to 500 µM EAE exposure, cells were treated with ENZO Oxidative Stress Detection Reagent, which fluoresces green in the presence of ROS. After 4 h of incubation, fluorescent signal was quantified and averaged over cell number. Bars represent mean ± SEM of 3 samples. Statistical significance is indicated by *. *$P < .05$; **$P < .01$; ***$P < .001$. B and C, Treatment with anti-oxidants limits EAE induced cell death. Endothelial cells were simultaneously treated with 500 µM

stress, and at what level as compared with cigarette smoke, we used a fluorescent based assay for total ROS generation. We chose a treatment concentration of 500 μM as it was capable of causing EAE induced cell death in our previous assays. Figure 5A shows the relative level of ROS generated by either 500 μM EAE or 500 μM CSE 4 h after exposure to extracts. EAE treatment generated significant levels of ROS (~4.5-fold upregulation over control) though still less than the levels generated by CSE at the same treatment concentration (~7.8-folds over control).

### Anti-Oxidant Treatment Prevents E-Cigarette Aerosol Induced Cell Death

As significant levels of ROS were generated in EAE treated endothelial cells prior to the induction of cell death, we hypothesized that ROS plays a causative role in EAE induced cell death. We subjected cells to 72 h of treatment with both 500 μM EAE and either 1 of 2 anti-oxidants that have been previously demonstrated to protect endothelial cells from oxidative stress: α-Toc and NAC (Bielli *et al.*, 2015). Figure 5B summarizes the results of flow cytometry analysis (Figure 5C) using Ax V as an apoptosis marker and ethidium III homodimer as a necrosis marker. EAE treatment significantly increased the number of cells that stain with either Ax V (1.43 ± 0.05% vs 10.69 ± 0.08%), ethidium III homodimer (1.39 ± 0.08 vs 5.9 ± 0.21%), or both (1.98 ± 0.23 vs 10.17 ± 0.13%). Treatment with either anti-oxidant significantly decreased the number of positively stained cells, α-Toc (Ax V: 6.75 ± 0.17%, ethidium III homodimer: 1.31 ± 0.06%, both: 2.86 ± 0.35%) proved significantly more effective at preventing both forms of cell death than NAC (Ax V Ax V : 9.20 ± 0.42%, ethidium III homodimer: 3.06 ± 0.11%, both: 4.83 ± 0.09%). The reduction in the number of Ax V positive cells was significantly weaker than the reduction in ethidium-III homodimer positive cells or doubly positive cells in both anti-oxidant treatments. Notably, α-Toc treatment is capable of reducing the number of ethidium-III homodimer positive cells or doubly positive cells in EAE treated samples to levels indistinguishable from control cells. These data indicate that sufficient anti-oxidant treatment is capable of preventing the necrotic cell death induced by e-cigarette aerosol. Anti-oxidant treatment can reduce but does not prevent e-cigarette aerosol induced apoptotic cell death.

## DISCUSSION

In this study, we have demonstrated that e-cigarette aerosol is capable of inducing reactive oxygen species, DNA damage, and cell death in HUVECs. In all cases, the effects of e-cigarette aerosol were less than those of cigarette smoke applied at the same consumed nicotine concentration. No significant differences were noted within or among different brands of e-cigarette. By demonstrating that e-cigarette aerosol can cause cleavage of caspase-3 and Ax V positivity as well as phosphorylation of MLKL and ethidium III homodimer positivity, we have provided strong evidence that the cytotoxic effect of e-cigarette aerosol is mediated by both apoptosis and programmed necrosis. Further, our partial prevention of e-cigarette aerosol induced cell death by treatment with anti-oxidants indicates that the increased levels of ROS observed play a causal role in e-cigarette aerosol induced cytotoxicity. That we have been able to use anti-oxidant treatment to prevent e-cigarette induced necrosis but not apoptosis provides evidence that e-cigarette aerosol induced cytotoxicity is a multifactorial process. This raises the possibility that distinct components of e-cigarette aerosol (nicotine, fine particles, flavor compounds, etc.) may be linked to distinct toxic effects, providing an avenue for potentially improving these systems by eliminating harmful components.

Over the last several years, e-cigarette use has risen dramatically among both youths and adults, making it vital that we understand the potential health consequences of e-cigarette aerosol exposure (Arrazola *et al.*, 2015; Weaver *et al.*, 2015). However, the general consensus is that the current data is not adequate to make sound, evidence based judgments. This decision stems not only from a lack of available data but also from concerns regarding the representative nature and reproducibility of the data collected (Callahan-Lyon, 2014; Pisinger and Døssing, 2014; West and Brown, 2014). The question of conflict of interest is particularly important in e-cigarette research (Etter, 2015). In the 2014 review by Pisinger and Døssing, it was estimated that 34% of authors publishing on the subject stated conflicts of interest, though the significance and interpretation of this issue have recently become matters of contention (Kosmider and Anastasi, 2016; which was responded to in Pisinger, 2016). Moreover, the varying toxicity profile of different e-cigarette liquids, and the fast rate of development of new flavors, provides an extra level of challenge to addressing the health consequences of e-cigarette aerosol (Allen *et al.*, 2015; Barrington-Trimis *et al.*, 2014; Zhu *et al.*, 2014).

In order to insure the relevance and representative nature of our results we have used a panel consisting of one design of tobacco flavored e-cigarette from 4 different manufacturers whose products make up a significant portion of sales in multiple markets (Maier, 2015). This approach ensures that the products we are testing are products actively being purchased by a significant portion of consumers. E-cigarette liquid aerosolization can result in chemical transformation and the formation of harmful substances (Hutzler *et al.*, 2014). Therefore, assaying pre-aerosolized e-cigarette liquid, or e-cigarette aerosol generated in non-standard fashions, may lead to variations in toxicity analysis. In order to minimize variation and increase the relevance of our study, we developed a laboratory apparatus (Figure 1C) capable of puffing either a conventional cigarette or e-cigarette. While e-cigarette puffing topography varies from conventional cigarette puff topography, there is no current standard operating procedure for e-cigarette puffing (Behar *et al.*, 2015; Farsalinos *et al.*, 2013; Robinson *et al.*, 2015). Consequently, we employed the WHO standard operating procedure for cigarette smoking for both e-cigarettes and conventional cigarettes (WHO, 2012).

There is little consistency in the composition of e-cigarette liquid in the market and high variations in the concentration used in studies across the literature (Cheng, 2014). Since nicotine cravings drive smoking behavior (Picciotto and Kenny, 2013), we

FIG. 5. Continued
EAE and either 50 μM α-Toc or 5 mM NAC. Treatment was continued daily over 72 h. After treatment, cells were trypsinized, washed, and incubated with Ax V and ethidium 3 Homodimer (Ethd III). Following incubation, stained cells were analyzed via flow cytometry, and markers were set by averaging the point at which 95% of cells were considered healthy across 3 no treatment controls. B, Bar graph of flow cytometry results. The height of the bars indicates the mean ± SEM percentage of positive cells in each staining condition (Ax V, Ethd III, or Both) for each treatment group across 3 repeats. Statistical significance is indicated as follows: * significant differences from Control; † significant differences from EAE; */†, $P < .05$; **/††, $P < .01$; ***/†††, $P < 0.001$. C, Representative flow cytometry log-scale graphs of green fluorescence (Ax V) versus red fluorescence (Ethd III).

have created and diluted extracts based on equivalent concentrations of consumed nicotine. This strategy allows us to normalize between e-cigarettes with higher or lower nicotine concentrations as well as between e-cigarette and conventional tobacco cigarettes. Although it is at times difficult to choose appropriately physiologically relevant concentrations for *in vitro* studies, we chose to keep the amount of aerosol used to <1 mM consumed nicotine concentration. The range of concentrations used across similar *in vitro* studies of e-cigarette aerosol cytotoxicity runs from about 2 μM to 25 mM (Farsalinos *et al.*, 2013; Lerner *et al.*, 2015; Romagna *et al.*, 2013; Schweitzer *et al.*, 2015; Teasdale *et al.*, 2016). This means that we are using some of the lowest concentrations of e-cigarette aerosol of any similar study at the time of this writing. For most of our experiments we rely on 25 μM consumed nicotine as a low concentration and 500 μM consumed nicotine as a high concentration. The concentration of 25 μM is particularly significant, as it is extremely close to the projected consumed nicotine concentration of the average smoker in the developed world per day (based on the data gathered by Ng *et al.*, 2014). 500 μM was selected as it was the concentration at which we saw consistent, reproducible harm from e-cigarette aerosol across all methods used in this study.

Our findings support and extend previous studies of e-cigarette induced cytotoxicity. Previous *in vitro* studies have focused on a range of cell types, with some of the most notable being embryonic fibroblasts and stem cells (Palpant *et al.*, 2015; Romagna *et al.*, 2013), oral/buccal cells (Sancilio *et al.*, 2016), lung epithelial cells (Cervellati *et al.*, 2014; Lerner *et al.*, 2015) and cardiovascular cells (Farsalinos *et al.*, 2013; Putzhammer *et al.*, 2016; Schweitzer *et al.*, 2015; Teasdale *et al.*, 2016). In all cases where e-cigarette aerosol was compared with cigarette smoke, e-cigarette aerosol was found to be significantly less harmful, which is consistent with our results. Of these studies, only Sancilio *et al.* attempted to look deeper the mechanism of e-cigarette aerosol induced cell death. They were able to positively confirm apoptosis through Ax V positivity and Bax expression. Interestingly, Sancilio *et al.* did not see the induction of necrosis in their study. This lack of necrosis in their study may indicate that programmed necrosis a cell type specific response to e-cigarette aerosol induced oxidative stress. Of the cardiovascular cell specific studies, Farsalinos *et al.* used MTT assays to detect reductions in cell viability in response to e-cigarette aerosol while Schweitzer *et al.* demonstrated the ability of e-cigarette liquid to increase oxidative stress and inhibit endothelial barrier function. Putzhammer *et al.* employed similar methods to the present study to analyze ROS generation and cytotoxicity in HUVECs, but their results proved highly variable. Our study extends on their work by exploring the nature of e-cigarette aerosol induced cytotoxicity and its relationship to oxidative stress. Teasedale *et al.* measured oxidative stress response in coronary artery endothelial cells and noted no effect of e-cigarette aerosol. However, they used a concentration of aerosol much lower than the other studies (2.16 μM), which our study indicates is below the threshold of e-cigarette aerosol induced cytotoxicity. As no single cell type can fully represent the highly heterogeneous nature of the cardiovascular system, there is a need for more comprehensive studies in the future.

*In vivo* studies of e-cigarette aerosol's effect on the cardiovascular system have shown a broad spectrum of potentially negative effects (Carnevale *et al.*, 2016; Hom *et al.*, 2016; Schweitzer *et al.*, 2015). The findings of Carnevale *et al.*, are particularly interesting as they have shown upregulation of oxidative stress related marker NOX-2 and dysregulation of lipid peroxidation marker 8-isoPGF2α in human serum after both conventional tobacco cigarette smoking and e-cigarette vaping. They were also able to show a reduction of serum α-Toc in the same subjects. A similar effect measured in mouse plasma by assaying levels of 8-OHdG was reported in Schweitzer *et al.*, 2015. Our study supports these findings by demonstrating that there is an increase of reactive oxygen species in endothelial cells as a response to e-cigarette aerosol, that HUVECs die through both programmed necrosis and apoptosis, and that endothelial cell necrosis can be prevented by the addition of α-Toc. The suggestion by Schweitzer *et al.* is that the majority of the consequences of e-cigarette aerosol exposure can be attributed to the effects of nicotine. Although nicotine has long been associated with cardiovascular disease (Benowitz and Burbank, 2016), nicotine independent effects of e-cigarette aerosol have been noted. For instance, the work of Hom *et al.*, demonstrates specific nicotine dependent and nicotine independent effects of e-cigarette aerosol on platelet activation *in vivo*. The nature and magnitude of the effect of nicotine in e-cigarette induced cytotoxicity remains unclear. Our study indicates that nicotine induced oxidative stress does not appear to be the sole cause of e-cigarette induced cell death in HUVECs. However, we cannot rule out effects of nicotine unrelated to oxidative stress or effects of e-cigarette aerosol unrelated to nicotine. Both of these topics deserve additional study if we are to fully understand the potential cardiovascular risks of e-cigarette use. While there is still a great deal of work to be done to understand the long-term health consequences of e-cigarette aerosol, our data contribute to the growing consensus that it is simultaneously significantly safer than cigarette smoke but far from safe.

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EXHIBIT G

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RESEARCH ARTICLE

# Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice

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**Olfert IM, DeVallance E, Hoskinson H, Branyan KW, Clayton S, Pitzer CR, Sullivan DP, Breit MJ, Wu Z, Klinkhachorn P, Mandler WK, Erdreich BH, Ducatman BS, Bryner RW, Dasgupta P, Chantler PD.** Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice. *J Appl Physiol* 124: 573–582, 2018. First published November 2, 2017; doi:10.1152/japplphysiol.00713.2017.—Proponents for electronic cigarettes (E-cigs) claim that they are a safe alternative to tobacco-based cigarettes; however, little is known about the long-term effects of exposure to E-cig vapor on vascular function. The purpose of this study was to determine the cardiovascular consequences of chronic E-cig exposure. Female mice (C57BL/6 background strain) were randomly assigned to chronic daily exposure to E-cig vapor, standard (3R4F reference) cigarette smoke, or filtered air ($n$ = 15/group). Respective whole body exposures consisted of four 1-h-exposure time blocks, separated by 30-min intervals of fresh air breaks, resulting in intermittent daily exposure for a total of 4 h/day, 5 days/wk for 8 mo. Noninvasive ultrasonography was used to assess cardiac function and aortic arterial stiffness (AS), measured as pulse wave velocity, at three times points (before, during, and after chronic exposure). Upon completion of the 8-mo exposure, ex vivo wire tension myography and force transduction were used to measure changes in thoracic aortic tension in response to vasoactive-inducing compounds. AS increased 2.5- and 2.8-fold in E-cig- and 3R4F-exposed mice, respectively, compared with air-exposed control mice ($P < 0.05$). The maximal aortic relaxation to methacholine was 24% and 33% lower in E-cig- and 3R4F-exposed mice, respectively, than in controls ($P < 0.05$). No differences were noted in sodium nitroprusside dilation between the groups. 3R4F exposure altered cardiac function by reducing fractional shortening and ejection fraction after 8 mo ($P < 0.05$). A similar, although not statistically significant, tendency was also observed with E-cig exposure ($P < 0.10$). Histological and respiratory function data support emphysema-associated changes in 3R4F-exposed, but not E-cig-exposed, mice. Chronic exposure to E-cig vapor accelerates AS, significantly impairs aortic endothelial function, and may lead to impaired cardiac function. The clinical implication from this study is that chronic use of E-cigs, even at relatively low exposure levels, induces cardiovascular dysfunction.

**NEW & NOTEWORTHY** Electronic cigarettes (E-cigs) are marketed as safe, but there has been insufficient long-term exposure to humans to justify these claims. This is the first study to report the long-term in vivo vascular consequences of 8 mo of exposure to E-cig vapor in mice (equivalent to ~25 yr of exposure in humans). We report that E-cig exposure increases arterial stiffness and impairs normal vascular reactivity responses, similar to other risk factors, including cigarette smoking, which contribute to the development of cardiovascular disease.

aortic stiffness; smoking; transthoracic echocardiography; vaping; vascular reactivity

## INTRODUCTION

Smoking is the most prevalent source of preventable mortality in modern history and accounts for one of every five deaths in the United States each year (2, 11). Electronic cigarettes (E-cigs), which are also known as electronic nicotine delivery devices, are advertised as a "safe" alternative to conventional tobacco cigarettes (45, 55). Proponents for E-cigs suggest that these devices should be considered a harm-reduction device to assist with smoking cessation (33, 46, 56), in part due to tobacco industry-sponsored animal studies that have concluded that E-cigs have no adverse effects on pulmonary structure and function (34, 54). However, meta-analysis and systemic reviews collectively state that there is limited robust evidence of the impact of the E-cigs on tobacco smoking cessation (18) and that there is even evidence to suggest that E-cigs may negatively impact smoking cessation (32). Accordingly, the value of E-cigs for smoking cessation remains controversial, particularly as it relates to nicotine dependence/addiction. Importantly, there is also considerable concern about

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the overall health consequences related to short- and long-term E-cig use.

Counter to the notion that E-cigs are safe is the recognition that E-cig vapor contains chemicals, such as nicotine, formaldehyde, acetaldehyde, acrolein, and acetone, as well as other compounds, that are known to have deleterious health effects in humans (4, 30, 35, 60). Indeed, in vitro studies have found that E-cigs are cytotoxic to epithelial cells (59, 74), increase oxidative stress in the lung (59), and likely suppress host defenses and promote the virulence of colonizing bacteria (28, 73). Animal studies are also finding evidence of oxidative stress in the lung following short-term daily exposure (i.e., 3–14 days) to E-cigs (41, 62). Studies of the acute effects of E-cig vapor in humans show increases in airway resistance (65) and diastolic blood pressure (17), greater sympathetic activity (50), higher oxidative stress (10, 50), acute increases in aortic arterial stiffness (AS) (68), and impaired flow-mediated dilation (FMD, a measure of arterial health and function) (10). The changes in AS and FMD are consistent with the development of premature or accelerated cardiovascular disease (CVD). While there is growing evidence of the long-term pulmonary toxicity related to E-cigs [see recent review (16)], to our knowledge there are no interventional studies that have reported on the long-term effects of E-cig exposure on cardiac and vascular function. Although observational studies (up to 24 mo) have reported few cardiovascular events in E-cig users (9, 19, 44), it is too early to know the consequences of decades of E-cig use in terms of human cardiovascular health. This issue is particularly important, as the most recent US Surgeon General report states that E-cigs have replaced all other forms of smoking or tobacco products to become the leading product used by 12- to 17-yr-old youths and that use of E-cigs among individuals in this age group increased 900% between 2011 and 2015 (1). While animal smoking models can be controversial (38), they have proven useful and broadly reflect the functional cardiopulmonary and vascular outcomes observed in humans (22, 71, 72). Given the rapid increase in popularity of E-cigs among young adults, and particularly in youth, it is critical to investigate and understand the long-term health consequences associated with habitual E-cig use before these devices are deemed safe. Therefore, the purpose of our study was to evaluate the cardiovascular effects following 8 mo of chronic E-cig exposure in young mice as they advance to middle age to gain insight into the long-term consequences and potential progression of CVD that humans might face after decades of E-cig use.

We hypothesized that if E-cigs are safe, we would not observe cardiovascular dysfunction in mice chronically exposed to E-cig vapor for 8 mo. Based on the life span of the mouse (~2 yr), an 8-mo exposure paradigm represents ~33% of the animal's life, which in human terms would equate to chronic exposure for a period of ~25 yr (assuming an average life expectancy of 78 yr). Since E-cigs were only first introduced in the United States in 2006–2007, the earliest possible time frame to study this level of exposure in humans would theoretically be ~2032 (assuming sufficient numbers of reliable early adopters from 2007 could be recruited and studied). Rather, it is more likely that it will be many decades (perhaps closer to 2050 or beyond) before a large enough study population of humans can be recruited to robustly determine the long-term impact of daily E-cig use on cardiovascular health.

## METHODS

*Study design.* For this environmentally controlled animal study, 10-wk-old female C57BL/6J mice ($n$ = 45) were purchased (stock no. 000664, Jackson Laboratory, Bar Harbor, ME) and randomly assigned ($n$ = 15/group) to chronic exposure to *1*) E-cig vapor (cappuccino-flavored, 18 mg/ml nicotine), *2*) 3R4F reference cigarette smoke, or *3*) filtered air. Mice were allowed 1 wk to adapt to the new vivarium before baseline testing (see below) and randomization to one of the three treatment groups. The starting age at exposure (~13–14 wk old) followed by 8 mo of exposure (ending at ~12 mo of age) represents exposure beginning at adolescence and continuing into adulthood. In human terms, this equates to an individual starting to smoke at ~11 yr of age and continuing to smoke until 35 yr of age, or similar to middle school age through early adulthood (assuming a total life span of 2 yr for mice and 78 yr for humans).

Mice were group-housed (4–5 animals per cage with the same exposure group) in a temperature-controlled (22 ± 4°C, relative humidity 39 ± 6%) pathogen-free vivarium room and maintained on a 12:12-h light-dark cycle. Standard chow (Teklad diet; 18% fat, 24% protein, and 58% carbohydrates) and tap water were provided ad libitum. All procedures were approved by the West Virginia University Institutional Animal Care and Use Committee.

*Exposure.* Mice were exposed as single respective treatment groups (i.e., E-cig, 3R4F cigarette, or filtered air) at the same time ($n$ = 15/group) in separate identical 15.1-liter whole body exposure chambers. E-cig vapor and tobacco smoke were gradually introduced during the first 8 wk, after which the mice were consistently exposed to four 1-h-exposure time blocks, with each exposure separated by 30-min intervals of fresh air breaks, resulting in an intermittent exposure pattern for a total exposure of 4 h/day (occurring over a 6-h window each day). The animals were subjected to this daily regimen for 5 days/wk for a total of 8 mo. Urine analysis for cotinine (cotinine ELISA, Calbiotech, El Cajon, CA) suggested that our daily exposure paradigm tended to produce higher nicotine exposure in 3R4F- than E-cig-exposed groups [47.4 ± 16.3 vs. 24.3 ± 0.6 (SD) ng/ml, $P$ = 0.07]. Urine cotinine levels were undetectable in the air-exposed (control) group.

The E-cig device, a third-generation, tank-style device purchased online (eGrip OLED, Joyetech, www.joyetech.com), was controlled using a custom-made electronic cradle (i.e., artificial hand and thumb) that allowed precise and reliable control of the frequency and duration of E-cig button activation without modifications to the E-cig device. The E-cig voltage was set to 4.9 V, and the device was activated every 99 s for a 5-s duration, resulting in ~38–39 puffs each hour.

The 3R4F reference cigarettes were purchased from the University of Kentucky Center for Tobacco Reference Products, stored at 4°C for the duration of the study, and set in room air 1 wk before use. One cigarette was loaded and lighted on the ventilator inlet every 10 min, resulting in smoke generated from six cigarettes each hour (for a total of 24 cigarettes each day over the 4-h exposure).

Vapor/smoke was generated and delivered to the respective chambers with independent, but identical, rodent ventilators (Harvard Apparatus, Natick, MA) using a 55-ml tidal puff volume. Control mice received filtered air (Carbon Cap 150, Whatman) from a central compressed air line. Each chamber had a bias flow of ~3 l/min, and all exposures occurred simultaneously each day.

*In vivo measurements.* A VisualSonics Vevo 2100 high-frequency, high-resolution micro-ultrasound system (with color-Doppler mode) was used to perform transthoracic echocardiography to assess cardiac function (64) and in vivo Doppler ultrasonography was used to assess AS (52) at three separate times points: *1*) before the exposure started, *2*) halfway through the exposure (i.e., at ~4 mo), and *3*) after 8 mo of exposure. Assessment of AS comprised imaging of the common carotid artery from its insertion on the aorta to the bifurcation of the common carotid artery at the internal and external branches to measure pulse wave velocity (PWV). The theory and details of PWV

measurements have been previously described (21, 52). Briefly, we used a 15-MHz linear-array transducer and color-flow Doppler probe (VisualSonics Vevo 2100) to scan the aorta to the bifurcation of the common carotid artery into its internal and external branches. Color-flow Doppler was employed to help locate the arteries and guide placement of the sample gate for obtaining pulse waveforms. The probe was directed parallel to blood flow. ECG and Doppler signals were then recorded simultaneously at a sweep speed of 200 mm/s for several cardiac cycles, and the data were stored for subsequent offline analysis. The distance ($D$, measured in mm) between the points of probe applanation over the aorta and the carotid bifurcation was measured using an on-screen digital caliper. The time intervals between the R wave of the ECG and the foot of the Doppler carotid and aortic waveforms were averaged over three cardiac cycles, and the pulse-transit time from the carotid to the aorta was calculated by subtracting the mean R wave-to-carotid foot time interval from the mean R wave-to-aortic foot time interval. PWV was then calculated as follows: PWV = $D/T$, where $D$ is the distance (in mm) and $T$ = R wave-to-aortic foot interval − R wave-to-carotid foot interval (in ms). During both assessments (i.e., cardiac function and AS), mice are lightly anesthetized with inhaled isoflurane, and body temperature was monitored and maintained at 37°C.

Noninvasive whole body in vivo plethysmography was used to measure respiratory parameters in awake, unanesthetized mice under basal (resting) conditions (26, 31) prior to and just before completion of the 8-mo exposure. Upon completion of the 8-mo exposure, animals were deeply anesthetized (intraperitoneal ketamine-xylazine), tracheally intubated, and ventilated after administration of a paralytic agent (pancuronium bromide, 0.8 mg/kg) to assess airway reactivity to an aerosolized methacholine challenge using a ventilator (flexiVent, Scireq, Montreal, QC, Canada). Thereafter, major body organs were removed, and the thoracic aorta was surgically dissected, sectioned into rings, and mounted onto an ex vivo wire tension myograph system.

*Ex vivo measurements.* Force transduction was used to measure changes in aortic tension in response to vasoactive compounds (i.e., phenylephrine, methacholine, and sodium nitroprusside). The thoracic aorta was removed, rinsed in physiological salt solution, cleared of surrounding tissue, and cut into 3-mm ring segments. Each ring was mounted in a myobath chamber between a fixed point and a force transducer (World Precision Instruments) and stretched to 0.5-g tension for 45 min for equilibration; then the final experimental baseline tension was adjusted to 0.25 g. The organ baths contained Krebs-Henseleit buffer at 37°C and were aerated with 95% $O_2$-5% $CO_2$. Ring viability and maximal constriction were tested with 50 mM KCl, which was washed out, and baseline tension was reestablished. Subsequently, endothelial function was assessed by preconstriction with phenylephrine ($10^{-7}$ M) followed by increasing concentrations ($10^{-9}$–$10^{-5}$ M) of methacholine; data are represented as percent return to baseline from preconstriction. A washout time of ≥10 min was allowed between pharmacological agents (and verified by return to baseline tension). Aortic rings were then exposed to increasing concentrations ($10^{-9}$–$10^{-5}$ M) of phenylephrine, and data are represented as percentage of KCl maximal constriction. After a final washout period and return to baseline tension, endothelium-independent relaxation was tested with phenylephrine ($10^{-7}$ M) preconstriction followed by sodium nitroprusside ($10^{-9}$–$10^{-5}$ M).

*Histological assessments.* Prior to fixation, both lungs were carefully excised from the chest cavity. One lung was clamped, tied off, removed, and flash-frozen. The remaining lung was fixed with 1 ml of fixative (4% paraformaldehyde), which was kept in the lung with use of a closed-off stopcock attached to the tracheal catheter. The entire lung was submerged in a 4% paraformaldehyde bath for 48–72 h and then processed at the West Virginia University Pathology Core Facility using standardized automated tissue-processing techniques. A pathologist with experience in evaluating mouse lung tissue and blinded to the treatment groups used the following scoring criteria to quantify the presence and severity of abnormalities in fixed lung tissue: 0, none; 1, minimal; 2, mild; 3, moderate; and 4, marked.

*Statistical analysis.* Initially, there were 15 mice in each group and no significant group differences in prestudy measures. Despite the appearance of health, it was expected that, over the course of the 8-mo study, some mice would die prematurely due to unexpected or unknown conditions. A total of eight mice died at varying time points during the study due to physical trauma/accidents ($n$ = 4) or unexplained causes ($n$ = 4). Postmortem examination of one mouse revealed nonsuppurative meningoencephalitis with mild hydrocephalus, but necropsies of the remaining three animals with unexplained death did not provide further insight into cause of death. Deaths occurred across all groups, resulting in a final number of mice (with data from all study time points) used for statistical analysis in each group as follows: 13 air, 11 E-cig, and 13 3R4F. Although there were a greater number of deaths in the E-cig group than the other two groups, no obvious pathological cause of death was identified at necropsy in E-cig mice.

Repeated-measures ANOVA was used to assess group and time differences and group × time interactions when multiple measures were obtained from the same animal. ANOVA was utilized for cross-sectional group comparison for single time-point data (e.g., aortic reactivity measured at the final time point). If significant main effects were observed, post hoc Student's $t$-test was used to identify individual group differences. Kruskal-Wallis (nonparametric) analysis was used for discontinuous variables (i.e., histological score rankings).

All data were analyzed without imputing missing data, when this occurred. Continuous variables are presented as means ± SE, unless otherwise noted. All analyses were conducted using the StatView software package (version 5.0.01, SAS Institute, Cary, NC). Significance was set with $\alpha \leq 0.05$.

## RESULTS

Body mass was not different between the groups before exposure (Table 1). 3R4F-exposed mice gained significantly less body mass ($P < 0.05$) and exhibited a tendency toward lower body mass after the 8-mo exposure than E-cig- and air-exposed mice. Body mass and weight gain between E-cig and air-exposed mice were not different (Table 1).

*Vascular responses.* Assessment of AS revealed no significant change in PWV between the first (prestudy) and halfway (after 4.5 mo of exposure) assessment time points (Fig. 1*A*) but a nearly threefold greater increase in E-cig- and 3R4F-exposed than air-exposed mice after 8 mo of exposure ($P < 0.05$, by repeated-measures ANOVA; Fig. 1).

Ex vivo assessment of the aortic vessel constrictor response to phenylephrine (an $\alpha_1$-adrenergic receptor agonist) was greater in E-cig- and 3R4F- than air-exposed mice ($P < 0.05$; Fig. 2*A*). The vasodilatory response to methacholine (i.e., a muscarinic receptor agonist) was reduced in E-cig- and 3R4F-exposed mice compared with air-exposed mice ($P < 0.05$), suggesting endothelium-dependent impaired dilation (Fig. 2*B*). However, the nitric oxide (NO) donor (sodium nitroprusside), representing a non-endothelial-derived source of NO, dilated aortic rings equally in all groups (Fig. 2*C*). At the highest respective dosage, the adrenergic constriction response was 52 ± 4% greater ($P < 0.05$), while the muscarinic vasodilatory effect was 24 ± 2% lower ($P < 0.05$), in E-cig- than air-exposed (control) mice, demonstrating significant vascular endothelial dysfunction in E-cig-exposed compared with control mice. Like E-cig-exposed mice, 3R4F-exposed mice also ex-

Table 1. *Mouse mass and echocardiography data*

| | Groups | | | |
|---|---|---|---|---|
| | Air | E-cig | 3R4F | *P* Value (ANOVA) |
| *n* | 8 | 9 | 12 | |
| Body mass, g | | | | |
| Pre | 19.6 ± 0.2 | 19.4 ± 0.3 | 19.8 ± 0.3 | 0.59 |
| Post | 28.9 ± 0.7 | 29.8 ± 1.0‡ | 27.1 ± 0.7 | 0.08 |
| Δ | 9.3 ± 0.5 | 10.2 ± 0.9† | 7.4 ± 0.6¶ | **0.02** |
| Heart mass, mg | 115.1 ± 3.2 | 118.5 ± 2.4 | 109.4 ± 2.5 | 0.09 |
| Heart-to-body mass ratio | 4.0 ± 0.1 | 4.2 ± 0.2 | 4.2 ± 0.1 | 0.65 |
| LV mass,[a] mg | 109 ± 10 | 126 ± 5†¶ | 102 ± 3 | **0.02** |
| LV corrected,[a] mg | 87 ± 8 | 101 ± 4†¶ | 83 ± 2 | **0.03** |
| Lung, mg | 84.8 ± 4.1 | 97.3 ± 8.7 | 91.2 ± 5.6 | 0.36 |
| Spleen, mg | 84.5 ± 3.7 | 89.3 ± 3.9† | 72.6 ± 2.7* | **<0.01** |
| *Echocardiography* | | | | |
| *n* | 11 | 12 | 13 | |
| Heart rate, beats/min | 461 ± 16 | 472 ± 18 | 477 ± 17 | 0.84 |
| Stroke volume, μl | 31.0 ± 3.0 | 26.9 ± 2.4 | 25.4 ± 2.3 | 0.33 |
| Cardiac output, ml/min | 12.5 ± 1.1 | 12.0 ± 1.4 | 12.8 ± 1.5 | 0.91 |
| Stroke volume-to-body mass ratio, μl/g | 1.10 ± 0.14 | 0.87 ± 0.08 | 0.94 ± 0.09 | 0.34 |
| Cardiac output-to-body mass ratio, ml/min/g | 0.44 ± 0.03 | 0.41 ± 0.05 | 0.47 ± 0.06 | 0.70 |
| FS, % | 24.9 ± 3.2 | 21.4 ± 2.5‡ | 15.2 ± 2.0* | **0.04** |
| EF, % | 72 ± 3 | 63 ± 3¶ | 56 ± 4* | **0.01** |
| Area, $mm^2$ | | | | |
| Systole | 8.40 ± 0.79 | 10.00 ± 1.03 | 11.67 ± 0.93 | 0.07 |
| Diastole | 18.61 ± 0.91 | 18.30 ± 1.14 | 19.13 ± 0.81 | 0.83 |
| Volume, μl | | | | |
| Systole | 13.5 ± 1.3 | 19.5 ± 2.1 | 20.4 ± 2.6 | 0.07 |
| Diastole | 44.0 ± 3.5 | 43.9 ± 4.3 | 45.9 ± 2.8 | 0.91 |

Values are means ± SE. E-cig, electronic cigarette; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; 3R4F, reference cigarette. Boldface indicates statistical significance. [a]Values are from echocardiographic analysis. *$P < 0.05$ vs. air, †$P < 0.05$ vs. 3R4F, ¶$P < 0.10$ vs. air, and ‡$P < 0.10$ vs. 3R4F.

hibited a higher constriction (63 ± 3%) and a lower relaxation (33 ± 2%) response than control mice ($P < 0.05$).

*Cardiac responses.* After 8 mo of exposure, whole heart mass was not statistically different but tended to be higher in E-cig- than air- and 3R4F-exposed mice. In support of this finding, the estimation of left ventricular (LV) mass from echocardiographic dimensional measurements revealed greater LV mass for E-cig-exposed than 3R4F-exposed mice ($P < 0.05$) and a tendency similar to air-exposed mice ($P < 0.10$; Table 1). Cardiac function from echocardiography revealed no effects on heart rate, stroke volume, and cardiac output but significantly lower fractional shortening (FS%) and ejection fraction (EF%) in 3R4F-exposed mice ($P < 0.05$), with a similar tendency only for EF% ($P < 0.10$) in E-cig-exposed mice, compared with air-exposed mice (Table 1).

*Pulmonary responses.* Respiratory function assessed by flexiVent measurements (assessing airway reactivity to methacholine challenge) revealed slightly greater respiratory compliance in 3R4F- than E-cig- or air-exposed groups, which is consistent with a developing emphysema phenotype (Fig. 3). Consistent with this finding, we observed *1*) a trend ($P = 0.08$) for peak expiratory flow to be lower in 3R4F- than E-cig- and air-exposed mice, as assessed via whole body plethysmography (Table 2), and *2*) histology of the fixed lung tissue demonstrating a higher emphysematous score (air space enlargement) and more pigmented macrophages in the lungs of 3R4F-exposed mice ($P < 0.05$) but no effect in E-cig-exposed compared with air-exposed mice (Table 3).

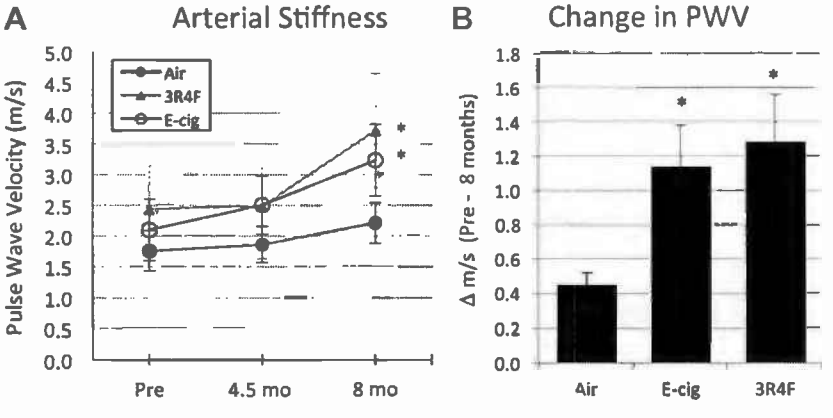


Fig. 1. B-mode Doppler ultrasound in vivo data from the carotid artery of mice under anesthesia (inhaled isoflurane) before, during (at ~4.5 mo), and after 8 mo of chronic exposure to electronic cigarette (E-cig) vapor and reference tobacco (3R4F) cigarette smoke. *A*: significant increase in arterial stiffness [measured as pulse wave velocity (PWV)] for E-cig and 3R4F groups following 8-mo exposure. *B*: significantly greater change in PWV (translating to greater arterial stiffness) after 8 mo in E-cig- and 3R4F-exposed than control (air-exposed) mice. Slight, nonsignificant, rise in PWV in control mice following 8 mo is consistent with the normal aging effect. $n = 5$–8 mice/group. *$P < 0.05$ vs. air.

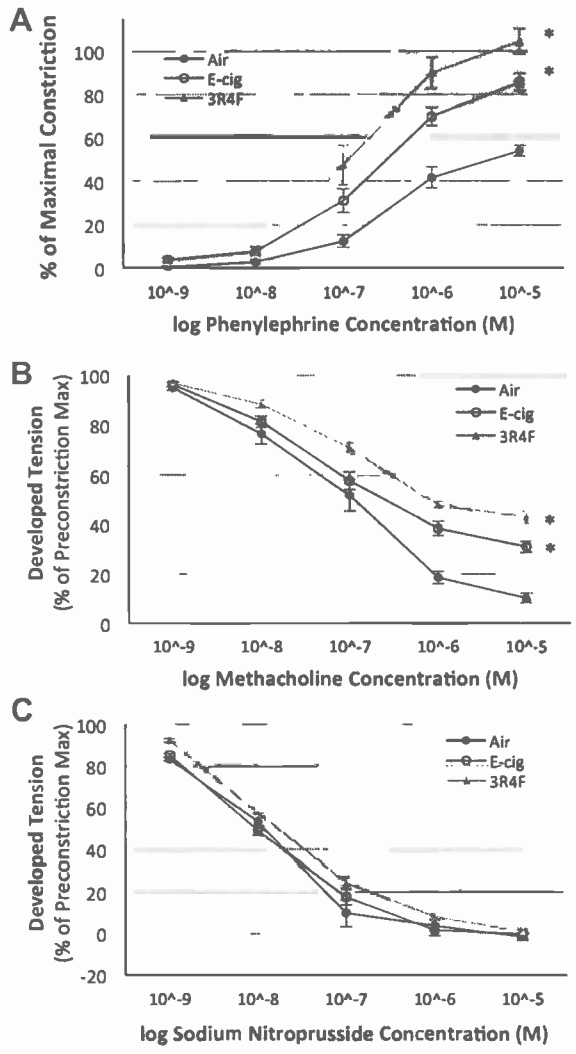


Fig. 2. Ex vivo dose-response curves for phenylephrine (*A*), methacholine (*B*), and sodium nitroprusside (*C*) obtained from thoracic aorta ring segments following 8 mo of exposure to E-cig vapor, reference tobacco (3R4F) cigarette smoke, and filtered air. α-Adrenergic vasoconstrictor response was greater (*A*), while the endothelium-mediated vasodilatory response was impaired (*B*). following 8 mo of exposure to E-cig vapor and 3R4F cigarette smoke. Non-endothelium-dependent response (*C*) to sodium nitroprusside was not altered or different between groups, demonstrating that exposure to E-cig vapor and 3R4F smoke resulted in direct harm to endothelial cell-mediated mechanisms. $n = 5$ mice/group. $^*P < 0.05$ vs. air.

## DISCUSSION

The principal finding of this study is that habitual E-cig use leads to vascular dysfunction, such as a significant increase in AS, reduced vascular relaxation to vasodilators, and enhanced responses to vascular constrictor agents. These findings are associated with the development of CVD (14) and qualitatively relate to other well-known CVD risk factors, including smoking traditional cigarettes.

*Vascular and cardiac responses.* The key observation from this study is that even low levels (see below) of exposure to E-cig vapor increased AS and impaired ex vivo vascular responses. The clinical relevance of our findings can be demonstrated by relating the degree of the arterial dysfunction we observed in the present study to other well-known CVD risk factors (Fig. 4). *1*) Previous reports indicate that smoking increases central AS from 0.6 to 1.1 m/s (6, 40, 57, 66). In context, a 1-m/s increase in central PWV corresponds to an age-, sex-, and risk factor-adjusted risk increase of 15% in cardiovascular and all-cause mortality (67). Thus the 1.14- and 1.28-m/s increase in AS (i.e., ΔPWV shown in Fig. 1*B*) we observed in the present study would reflect an ~17–19%

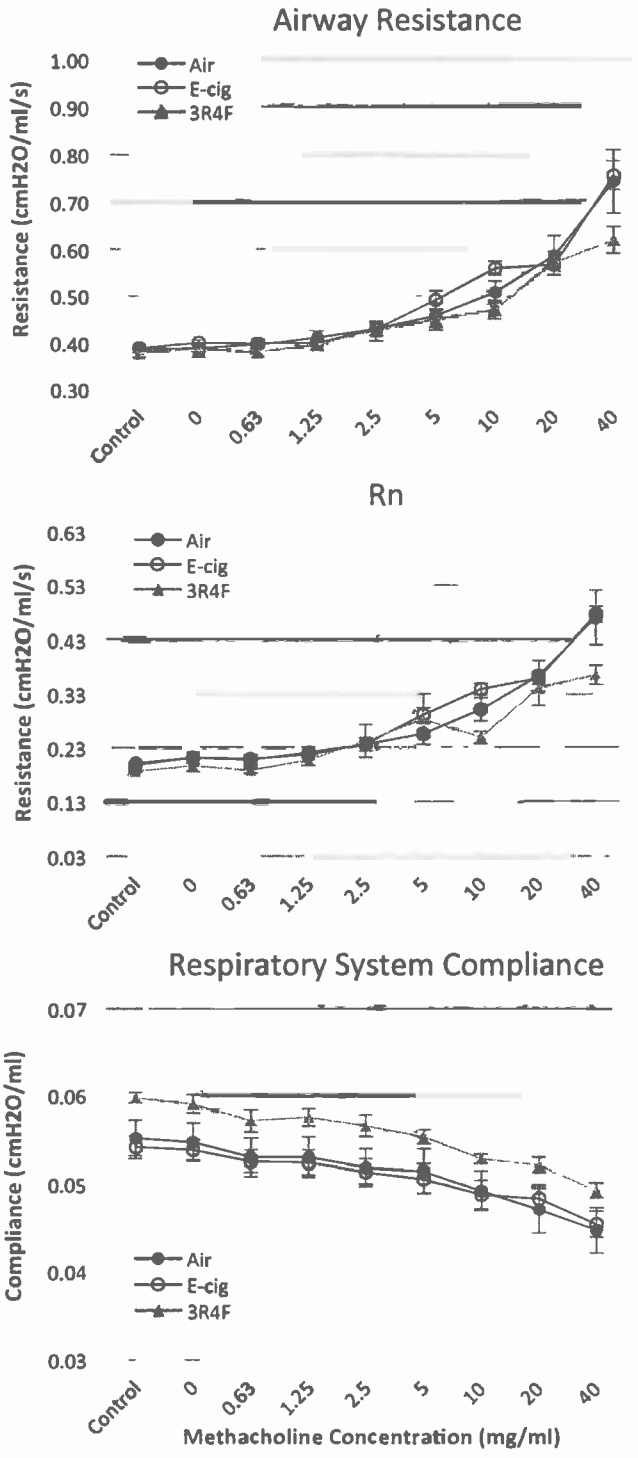


Fig. 3. Airway resistance, lung compliance (Rn), and respiratory system compliance in response to inhaled methacholine dose challenge in anesthetized (intraperitoneal ketamine-xylazine) and paralyzed mice on a flexiVent ventilator following 8 mo of exposure to E-cig vapor, reference tobacco (3R4F) smoke, and/or filtered air. $n$ = 8–10 mice/group.

Table 2. *Respiratory function measured in awake, resting, mice at ~8 mo of chronic exposure*

| | Groups | | | *P* Value |
|---|---|---|---|---|
| | Air ($n$ = 13) | E-cig ($n$ = 11) | 3R4F ($n$ = 13) | (ANOVA) |
| Frequency, breaths/min | 384 ± 10 | 408 ± 10 | 384 ± 9 | 0.15 |
| Tidal volume, ml | 0.72 ± 0.08 | 0.70 ± 0.04 | 0.59 ± 0.05 | 0.26 |
| Minute ventilation, ml min | 271 ± 28 | 281 ± 12 | 223 ± 18 | 0.12 |
| Peak inspiratory flow | 16.8 ± 1.4 | 18.1 ± 0.8 | 15.0 ± 0.8 | 0.14 |
| Peak expiratory flow | 17.4 ± 0.6 | 18.6 ± 0.9 | 15.5 ± 1.2 | 0.08 |

Values are means ± SE.

increased risk of all-cause mortality with chronic use of E-cigs and conventional cigarettes in humans. *2*) When comparing our results with other studies assessing aortic reactivity in rodents with either overt CVD (e.g., hypertension and atherosclerosis) or known CVD risk factors (e.g., stress, hyperlipidemia, and diabetes), we found that E-cig exposure for 8 mo created a risk for CVD similar to several other well-known risk factors, including smoking (Fig. 4).

Because a measurable deficit in AS was only seen in our mice after 4.5 mo of exposure, it might be tempting to think that the same relative duration in humans (i.e., ~15 yr) will be the time frame needed to achieve vascular dysfunction in humans. This notion should be viewed with caution, since it is likely that our mice experienced much lower levels of E-cig vapor than are likely to occur in humans. For chronic smoke chamber studies, daily total particulate matter (TPM) in a range of 100–250 mg/m$^3$ has typically been used to induce chronic obstructive pulmonary disease (COPD) in small animals (20, 39, 69). Our average daily chamber TPM from the E-cigs was much lower [i.e., 59 ± 14 (SD) mg/m$^3$]. This means that our exposure was less than half of the average concentration range typically used to elicit COPD symptoms with cigarette smoke in animal studies (20, 39, 69). Yet the fact that vascular dysfunction was observed, despite the lower exposure level, suggests that the threshold to induce vascular injury may be "very low." This finding is consistent with evidence showing a marked increase in cardiovascular risk even at "low levels" of cigarette exposure (7, 37). A recent study in humans also reports an acute effect of E-cig exposure (to just 9 puffs) of impaired FMD, a noninvasive measure of endothelium-mediated vascular function linked to NO bioavailability that is frequently used to assess vascular function in humans (10). Given that endothelial dysfunction, even if temporary, can be seen early in atherogenesis (14), we believe that these data collectively suggest that the threshold for damage from E-cigs is likely low with respect to the vascular system, similar to that observed with traditional cigarettes (7, 37).

A potential explanation for increased risk from low-level exposures may be that E-cigs produce elevated levels of particulate matter (PM) in the ultrafine (<100 nm) and $PM_{2.5}$ (<2.5 μm) range (47, 48). While some studies indicate similar distribution of the particle size from E-cigs and tobacco cigarettes in the submicrometer range (~125–160 nm) (29, 70, 76), there is also evidence that E-cigs deliver more ultrafine (<1-μm) particles (23, 42, 43). Ultrafine and submicrometer particles are more easily brought into and out of the lung and penetrate more deeply than larger (micrometer) particles (42). Nanoparticles also easily traverse the alveolar-capillary interface and gain direct access to vascular endothelial cells and the bloodstream, which could explain the robust and rapid systemic vascular effects that have recently been observed in response to acute E-cig use (10).

One aspect that cannot be addressed by our study is determination of the component(s) in E-cig vapor responsible for mediating these vascular effects. For example, nicotine is known to induce significant effects on the cardiovascular system. In humans and rodents, nicotine increases blood pressure and has been linked to arterial remodeling (75). The arterial response to phenylephrine-induced contraction is greater in nicotine-treated than control rats, and nicotine-treated animals showed impaired endothelium-dependent relaxation to acetylcholine compared with control rats (75), demonstrating that nicotine alone is capable of inducing vascular dysfunction. However, the role of nicotine in CVD risk is controversial, since CVD risk is low (or lower) in individuals who use nicotine medications or smokeless tobacco products compared with active smokers (5). However, very few long-term exposure studies have been conducted with inhaled or aerosolized nicotine, and this route involves less contact with other cells and nicotine metabolism before contact with the vascular endothelium. So, while nicotine replacement therapies do not appear to increase CVD risk, the long-term effects of inhaling nicotine (in the absence of combustion of tobacco) are still poorly understood.

Since nicotine is capable of acutely increasing vascular wall stiffness (due to its effects on the central nervous system), temporal increases in AS that do not reflect vessel remodeling can be observed immediately following acute exposures. However, the changes in AS and ex vivo aortic ring tension

Table 3. *Histological scoring of fixed lung tissue*

| | Groups | | | *P* Value (Kruskal-Wallis) |
|---|---|---|---|---|
| | Air ($n$ = 12) | E-cig ($n$ = 11) | 3R4F ($n$ = 13) | |
| Inflammation | | | | |
| Acute | 0.0 ± 0.0 | 0.09 ± 0.09 | 0.15 ± 0.10 | 0.805 |
| Chronic | 1.33 ± 0.26 | 1.09 ± 0.16 | 1.62 ± 0.27 | 0.462 |
| Emphysematous changes (air space enlargement) | 0.0 ± 0.0 | 0.18 ± 0.12 | 1.00 ± 0.28*§ | **0.019** |
| Alveolar macrophages | 1.3 ± 0.3 | 1.0 ± 0.0 | 1.6 ± 0.2 | 0.132 |
| Pigmented macrophages | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.69 ± 0.26*§ | **0.0002** |
| Smooth muscle hyperplasia (main stem bronchus) | 0.08 ± 0.08 | 0.27 ± 0.14 | 0.62 ± 0.24 | 0.251 |
| Fibrosis | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.08 ± 0.08 | 0.931 |

Values are means ± SE. Scoring criteria are as follows: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked. Boldface indicates statistical significance. *$P$ < 0.05 vs. air and §$P$ < 0.05 vs. E-cig.

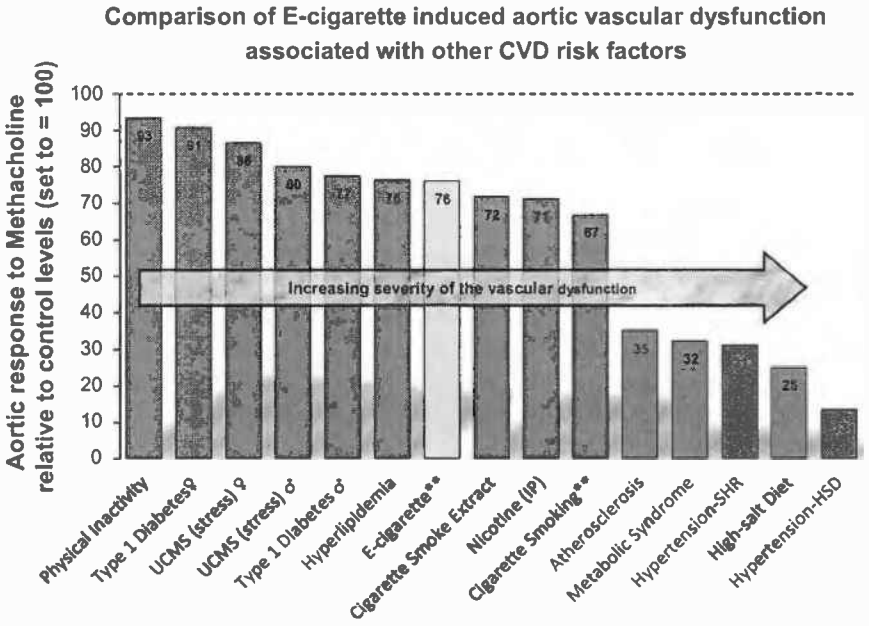


Fig. 4. Magnitude of aortic vascular dysfunction from E-cig vapor compared with other rodent studies (using the same or similar ex vivo methodology) evaluating aortic responses to various known cardiovascular disease (CVD) risk factors and/or overt CVD (e.g., atherosclerosis and hypertension). All data represent maximal methacholine dose reported (e.g., $10^{-5}$ M) compared with control conditions within each respective study, where controls were set to equal 100 and change in the treatment/disease condition is calculated. UCMS, unpredictable chronic mild stress; IP, intraperotineal; SHR, spontaneously hypertensive rats; HSD, high-salt diet. **Data from the present study.

observed in our study are not likely related to the acute exposure effects, since all our assessments were made ≥24 h after smoke/vapor exposure. Moreover, the halfway time-point assessment for AS (i.e., PWV; Fig. 1) does not show significant change compared with baseline; therefore, we do not believe that our data are the result of lingering acute effects. Also, the level of urine cotinine (a stable by-product of nicotine used as a biomarker for nicotine due to its short half-life) in our E-cig-exposed mice was almost half that in 3R4F-exposed mice, yet the degree of vascular dysfunction was very similar between the groups. This could suggest that some component of the E-cig liquid (other than nicotine) may have a greater influence on vascular impairment. Further studies are required to elucidate these effects.

Our chronic exposure resulted in small, but statistically significant, decreases in FS% and EF% in 3R4F-exposed mice, with a similar (although not significant) trend for E-cig-exposed mice ($P < 0.10$; Table 1). Based on these data, this level of E-cig exposure did not result in overt cardiac dysfunction. However, LV mass was greater in the E-cig- than air- or 3R4F-exposed mice. Although this finding can signify cardiac remodeling, its significance is unclear, as reduced cardiac performance was observed only in 3R4F-exposed mice (in which LV mass was not different from controls). It may be tempting to speculate that E-cigs have little impact on cardiac function, but we cannot rule out the possibility that the subtle changes we observed could progress to pathological outcomes with more intense and/or longer exposure. Further research is needed to elucidate such effects, as well as other responses (i.e., development of hypertension and histological assessment of aortic remodeling) that was not determined from our present study.

*Respiratory responses.* We observed changes in lung histology (Table 3) and respiratory system compliance (Fig. 4) in 3R4F-exposed, but not E-cig- or air-exposed, mice. The changes in 3R4F-exposed mice (i.e., higher compliance, more pigmented macrophages, and higher emphysema score) are consistent with the well-known effects of cigarette smoke. While our pulmonary findings are consistent with findings from at least one other study that used the C57BL/6 murine model (54), studies using other mouse strains (e.g., A/J or BALB/c) demonstrate that E-cig vapor does induce histological changes and impairment in airway and lung mechanical properties similar to a COPD phenotype (24, 36). Thus it is possible that the lack of pulmonary effects from E-cigs in our study may be due to *1*) the selected inbred mouse strain we used and/or *2*) the relatively mild TPM exposure in our paradigm (see above). Given growing evidence from cellular and in vivo animal studies, as well as acute studies in humans, showing the toxicity of E-cig vapor in airway cells and respiratory function [see review (16)], we would caution against the interpretation (based on our data) that E-cigs are safe for the lung.

*Clinical relevance to humans and study limitations.* Some might argue that intermittent E-cig use for a total of 4 h/day seems too high or unrealistic for the average E-cig user. However, when examining data anonymously volunteered from >180,000 Evolv DNA-series E-cig devices via the ECig-Stats data collection program (www.ecigstats.org, accessed April 6, 2017) that records user usage characteristics, we found that the average number of puffs per day reported across all devices is 172 ± 131 (SD). This is actually higher than the 152–156 puffs/day used in this study (38–39 puffs per hour × 4 h). However, the ECigStats data also report that devices/users have an average puff duration time of 2.31 ± 2.11 (SD) s. The nearly identical mean and SD of the puff duration (2.31 and 2.11, respectively) indicates a wide variability in individual usage characteristics, with nearly one-third of the average users adopting long (up to 4.4-s) puff durations. Our E-cig usage characteristics (i.e., 5-s, 55-ml puff with the device set at 4.9 V, 14.1 W) are actually similar to those of several recent scientifically controlled studies examining E-cig puff topography, showing that the average "experienced" E-cig user adopts longer (e.g., 4–8 s) puff durations (61, 63). Moreover, an "average-experienced" E-cig user (with a 4-s puff duration) is reported to generate 29.4–152.7 mg of TPM, depending on

puff velocity and voltage (i.e., 3.3 vs. 5.2 V, respectively), but an "extremely experienced" E-cig user (with an 8-s puff duration) can generate 68.8–333.2 mg of TPM (63). So, when comparing the total daily TPM achieved by experienced E-cig users with our chamber exposure, we find that the mice (with a daily TPM average of 59 mg) received the lower end of TPM compared with most experienced E-cig users. Thus, despite the similarities in E-cig topology between our animal exposure and human usage, it is possible that our exposure paradigm underestimates the effect that will be experienced by a human E-cig user. Our use of a chamber exposure paradigm may also have a dampening effect on our outcomes, because rodents are obligate nasal breathers, and the nose can effectively filter many airborne particles compared with direct inhalation via the mouth (as humans would experience). Together with the caveat that our animals experienced a lower level of TPM exposure than the average E-cig user, this could also potentially explain why we saw minimal pulmonary abnormalities in our study. Nevertheless, the mice developed significant vascular dysfunction, suggesting that the degree of vascular dysfunction may not be substantially different between E-cigs and conventional cigarettes. The fact that we observed minimal cardiac and pulmonary changes could mean that these organs have a greater threshold and/or resilience to functional impairments and that endothelial and vascular dysfunction is simply the first step and harbinger in the etiology of CVD (15, 25, 49).

An additional clinical consideration is that we studied only female mice. The chamber exposure method we used necessitated the use of female mice to reduce fighting and injury when mice (otherwise housed in separate cages) were temporarily grouped communally for several hours each day during the exposures. We do not know if male mice would have exhibited the same level of vascular dysfunction. Consistent with human studies (53), rodent studies have also found that females are less sensitive than males to the pharmacological effects of nicotine (27, 58), in part because of protective effects of female hormones (8). Thus one could speculate that female mice may have a dampened response and that E-cig exposure in males may result in greater CVD risk and worse outcomes (3, 8). Future studies must include both sexes in these evaluations.

A broad concern is the relevance of E-cig-related cardiovascular responses in mice compared with humans, especially given the potential difference we have noted with respect to mouse strains. Indeed, it is too early to know if vaping effects in rodents will faithfully recapitulate those ultimately seen in humans. Given the wide variations and options possible with E-cig vapor exposures (i.e., varying quality of devices, different device settings, and varying concentration of constituents in the E-cig liquid and/or formulation of the base solution), it is likely that varying and, possibly, divergent outcomes may be observed, depending on the exposure paradigm. However, broadly speaking, we would emphasize that decades of evidence from cigarette smoking demonstrate good fidelity with pulmonary and cardiovascular outcomes observed between rodents and humans (22, 71, 72). We would also suggest, even given the limited evidence that currently exists, from the preponderance of data obtained from interventional studies (including this study), that it seems counterintuitive to believe or expect that long-term use of E-cigs will likely prove to be safe in terms of overall human health.

*Conclusions.* To our knowledge, these are the first interventional data to show the long-term cardiovascular health consequences of chronic E-cig use. While our exposure paradigm resulted in no significant changes in the pulmonary outcomes we measured in E-cig-exposed mice and only a small change in cardiac function, we caution that the potential interpretation that E-cigs are safe is likely short-sighted, especially given the relatively low level of exposure with our exposure paradigm. Rather, despite the relatively low daily exposure level, we found significant increases in AS and adrenergic-mediated vasoconstriction and impaired endothelium-dependent vasodilation. These data indicate significant vascular dysfunction induced by E-cig vapor exposure. Based on existing evidence, the level of vascular dysfunction is similar to that observed for other known risk factors leading to CVD, including smoking tobacco cigarettes.

The clinical implication is that chronic use of E-cigs impairs vascular function. Future animal studies will be able to determine the potential time-course effects of varying exposure usage levels and the contribution of specific components of the E-cig liquid/vapor (e.g., carbonyl compounds, nicotine, and flavorings) to the etiology toward arterial dysfunction. These data should be viewed as a harbinger of the potential effects on humans, such that E-cig use should not be considered safe and perhaps even questionable as a harm-reduction device, given the low potential threshold for inducing vascular injury. Diligent clinical monitoring of vascular health should be encouraged in adolescent and adult E-cig users.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

I.M.O., Z.-X.W., P.K., B.H.E., R.W.B., and P.D. conceived and designed research; I.M.O., E.D., H.H., K.W.B., S.C., C.R.P., D.P.S., M.J.B., Z.-X.W., W.K.M., B.H.E., B.S.D., and P.D.C. analyzed data; I.M.O., E.D., K.W.B., D.P.S., M.J.B., Z.-X.W., W.K.M., B.S.D., R.W.B., and P.D.C. interpreted results of experiments; I.M.O. and E.D. prepared figures; I.M.O. drafted manuscript; I.M.O. and P.D.C. edited and revised manuscript; I.M.O., E.D., H.H., K.W.B., S.C., C.R.P., D.P.S., M.J.B., Z.-X.W., P.K., W.K.M., B.H.E., B.S.D., R.W.B., P.D., and P.D.C. approved final version of manuscript; E.D., H.H., K.W.B., S.C., C.R.P., M.J.B., and B.H.E. performed experiments.

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# EXHIBIT B

CM-010

ATTORNEY OR PARTY WITHOUT ATTORNEY (Name, State Bar number, and address):
Steven W. Ritcheson, Esq. (SBN 174062)
Insight PLLC, 9800 D Topanga Canyon Blvd#347, Chatsworth, California 91311

TELEPHONE NO.: 818-882-1030 FAX NO.: 818-337-0383
ATTORNEY FOR (Name): Timothy Malaney, Brendan Gorman, and the Class

FOR COURT USE ONLY

FILED
Superior Court of California
County of Los Angeles
OCT 26 2018
Sherri R. Carter, Executive Officer/Clerk
By _____, Deputy
Steven Drew

SUPERIOR COURT OF CALIFORNIA, COUNTY OF Los Angeles
STREET ADDRESS: 111 North Hill Street
MAILING ADDRESS: 111 North Hill Street
CITY AND ZIP CODE: Los Angeles, CA 90012
BRANCH NAME: Stanley Mosk Courthouse

CASE NAME:
Malaney v. JUUL

CASE NUMBER: 18STCV02948

JUDGE:

DEPT:

**CIVIL CASE COVER SHEET**

☑ Unlimited (Amount demanded exceeds $25,000) ☐ Limited (Amount demanded is $25,000 or less)

**Complex Case Designation**

☐ Counter ☐ Joinder

Filed with first appearance by defendant (Cal. Rules of Court, rule 3.402)

*Items 1–6 below must be completed (see instructions on page 2).*

1. Check **one** box below for the case type that best describes this case:

**Auto Tort**
- ☐ Auto (22)
- ☐ Uninsured motorist (46)

**Other PI/PD/WD (Personal Injury/Property Damage/Wrongful Death) Tort**
- ☐ Asbestos (04)
- ☑ Product liability (24)
- ☐ Medical malpractice (45)
- ☐ Other PI/PD/WD (23)

**Non-PI/PD/WD (Other) Tort**
- ☐ Business tort/unfair business practice (07)
- ☐ Civil rights (08)
- ☐ Defamation (13)
- ☐ Fraud (16)
- ☐ Intellectual property (19)
- ☐ Professional negligence (25)
- ☐ Other non-PI/PD/WD tort (35)

**Employment**
- ☐ Wrongful termination (36)
- ☐ Other employment (15)

**Contract**
- ☐ Breach of contract/warranty (06)
- ☐ Rule 3.740 collections (09)
- ☐ Other collections (09)
- ☐ Insurance coverage (18)
- ☐ Other contract (37)

**Real Property**
- ☐ Eminent domain/Inverse condemnation (14)
- ☐ Wrongful eviction (33)
- ☐ Other real property (26)

**Unlawful Detainer**
- ☐ Commercial (31)
- ☐ Residential (32)
- ☐ Drugs (38)

**Judicial Review**
- ☐ Asset forfeiture (05)
- ☐ Petition re: arbitration award (11)
- ☐ Writ of mandate (02)
- ☐ Other judicial review (39)

**Provisionally Complex Civil Litigation (Cal. Rules of Court, rules 3.400–3.403)**
- ☐ Antitrust/Trade regulation (03)
- ☐ Construction defect (10)
- ☐ Mass tort (40)
- ☐ Securities litigation (28)
- ☐ Environmental/Toxic tort (30)
- ☐ Insurance coverage claims arising from the above listed provisionally complex case types (41)

BY FAX

**Enforcement of Judgment**
- ☐ Enforcement of judgment (20)

**Miscellaneous Civil Complaint**
- ☐ RICO (27)
- ☐ Other complaint *(not specified above)* (42)

**Miscellaneous Civil Petition**
- ☐ Partnership and corporate governance (21)
- ☐ Other petition *(not specified above)* (43)

2. This case ☑ is ☐ is not complex under rule 3.400 of the California Rules of Court. If the case is complex, mark the factors requiring exceptional judicial management:
   a. ☑ Large number of separately represented parties
   b. ☑ Extensive motion practice raising difficult or novel issues that will be time-consuming to resolve
   c. ☑ Substantial amount of documentary evidence
   d. ☑ Large number of witnesses
   e. ☐ Coordination with related actions pending in one or more courts in other counties, states, or countries, or in a federal court
   f. ☑ Substantial postjudgment judicial supervision
3. Remedies sought *(check all that apply)*: a. ☑ monetary b. ☑ nonmonetary; declaratory or injunctive relief c. ☑ punitive
4. Number of causes of action *(specify)*: 11
5. This case ☑ is ☐ is not a class action suit.
6. If there are any known related cases, file and serve a notice of related case. *(You may use form CM-015.)*

Date: 10/26/2018
Steven W. Ritcheson
(TYPE OR PRINT NAME)

S W Ritcheson
(SIGNATURE OF PARTY OR ATTORNEY FOR PARTY)

**NOTICE**
- Plaintiff must file this cover sheet with the first paper filed in the action or proceeding (except small claims cases or cases filed under the Probate Code, Family Code, or Welfare and Institutions Code). (Cal. Rules of Court, rule 3.220.) Failure to file may result in sanctions.
- File this cover sheet in addition to any cover sheet required by local court rule.
- If this case is complex under rule 3.400 et seq. of the California Rules of Court, you must serve a copy of this cover sheet on all other parties to the action or proceeding.
- Unless this is a collections case under rule 3.740 or a complex case, this cover sheet will be used for statistical purposes only.



Form Adopted for Mandatory Use
Judicial Council of California
CM-010 [Rev. July 1, 2007]

CIVIL CASE COVER SHEET

Cal. Rules of Court, rules 2.30, 3.220, 3.400–3.403, 3.740;
Cal. Standards of Judicial Administration, std. 3.10
www.courtinfo.ca.gov

# EXHIBIT C

SUM-100

# SUMMONS
## (CITACION JUDICIAL)

**NOTICE TO DEFENDANT:**
***(AVISO AL DEMANDADO):***

JUUL LABS, INC; and PAX LABS, INC

**YOU ARE BEING SUED BY PLAINTIFF:**
***(LO ESTÁ DEMANDANDO EL DEMANDANTE):***

Timothy Malaney; and Brendan Gorman

*FOR COURT USE ONLY*
*(SOLO PARA USO DE LA CORTE)*

**FILED**
Superior Court of California
County of Los Angeles

OCT 26 2018

Sherri R. Carter, Executive Officer/Clerk
By _____, Deputy
Steven Drew

**NOTICE!** You have been sued. The court may decide against you without your being heard unless you respond within 30 days. Read the information below.

You have 30 CALENDAR DAYS after this summons and legal papers are served on you to file a written response at this court and have a copy served on the plaintiff. A letter or phone call will not protect you. Your written response must be in proper legal form if you want the court to hear your case. There may be a court form that you can use for your response. You can find these court forms and more information at the California Courts Online Self-Help Center (*www.courtinfo.ca.gov/selfhelp*), your county law library, or the courthouse nearest you. If you cannot pay the filing fee, ask the court clerk for a fee waiver form. If you do not file your response on time, you may lose the case by default, and your wages, money, and property may be taken without further warning from the court.

There are other legal requirements. You may want to call an attorney right away. If you do not know an attorney, you may want to call an attorney referral service. If you cannot afford an attorney, you may be eligible for free legal services from a nonprofit legal services program. You can locate these nonprofit groups at the California Legal Services Web site (*www.lawhelpcalifornia.org*), the California Courts Online Self-Help Center (*www.courtinfo.ca.gov/selfhelp*), or by contacting your local court or county bar association. **NOTE:** The court has a statutory lien for waived fees and costs on any settlement or arbitration award of $10,000 or more in a civil case. The court's lien must be paid before the court will dismiss the case.

*¡AVISO! Lo han demandado. Si no responde dentro de 30 días, la corte puede decidir en su contra sin escuchar su versión. Lea la información a continuación.*

*Tiene 30 DÍAS DE CALENDARIO después de que le entreguen esta citación y papeles legales para presentar una respuesta por escrito en esta corte y hacer que se entregue una copia al demandante. Una carta o una llamada telefónica no lo protegen. Su respuesta por escrito tiene que estar en formato legal correcto si desea que procesen su caso en la corte. Es posible que haya un formulario que usted pueda usar para su respuesta. Puede encontrar estos formularios de la corte y más información en el Centro de Ayuda de las Cortes de California (www.sucorte.ca.gov), en la biblioteca de leyes de su condado o en la corte que le quede más cerca. Si no puede pagar la cuota de presentación, pida al secretario de la corte que le dé un formulario de exención de pago de cuotas. Si no presenta su respuesta a tiempo, puede perder el caso por incumplimiento y la corte le podrá quitar su sueldo, dinero y bienes sin más advertencia.*

*Hay otros requisitos legales. Es recomendable que llame a un abogado inmediatamente. Si no conoce a un abogado, puede llamar a un servicio de remisión a abogados. Si no puede pagar a un abogado, es posible que cumpla con los requisitos para obtener servicios legales gratuitos de un programa de servicios legales sin fines de lucro. Puede encontrar estos grupos sin fines de lucro en el sitio web de California Legal Services, (www.lawhelpcalifornia.org), en el Centro de Ayuda de las Cortes de California, (www.sucorte.ca.gov) o poniéndose en contacto con la corte o el colegio de abogados locales. AVISO: Por ley, la corte tiene derecho a reclamar las cuotas y los costos exentos por imponer un gravamen sobre cualquier recuperación de $10,000 ó más de valor recibida mediante un acuerdo o una concesión de arbitraje en un caso de derecho civil. Tiene que pagar el gravamen de la corte antes de que la corte pueda desechar el caso.*

The name and address of the court is:
*(El nombre y dirección de la corte es):* Stanley Mosk Courthouse
111 N. Hill St., Losa Angeles, CA 90012

CASE NUMBER:
*(Número del Caso):* 18STCV02948

The name, address, and telephone number of plaintiff's attorney, or plaintiff without an attorney, is:
*(El nombre, la dirección y el número de teléfono del abogado del demandante, o del demandante que no tiene abogado, es):*
Steven W. Ritcheson, 9800 D Topanga Canyon Blvd. #347, Chatsworth, California 91311, 818-882-1030

DATE: 10/26/18 *(Fecha)*
Sherri R. Carter, Clerk
Clerk, by *(Secretario)* _____, Deputy *(Adjunto)*

*(For proof of service of this summons, use* Proof of Service of Summons *(form POS-010).)*
*(Para prueba de entrega de esta citatión use el formulario* Proof of Service of Summons, *(POS-010)).*

STEVEN DREW

[SEAL]

**NOTICE TO THE PERSON SERVED:** You are served
1. ☐ as an individual defendant.
2. ☐ as the person sued under the fictitious name of *(specify):*
3. ☑ on behalf of *(specify):*
   under: ☑ CCP 416.10 (corporation)
   ☐ CCP 416.20 (defunct corporation)
   ☐ CCP 416.40 (association or partnership)
   ☐ other *(specify):*
   ☐ CCP 416.60 (minor)
   ☐ CCP 416.70 (conservatee)
   ☐ CCP 416.90 (authorized person)
4. ☐ by personal delivery on *(date):*



Form Adopted for Mandatory Use
Judicial Council of California
SUM-100 [Rev. July 1, 2009]

**SUMMONS**

Code of Civil Procedure §§ 412.20, 465
www.courtinfo.ca.gov

# EXHIBIT D

**SUPERIOR COURT OF CALIFORNIA**
**COUNTY OF LOS ANGELES**

COURTHOUSE ADDRESS:
Spring Street Courthouse
312 North Spring Street, Los Angeles, CA 90012

Reserved for Clerk's File Stamp

FILED
Superior Court of California
County of Los Angeles
10/26/2018
Sherri R. Carter, Executive Officer / Clerk of Court
By: Steve Drew Deputy

# NOTICE OF CASE ASSIGNMENT

# UNLIMITED CIVIL CASE

**Your case is assigned for all purposes to the judicial officer indicated below.**

CASE NUMBER:
18STCV02948

**THIS FORM IS TO BE SERVED WITH THE SUMMONS AND COMPLAINT**

| | ASSIGNED JUDGE | DEPT | ROOM | | ASSIGNED JUDGE | DEPT | ROOM |
|---|---|---|---|---|---|---|---|
| ✓ | Kenneth R. Freeman | 14 | | | | | |

Given to the Plaintiff/Cross-Complainant/Attorney of Record

Sherri R. Carter, Executive Officer / Clerk of Court

on 10/30/2018 (Date)

By Steve Drew, Deputy Clerk

# EXHIBIT E

# SUPERIOR COURT OF CALIFORNIA, COUNTY OF LOS ANGELES

**Civil Division**

Central District, Spring Street Courthouse, Department 14

**18STCV02948** November 13, 2018
**TIMOTHY MALANEY, et al. vs JUUL LABS, INC., et al.** 1:00 PM

Judge: Honorable Kenneth R. Freeman CSR: None
Judicial Assistant: R. Arraiga ERM: None
Courtroom Assistant: D. McKinney Deputy Sheriff: None

APPEARANCES:

For Plaintiff(s): No Appearances

For Defendant(s): No Appearances

**NATURE OF PROCEEDINGS:** Court Order Regarding Newly Filed Class Action;

By this order, the Court determines this case to be Complex according to Rule 3.400 of the California Rules of Court. The Clerk's Office has randomly assigned this case to this department for all purposes.

By this order, the Court stays the case, except for service of the Summons and Complaint. The stay continues at least until the Initial Status Conference. Initial Status Conference is set for 02/08/2019 at 10:00 AM in this department. At least 10 court days prior to the Initial Status Conference, counsel for all parties must discuss the issues set forth in the Initial Status Conference Order issued this date. The Initial Status Conference Order is to help the Court and the parties manage this complex case by developing an orderly schedule for briefing, discovery, and court hearings. The parties are informally encouraged to exchange documents and information as may be useful for case evaluation.

Responsive pleadings shall not be filed until further Order of the Court. Parties must file a Notice of Appearance in lieu of an Answer or other responsive pleading. The filing of a Notice of Appearance shall not constitute a waiver of any substantive or procedural challenge to the Complaint. Nothing in this order stays the time for filing an Affidavit of Prejudice pursuant to Code of Civil Procedure Section 170.6.

Counsel are directed to access the following link for information on procedures in the Complex litigation Program courtrooms: http://www.lacourt.org/division/civil/CI0037.aspx

According to Government Code section 70616 subdivisions (a) and (b), each party shall pay a fee of $1,000.00 to the Los Angeles Superior Court within 10 calendar days from this date.

The plaintiff must serve a copy of this minute order and the attached Initial Status Conference

**SUPERIOR COURT OF CALIFORNIA, COUNTY OF LOS ANGELES**
**Civil Division**
Central District, Spring Street Courthouse, Department 14

**18STCV02948** November 13, 2018
**TIMOTHY MALANEY, et al. vs JUUL LABS, INC., et al.** 1:00 PM

Judge: Honorable Kenneth R. Freeman CSR: None
Judicial Assistant: R. Arraiga ERM: None
Courtroom Assistant: D. McKinney Deputy Sheriff: None

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Order on all parties forthwith and file a Proof of Service in this department within 7 days of service.

Certificate of Mailing is attached.