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Early View

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E-cigarette Use and Respiratory Disorder: An Integrative Review of Converging Evidence from Epidemiological and Laboratory Studies

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Second revision

E-cigarette Use and Respiratory Disorder: An Integrative Review of Converging Evidence from Epidemiological and Laboratory Studies

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Abstract

Background. Use of electronic cigarettes (e-cigarettes) is prevalent among adolescents and young adults but there has been limited knowledge about health consequences in human populations. We conduct a systematic review and meta-analysis of results on respiratory disorder from studies of general-population samples and consider the mapping of these results to findings about biological processes linked to e-cigarettes in controlled laboratory studies.

Method. We conduct a literature search and meta-analysis of epidemiological studies on the association of e-cigarette use with asthma and with chronic obstructive pulmonary disease (COPD). We then discuss findings from laboratory studies about effects of e-cigarettes on four biological processes: cytotoxicity, oxidative stress/inflammation, susceptibility to infection, and genetic expression.

Results. Epidemiological studies, both cross-sectional and longitudinal, show a significant association of e-cigarette use with asthma and COPD, controlling for cigarette smoking and other covariates. For asthma (n = 15 studies), the pooled adjusted odds ratio (AOR) was 1.39 (CI 1.28-1.51); for COPD (n = 9 studies) the AOR was 1.49 (CI 1.36-1.65). Laboratory studies consistently show an effect of e-cigarettes on biological processes related to respiratory harm and susceptibility to illness, with e-cigarette conditions differing significantly from clean-air controls though sometimes less than for cigarettes.

Conclusions. The evidence from epidemiological studies meets established criteria for consistency, strength of effect, temporality, and in some cases a dose-response gradient. Biological plausibility is indicated by evidence from multiple laboratory studies. We conclude that e-cigarette use has consequences for asthma and COPD, which is of significant concern for respirology and public health.

Introduction

Use of electronic cigarettes (hereafter, e-cigarettes) and other types of electronic nicotine delivery systems is currently prevalent among adolescents and young adults [1, 2]. Recent surveys show nicotine-based e-cigarette use is common [3] and indicate that 27.5% of US high school students are current e-cigarette users [4]. This prevalence has raised concern among a broad range of public health researchers and laboratory scientists [5-8].

While there has been considerable research on the correlates of e-cigarette use [9, 10], there has been less knowledge about health consequences in human populations. A report compiled early in 2017 concluded there was no definitive evidence on whether e-cigarettes cause respiratory disease in humans [11]. However, since then there has been considerable evidence on health variables from epidemiological investigations of large general-population samples, from laboratory studies of biological processes linked to e-cigarettes, and from case reports based on single patients that have provided examples of respiratory disease associated with e-cigarette use [12-17].

The aim of this paper is to provide a comprehensive review and meta-analysis of evidence from epidemiological studies about the association of e-cigarette use with asthma and chronic obstructive pulmonary disease (COPD) in human populations and to discuss this evidence in relation to findings from controlled laboratory studies of biological processes affected by e-cigarette use. Epidemiological studies indicate findings that occur in the natural environment of the participants and allow control for potential confounders. Laboratory studies have particular evidentiary value because they use experimental methods that allow for strict causal conclusions. Our review considers data from adolescents as well as adults because of the prognostic significance of early respiratory symptomatology for lung disease at later ages [18-20]. We do not consider research on e-cigarettes and cardiovascular disease [21, 22] and we do not cover research on epigenetic and intergenerational effects [23-26].

For the epidemiological evidence we provide a comprehensive review of all available studies and provide a meta-analysis of aggregate effect sizes across studies of asthma and COPD. For laboratory research we discuss selected studies that are most relevant for interpreting the epidemiological findings on respiratory outcomes, as detailed reviews of laboratory research are available in focused areas [27, 28]. The present paper is the first to conceptually link the epidemiological findings to evidence from laboratory research and to discuss the implications of these bodies of research when considered together.

Evidence from Epidemiological Studies

Epidemiological studies on the association of e-cigarettes (or synonyms) with asthma and COPD (or synonyms) in general populations were identified through searches on PsycInfo and PubMed, contacts with other investigators, and searching abstracts from recent research meetings. The search was conducted in March 2020.

The PRISMA charts (Figure 1) show how exclusion and inclusion criteria were applied for entries identified on asthma and COPD. Entries were coded and evaluated for appropriateness. To be included in the review, a study had to have a large representative sample, reasonable measures of e-cigarette use and cigarette smoking, a reasonable measure of respiratory disorder, and a comparison group of nonusers of e-cigarettes and combustible cigarettes (or repeated measures of the participants). Two of the authors (TW and SS) independently examined all entries and agreed on how studies met the criteria. Of 875 entries identified for asthma, 15 studies met all inclusion criteria; of 855 entries identified for COPD, 9 studies met all criteria.

Four general issues are important for interpretation of this literature. First, because combustible cigarette smoking is correlated with e-cigarette use [29-31] and is a risk factor for respiratory disease, it is crucial to control for this correlation in multivariable analyses. As noted in the tables, most of the studies did control for cigarette smoking, indicating that observed effects for e-cigarettes are not

attributable to confounding with smoking. Second, when e-cigarette use and cigarette smoking are entered together in a multivariable analysis, if they both show significant contributions to respiratory disease (i.e., additive effects) then the implication is that persons who both use e-cigarettes and smoke cigarettes will be worse off than exclusive e-cigarette users or exclusive smokers. Notation about additive effects is provided in the tables. Third, it is possible that the association between smoking and respiratory disease is different for persons who use e-cigarettes (i.e., interaction effect). This may be tested by a stratified analysis or by entering a cross-product term for e-cigarettes and smoking in a multivariable analysis in addition to their main effects. Interaction tests are noted in the tables. An interaction with OR > 1 would indicate that the association of e-cigarette use with respiratory outcomes is greater among those who smoke (i.e., synergistic effect); an OR < 1 would that e-cigarette use has a greater effect among nonsmokers (i.e., inverse interaction). Fourth, with cross-sectional data the finding of a positive association for e-cigarettes and respiratory disease could be interpreted as meaning that persons who develop disease quit smoking cigarettes and take up e-cigarettes (i.e., reverse causation). This possibility may be addressed in cross-sectional data through internal analyses that logically would work against an interpretation of reverse causation. Alternatively, longitudinal data showing that e-cigarette use precedes disease development in time would work against a reverse-causation interpretation. This issue is addressed in the review of the studies.

Epidemiological Studies of Asthma

Characteristics of studies of asthma are presented in Table 1. Studies of adolescents typically used school-based data collection and criterion variables indicating diagnosis of asthma by a health professional. The participants in these studies were mostly high school students (15-18 years of age). Studies of adults used direct interview and telephone survey methods. Multivariable analyses typically adjusted for demographics, cigarette smoking, and other relevant covariates. (Table 1 follows)

Table 1: Epidemiological Studies of E-cigarette Use and Asthma / Bronchitis

Ref	Sample N, age	E-cig measure	Respiratory measure	Covariates	Findings	Smoking control	Additive effects	Inter-action
	ADOLESCENT STUDIES							
[32]	35,904 (10^{th}-12^{th} graders)	Ever use, 30-day use	Dx with asthma by doctor (past 12 mo.)	Smoking, demographics, obesity, SHS	AOR = 2.74 for current e-cig use (never smokers)	Yes	Yes	INV
[33]	216,056 (7^{th}-12^{th} graders)	30-day use	Dx with asthma by doctor (ever, past 12 mo.)	Smoking, age, demographics, region, obesity, SHS, exercise	AOR = 1.13 for current e-cig use for past-year asthma	Y es	Yes	n.a.
[34]	58,336 (7^{th}-12^{th} graders)	Ever use	Dx with asthma by doctor (past 12 mo.)	Demographics, age, SES, region, obesity, physical activity, SHS	AOR = 1.23 for past-year asthma	Yes	Yes	n.a.
[35]	45,128 (7^{th}-12^{th} graders)	30-day use	Cough or phlegm, 3 consecutive mo. in past 12 mo.	Smoking, demographics, SHS	AOR = 2.06 for current e-cig use (never smokers)	Yes	Yes	INV
[36]	36,085 (9^{th}-12^{th} graders)	Ever use, 30-day use	Ever Dx with asthma; still have asthma	Smoking, SHS, metro status, demographics	AOR = 2.20 for current e-cig use for current asthma	Yes	n.a.	n.a.
[37]	32,921 (9^{th}-12^{th} graders)	30-day use	Ever Dx with asthma + still have asthma	Demographics	AOR = 1.34 for current e-cig use, current asthma	No	n.a.	n.a.
[38]	11,380 (6^{th}-12^{th} graders) (with asthma)	30-day exposure to e-cig aerosol in house or car	Did you have an asthma attack (past 12 mo.)	Demographics, individual tobacco product use, SHS	AOR = 1.27 for recent aerosol exposure, recent asthma attack	Yes	Yes	EQ[A]
[39]	2,840 (9^{th}-12^{th} graders)	E-cig use, past 12 mo.	Ever Dx with asthma by doctor, nurse	Demographics, SES	AOR = 1.78 for recent e-cig use	No	n.a.	n.a.

[40]	6,089 (9^{th}-12^{th} graders)	Ever use, 30-day use	Ever Dx with asthma by doctor; still have asthma	Demographics, smoking, BMI, marijuana use, educational plans	AOR = 1.48 for current e-cig use, current asthma	Yes	Yes	EQ
[41]	14,765 (9^{th}-12^{th} graders)	Ever use, 30-day use	Ever Dx with asthma by health professional	Demographics, smoking, obesity, marijuana use	AOR = 1.30 for current e-cig use	Yes	Yes	EQ
[42]	2,086 (high school)	Ever use, 30-day use	Chronic bronchitis past 12 mo., wheezing or whistling in chest	Demographics, smoking, SHS, parental education, housing conditions	AOR = 1.71 for past e-cig use, bronchitis	Yes	Yes	n.a.
	ADULT STUDIES							
[43]	39,747 (≥18 years)	30-day use	Ever Dx with asthma by health prof.	Demographics, smoking, CHD	AOR = 1.38 for current exclusive e-cig use	Yes	Yes	n.a.
[44]	8,087 (18-79 years)	Ever use, current use	Ever Dx with asthma by health prof.	Demographics, smoking, obesity, SHS	AOR = 1.33 for current e-cig use in nonsmokers	Yes	No	INV
[45]	402,822 (> 18 years)	Ever use + current use	Ever Dx with asthma + still have asthma	Demographics, BMI	AOR = 1.39 for never smokers	Yes	n.a.	NEV
[46]	23,760 (18-65 years)	Ever use, current use	Dx asthma by health prof. ever (W1), past 12 mo. (W2, W3)	Demographics, smoking, poverty status, clinical variables	AOR = 1.56 for incident asthma for current e-cig use	Yes	Yes	n.a.

Note: E-cig = e-cigarette; Dx = diagnosed; mo. = month; prof. = professional; SHS = second-hand smoke exposure; BMI = body mass index; n.a. = not available (data not available or test not performed). For Interaction column, SYN indicates synergistic interaction (effect of e-cigarettes greater among smokers), INV = inverse interaction (effects of e-cigarettes greater among nonsmokers), EQ = effect of e-cigarettes equal in smokers and nonsmokers; NEV = analysis performed only for nonsmokers.

[A] This study tested interactions of e-cig aerosol exposure with second-hand smoke exposure and current cigarette smoking.

Asthma among East Asian adolescents. All four studies [32-35] found the likelihood of respiratory symptoms to be significantly higher among e-cigarette users, with additive effects for e-cigarettes and smoking. Cho [32] also reported that e-cigarette users had more days absent from school because of asthma, an external validation. Kim et al. [33] and Lee et al. [34] found significant associations of e-cigarettes with asthma in pooled samples of middle and high school students. Cho and Paik [32] performed a cross-product test and found an inverse interaction: The association of e-cigarette use with asthma was significant among never smokers but was nonsignificant among smokers. Confirming these results, Wang [35] reported a stronger association of e-cigarette use with respiratory symptoms among never smokers. In these studies, the finding of a significant association with respiratory disease among never smokers works against an interpretation of reverse causation.

Statewide surveys of asthma in Florida. Choi and Bernat [36] reported a stronger association of e-cigarettes with asthma for current (30-day) use (AOR = 2.20) than for lifetime use (AOR = 1.72). Another study [37] reported a significant association of e-cigarette use with asthma in the whole sample in a Florida survey conducted in a different year. Two studies focusing on adolescents with asthma found own e-cigarette use [36] or second-hand exposure to e-cigarette aerosol [38] associated with higher likelihood of having had an asthma attack during the past year.

Regional and national surveys on asthma. A Canadian study [39] noted a significant association of ever e-cigarette use with lifetime asthma [39] and study in Hawaii [40] found a significant association of e-cigarette use with current asthma, controlling for cigarette smoking and other covariates (e.g., obesity). A study with a US national sample [41] similarly indicated a significant association of e-cigarette use with asthma controlling for cigarette smoking, marijuana use, and other covariates. In two studies [40, 41], e-cigarette use and cigarette smoking made additive contributions to likelihood of asthma but cross-product tests for

interaction between e-cigarette and cigarette smoking were mostly nonsignificant.

Bronchitis among high school students. In a California study [42], chronic bronchitis was coded if in the previous 12 months a participant had daily cough, congestion, or phlegm for 3 months in a row other than when having a cold. An analysis for the total sample showed significant associations of both e-cigarette use and smoking with chronic bronchitis and also showed a dose-response effect: the likelihood of bronchitis was higher with more frequent e-cigarette use. A significant association of e-cigarette use with bronchitis among never smokers was noted but a comparable analysis for smokers was not reported.

E-cigarette use and asthma among adults. In a national web-based survey conducted from 2013 through 2017 [43], current e-cigarette use was positively associated with a diagnosis of asthma and also with a breathing-difficulty score. In Hawaii data from the Behavioral Risk Factor Surveillance Survey (BRFSS), e-cigarette use was significantly associated with asthma only among nonsmokers [44], similar to findings from three adolescent studies [32, 35, 42]. Osei et al. [45] pooled data from two years of national BRFSS data and noted a significant association of current e-cigarette use with current asthma among persons who had never smoked. They also noted a dose-response effect, a greater likelihood of asthma with more frequent e-cigarette use. Bhatta and Glantz [46] used longitudinal data from a national household interview study, the Population Assessment of Tobacco and Health (PATH), to predict incident (i.e., new) asthma at Waves 2 and 3 among persons who were free of asthma at Wave 1. There was a significant relation of baseline e-cigarette use to incident asthma in this prospective analysis.

Epidemiological Studies of COPD

In studies of respiratory disorder among adults (Table 2), the criterion variable typically involved having been diagnosed with COPD (and sometimes other respiratory conditions) by a doctor, nurse, or other health professional. Seven studies were cross-sectional and two were longitudinal. Multivariable analyses adjusted for covariates similar to those used for asthma,

including demographics, cigarette smoking, and obesity.

US national sample (PATH). In an analysis of PATH data [47], participants were classified as nonusers, exclusive e-cigarette users, exclusive smokers, or dual users. Respiratory disease was coded if a respondent said they been told by a doctor they had any of COPD, chronic bronchitis, emphysema, or asthma. Results showed that current exclusive e-cigarette users had a higher likelihood of respiratory disease compared with nonusers, and dual users had an even higher likelihood. Another analysis of PATH data using a propensity-matching design to control for a range of confounders [48] showed that current e-cigarette users had a higher likelihood of COPD compared with matched controls and a stratified analysis showed an inverse interaction: a much stronger association of e-cigarette use with COPD among nonsmokers compared with the rest of the sample. Li et al. [49] analyzed 7 specific symptoms of respiratory illness (e.g., wheezing, dry cough) in Wave 2 PATH data. They found that exclusive e-cigarette use was positively related to most of the symptoms and this was not accounted for by smoking history. Dual users had greater risk for respiratory symptomatology compared with solo e-cigarette users (i.e., additive effects). (Table 2 follows)

Table 2

Epidemiological Studies of E-cigarette Use and Respiratory Disorder

Ref	Sample N, age	E-cigarette measure	Respiratory measure	Covariates	Findings	Smoking control	Additive effects	Inter-action
[43]	39,747 (≥ 18 years)	30-day use	Ever Dx with COPD by doctor or nurse	Demographics, smoking, CHD	AOR = 1.53 for current exclusive e-cig users	Yes	Yes	n.a.
[44]	8,085 (≥ 18 years)	Ever use, current use	Ever Dx with COPD by doctor, nurse, other health professional	Demographics, smoking, SHS, BMI, stress	AOR = 2.58 for whole sample, 2.98 for nonsmokers	Yes	Yes	INV
[47]	32,320 (≥ 18 years)	Current established user (cig or e-cig)	Ever Dx by doctor, other health prof. with COPD, chronic bronchitis, asthma, or emphysema	Demographics, other tobacco product use, marijuana use	AOR = 1.39 for solo e-cig users, AOR = 2.07 for dual users	Yes	Yes	EQ[A]
[48]	2,727 for case control (18-64 years)	Current e-cig user	Ever Dx with by health prof. with bronchitis, emphysema, or COPD	SHS, BMI, other tobacco product use, health measures	AOR = 1.47 for total sample, AOR = 2.94 for nonsmokers	Yes	n.a.	INV
[49]	28,171 (≥18 years)	Current established e-cig user	Wheezing, whistling, coughing, past 12 mo. (7 items)	Demographics, BMI, SHS, asthma, mental/ physical health	Solo e-cig users at more risk than nonusers, AORs 1.37 to 1 .78; for dual users, AORs 2.32 to 3.58	Yes	Yes	n.a.

[50]	705,159 (≥ 18 years)	Current e-cig use	Ever Dx by health prof. with emphysema, bronchitis, or COPD	Demographics, poverty status	AOR = 1.75 for all cases, AOR = 2.64 for never smoker / daily user	Yes	Yes	INV
[51]	6519 and 23,753 (ages 20-75 years)	Use daily or sometimes	Long-standing cough, phlegm or wheeze in past 3 mo., 12 mo.	Demographics, age, survey	For any respiratory symptom, AOR = 1.46 for exclusive e-cig users; AOR = 4.03 for dual users	Yes	Yes	n.a.
[46]	23,760 (18-65 years)	Ever use, current use	Dx by doctor, other health prof. with emphysema, bronchitis, or COPD ever (W1), past 12 mo. (W2, W3)	Demographics, smoking, poverty status, clinical variables	AOR = 1.29 for incident respiratory disease for current e-cig use at W1	Yes	Yes	n.a.
[52]	3,536 (45-80 years)	Ever use, current (monthly, weekly, daily) use	Repeated measures of spirometry, bronchitis, COPD exacerbations	Demographics, smoking, baseline clinical variables	E-cig users had more bronchitis, more COPD exacerbations, decline in lung function over time	Yes	Yes	n.a.

Note: E-cig = e-cigarette; Dx = diagnosed; prof. = professional; mo. = month; SHS = second-hand smoke exposure; BMI = body mass index; n.a. = not available (data not available or test not performed). For Interaction column, SYN indicates synergistic interaction (effect of e-cigarettes greater among smokers); INV = inverse interaction (effect of e-cigarettes greater among nonsmokers); EQ = effect of e-cigarettes equal in smokers and nonsmokers; NEV = analysis performed only for nonsmokers.

[A] In this study, interactions of marijuana with e-cigarette use were tested.

COPD in US samples. Data from a US national sample [43] indicated current exclusive e-cigarette use was significantly associated with diagnosed COPD, with the greatest likelihood of COPD found among dual users. A study with BRFSS data from Hawaii [44] found a significant inverse interaction: The association of exclusive e-cigarette use with COPD was stronger among nonsmokers compared with smokers. Analysis of national BRFSS data based on both ever and current e-cigarette use [50] also showed an association of e-cigarette use with COPD that was stronger among nonsmokers than among smokers. This study reported a dose-response effect and also emphasized that dual users were notably worse off for COPD.

Respiratory symptoms in regional samples from Sweden. Data from Sweden [51] included measures tapping occurrence of five specific respiratory symptoms (e.g., long-standing cough, sputum production). Among never smokers, the association of e-cigarette use with likelihood of respiratory symptoms was marginally significant but this may have been influenced by small cell size as the overall rate of e-cigarette use in this sample was relatively low (2% of the population). Stratified analyses suggested e-cigarette use adding to risk among both former smokers and current smokers but direct tests for additive effects were not conducted.

Longitudinal studies of respiratory disease. Prospective analyses of Wave 1 through Wave 3 PATH data [46] tested the relation of e-cigarette use at baseline to new disease at follow-up (chronic bronchitis, emphysema, or COPD) among persons who were free of disease at Wave 1. Significant predictive effects were found for both prior e-cigarette use and current e-cigarette use. Tests for additive effects indicated dual users were significantly worse off than exclusive e-cigarette users or exclusive smokers (AOR = 3.30). In the COPDGene study [52], participants were ages 45-80 years and had at least 10 pack-years of smoking history. Respiratory disease status was indexed at baseline through lung function tests and self-report of chronic bronchitis and COPD; follow-up measures were obtained at 6-month intervals. Longitudinal analyses controlling for baseline clinical variables indicated e-cigarette use was related to a higher

prevalence of chronic bronchitis and an increased number of COPD exacerbations. Participants who used e-cigarettes were more likely to have progression of lung disease on lung function tests though this was nonsignificant with adjustment for covariates.

Meta-Analysis

The meta-analysis was based on adjusted odds ratios (AORs) for e-cigarette use that controlled for cigarette smoking and other disease-related risk factors [cf. 10]. The meta-analysis for asthma as based on 11 studies of adolescents and 4 studies of adults (Table 1) having a total of 971,278 participants. A random effects meta-analysis indicated the pooled AOR for asthma was 1.39 (95% CI 1.28-1.51) for e-cigarette users compared to non-e-cigarette users (Figure 2A). We observed moderate heterogeneity in the results ($Q_{14} = 28.20$, $p = 0.01$; $I^2 = 50\%$) because the international studies exhibited greater heterogeneity than US-based studies. A separate meta-analysis of the four adult studies indicated a significant AOR of 1.40 (CI 1.23-1.58, data not shown) hence it was appropriate to include these with the adolescent studies.

The meta-analysis for COPD or composite respiratory symptoms was based on 9 studies of adults (Table 2) having a total of 1,023,494 participants. A random-effects meta-analysis indicated the pooled AOR for respiratory disease was 1.49 (95% CI 1.36-1.65) for e-cigarette users compared to non-e-cigarette users (Figure 2B). We observed no significant evidence of heterogeneity in these studies ($Q_8 = 6.41$, $p = 0.60$; $I^2 = 0\%$). (A sensitivity analysis supporting these findings is presented in Supplementary Material.)

Summary of Epidemiological Studies

A significant association of e-cigarette use with respiratory disorder was found across 23 of the 24 studies reviewed, and e-cigarette use typically added independently to risk derived from cigarette smoking. The studies had large representative samples drawn from multiple states and countries, and the analyses included a number of covariates so as to rule out several possible types of confounding. Methodological characteristics of the research were generally strong and

independent methodological studies have supported both the validity of self-reports of substance use[e.g., 53, 54] and the reliability and validity of health measures in large-scale surveys for adolescents [55, 56] and for adults [57-59]. Moreover, several studies provided external validation for self-report findings (e.g., through school absences); this makes interpretation of the findings as deriving from an "ill worker's effect" (i.e., persons with disease simply reporting a stereotypic cause) not very plausible. A limitation that could be noted is that most studies were cross-sectional. However, a reverse-causality interpretation is not very plausible because several cross-sectional studies showed a significant association of e-cigarette use with respiratory disease among never smokers, and longitudinal studies showing e-cigarette use to predict onset of respiratory disease from a disease-free baseline [46, 52] also make reverse causation unlikely.

Evidence from Laboratory Studies

Laboratory studies provide experimental evidence about effects of e-cigarettes on four types of biological processes that are linked to respiratory outcomes. While other processes are possibly implicated, such as fine particulate matter [60, 61], these are the areas where the most direct evidence is available. We discuss representative laboratory studies on these topics because extensive narrative reviews are available elsewhere [27, 28, 62]. We note that though nicotine itself has adverse effects on pulmonary variables [63, 64], in a number of studies the effects observed for e-cigarettes are independent of nicotine content hence are attributable to other components of e-cigarette liquid or aerosol. In our discussion we do not address the question of whether e-cigarettes have lower levels of carcinogenic toxicants associated with combustible cigarettes. While this tends to be the case for known carcinogens [e.g., 65], there are conditions where effects of e-cigarettes on other biological processes are comparable to those of cigarettes and there is evidence that new types of toxicants may emerge from the mixing and heating of e-cigarette humectants and flavorings [66]. Thus we believe a key question is whether effects of e-cigarettes on biological processes differ significantly from clean-air controls, indicating actual

damage to lung/airway tissues. We also consider how levels of biological effects differ for e-cigarettes and cigarettes.

Cytotoxic Effects (Supplementary Table 5)

In [67], cells exposed for 1-48 hours to e-cigarette aerosol extracts showed concentration-dependent cytotoxic effects and reduced cell proliferation for 5 of the 11 products tested. Leigh et al. [68] found that cell metabolic activity and viability were both decreased in the e-cigarette condition compared to an air control. Another study [69] found that all e-cigarette brands tested had cytotoxic effects. In one study [67], effects for e-cigarettes were sometimes less than for cigarettes but in another [69] the effect for DNA damage was comparable to that for cigarettes. Rowell et al. [70] found e-cigarettes produced decreases in cell viability, proliferation, and metabolism compared with the control condition, and a study with JUUL brand e-cigarettes showed that pod fluids were cytotoxic in two assays for all flavors [71]. In these studies, cinnamon, menthol, vanilla and berry or fruit flavorings were found to have particularly cytotoxic effects.

In one recent study, most of the 20 popular e-liquids screened showed evidence of significant toxicity [72]. Escobar et al. [73] tested the effects of three aerosolized humectants (propylene glycol, glycerol, and PG + GLY) with no flavorings added. Evidence of cytotoxicity was found for aerosolized humectants and evidence was found for increases in two pro-inflammatory cytokines, IL-6 and IL-8, and indices of cellular stress. Thus, evidence was found for biological effects of basic constituents of e-cigarettes, aside from contributions for flavorings.

Oxidative Stress and Inflammation (Supplementary Table 6)

Oxidative stress is an important process in the etiology of lung disease [74] and a number of studies have shown e-cigarettes related to indices of oxidative stress. In studies including both human cells and animal models [75], oxidative stress was increased and cell viability decreased in the e-cigarette condition compared to a clean-air control. Other studies have also found an

impact of e-cigarettes on oxidative stress and effects for disrupting lung functioning, with some effects independent of flavorings [76, 77]. Studies by Lerner et al. [78] showed that exposure to e-cigarettes reduced cell viability, increased reactive oxygen species, and produced an increase in the inflammatory cytokines IL-6 and IL-8. Larcombe et al. [80] found that mice exposed to e-cigarette aerosol had impaired lung function and changes in airway reactivity. Effects for e-cigarettes in [75, 76] were less than for cigarettes but in [78, 80] some effects were comparable to or greater than those for cigarettes. An *in vivo* study based on human nonsmokers [82] found increases over time in blood markers for oxidative stress and inflammation and evidence that oxidants were released into the blood. A cell study with human alveolar macrophages [83] found a dose-dependent reduction in cell viability (i.e., e-cigarettes increased cytotoxicity) together with increase in the production of reactive oxygen species and pro-inflammatory cytokines (e.g., IL-6, TNFα) and decrease in phagocytosis (i.e., bacteria-killing) ability. Notably, some studies found that heating of e-liquids increased the magnitude of adverse effects.

Linkages to Immune Function and Susceptibility to Infection (Supplementary Table 7)

In a mixed-methods study [84], cells cultured with various concentrations of e-liquid and inoculated with human rhinovirus had higher levels of viral load and decreased host defense molecule expression, and infected mice exposed to e-cigarettes showed higher viral load. Studies with macrophages and an animal model [85] indicated that e-cigarette exposure reduced antimicrobial activity, and a controlled-infection study with mice indicated greater MRSA bacterial burden and higher mortality in the e-cigarette condition. In a series of cell studies and *in vivo* studies [86], lungs of mice exposed to e-cigarette aerosol and infected with *Streptococcus pneumoniae* showed increased bacterial burden, and mice infected with influenza virus showed a higher rate of mortality in the e-cigarette condition compared to a clean-air control. Similarly, Gilpin [87] and Gomez [88] exposed macrophages and several types of bacteria (e.g., influenza, pneumonia) to e-cigarette aerosol extract and found increased bacterial virulence and

inflammatory potential as well as decreased bacteria-killing ability. A mouse study [89] found similar effects for macrophages and increased morbidity and mortality among influenza-infected animals, independent of nicotine. Human studies have found that e-cigarette users showed markers for increased oxidative stress and inflammatory response and aberrant neutrophil activation and mucus ratios, all of which could be involved in respiratory disease [91]. In another study, proteins associated with membrane formation and mucus formation were uniquely affected in e-cigarette users so as to increase susceptibility to respiratory infections [92]. Clapp et al. [93] found a suppressed host defense mechanism: exposure to one e-liquid reduced the motility and the beat frequency of lung cilia, hence impairing an essential respiratory defense mechanism, similar to effects found in [90]. Though these studies demonstrated effects of flavorings, some also found significant adverse effects for humectants alone. In several studies [87, 88, 89, 91, 92] some effects of e-cigarettes on immune function were less than for cigarettes but some were equal to cigarettes.

In the most recent studies, four common flavoring chemicals affected human neutrophils, an important part of the innate immune response, in a dose-dependent manner [94] and three of the four flavorings impaired defense against *Staphyloccus aureus*. Similarly, Corriden [95] exposed neutrophil cells and mice to e-cigarette aerosol and found that exposure reduced several measures of neutrophil function and increased number of bacteria found at an infection site. In a related study [96], proteases linked to respiratory disease were elevated in both e-cigarette users and smokers. Related effects were found in two other studies [97,98].

Genetic Effects (Supplementary Table 8)

Yu et al. [99] found that exposure to e-cigarette aerosol produced increased cell death and DNA damage compared to untreated cells. A human study [100] found that of 543 genes available for comparison, 358 genes were differentially expressed when comparing e-cigarette users with nonusers, the differences generally being consistent with immune suppression. Some

effects were six times greater for e-cigarettes than for cigarettes. A comparison of cigarette smokers and e-cigarette users [101] showed that genes downregulated for both groups (i.e., common effects) tended to be ones involved in cilia assembly and movement. PCR validation indicated that both e-cigarettes and cigarettes interfered with ciliated cells in the airway epithelium. Ganapathy et al. [102] found a dose-dependent effect of e-cigarettes on DNA damage. Two related studies [103, 104] confirmed effects for impaired cell functioning and increased interference with DNA repair mechanisms. Two of the studies [99, 102] found the effect of e-cigarettes on DNA damage and other processes was comparable to effects observed for cigarettes.

In the most recent work, an *in vivo* human study [105] found larger numbers of differentially expressed transcripts in exclusive smokers and e-cigarette users compared to controls (1726 vs.1152). Only 299 of the differences were common to smokers and e-cigarette users, indicating their effects were through largely different mechanisms. Song et al. [106] analyzed cells from bronchoscopies and found a large number of differentially expressed transcripts (2,452) for e-cigarette users and smokers compared to nonsmokers. Inflammation processes were implicated in that e-cigarette users had higher inflammatory infiltrates than nonsmokers but levels tended to be lower than for smokers.

Summary of Laboratory Studies

Laboratory studies have shown e-cigarettes to have effects on four biological processes that are relevant for respiratory disease. Evidence is found for exposure to e-cigarette liquid or aerosol producing cytotoxic effects and oxidative stress. Results for inflammation are less consistent but effects on cytokines and other indices of inflammation have been found in several studies. Both cell studies and animal models indicate that bacterial virulence and indices of susceptibility to infection are increased by e-cigarette exposure and that bacteria- and virus-infected animals show higher morbidity and mortality when they are exposed to e-cigarette

aerosol. Finally, studies of genetic variables have found e-cigarettes to cause DNA damage and e-cigarette use to suppress genes involved in immune function, with pathways that can be distinct from those found for cigarettes. While comments have been made about specific aspects for some of the studies (107-110), the finding of biological effects for e-cigarettes across four outcome domains in both cell cultures, animal models, and human studies shows a replicable body of findings linking e-cigarettes to several biological processes involved in the pathogenesis of respiratory disease in humans.

General Discussion

The aim of this paper is to provide an integrative review of the relation between e-cigarette use and respiratory health outcomes by considering findings from epidemiological studies together with evidence from laboratory studies. Our epidemiological review has demonstrated a consistent association of e-cigarette use with respiratory disorder in multiple independent studies with representative samples of adolescents and adults. Laboratory studies show e-cigarette effects on four biological processes relevant for respiratory disorder and include both *in vitro* and *in vivo* studies. Risk-promoting effects have been found consistently across four biological domains using fairly different paradigms. Thus, there is considerable evidence for a relation between e-cigarette use and respiratory disorder. In the following sections we discuss methodological issues relevant for drawing conclusions.

Alternative Explanations

Studies have dealt with several alternative explanations for findings about e-cigarettes. Epidemiological studies have controlled for a number of covariates (e.g., age, sex, race/ethnicity, obesity, own smoking, secondhand smoke exposure) and these were somewhat different ones across studies, hence an argument of potential omitted-variable bias is difficult to sustain. Also, significant findings from longitudinal studies and findings of associations of e-cigarette use with respiratory disease among persons who had never smoked cigarettes work against interpretations

of reverse causation. While it has sometimes been suggested that persons with respiratory disease might use e-cigarettes for therapeutic purposes, it is difficult to see why they would do this given that e-cigarette aerosol has lung irritant effects [e.g., 69, 80, 84, 91, 92, 111].

Difference from Controls and from Cigarettes

Laboratory studies consistently find e-cigarette conditions significantly elevated on adverse biological effects compared with clean-air or comparable control conditions, and a number of studies show e-cigarette effects comparable to those for cigarette smoke (Supplementary Tables 5-8). Thus, there is consistent evidence from controlled experiments that e-cigarettes, while not having the high levels of known carcinogens associated with cigarettes [65], still can have adverse consequences from a respiratory standpoint. These concerns are supported in the present review by data showing a consistent association of e-cigarette use with respiratory disorder in large general-population samples of adolescents and adults (Tables 1 and 2).

Relation to Hill's Criteria

Bradford Hills's criteria were developed to provide guidance for inferring causality from epidemiological research [112] and have had an enduring impact on multiple areas of research [113-115]. Our summary on how the evidence meets these criteria is as follows.

Consistency. Our epidemiological review shows a significant association between e-cigarette use and respiratory disorder in 23 of 24 studies, making this a highly consistent finding. In laboratory research, e-cigarettes have been found to affect disease-related biological processes relevant in 35 independent studies using different methods and paradigms. While nonsignificant conditions and null studies can be found, the consistency of confirmatory evidence is substantial.

Temporality: Finding the predictor to occur before the onset of a disease condition is a crucial criterion [112]. Prospective analyses have shown that e-cigarette use predicts onset of asthma or COPD among initially disease-free cases or worsening of respiratory symptoms over time among those with illness, controlling for baseline level [46, 52]. Together with findings

from laboratory experiments where the exposure precedes the outcome, this evidence gives support for meeting the temporality criterion.

Dose-response gradient: A graded relation between level of exposure and probability of illness is another important criterion. In the present review we have noted many instances of dose-response relationships in laboratory studies. Epidemiological studies typically do not have continuous exposure data but several have noted more recent use or greater number of days used in past month to be related to higher likelihood of respiratory disease [32, 42, 47, 51, 52]. Thus, this criterion is met to some extent though not uniformly across the types of studies discussed.

Biological plausibility: We have shown in detail how e-cigarettes affect biological processes known to be important in the pathogenesis of human respiratory disease. This is based on experimental studies testing specific biological processes and controlled-infection studies using pathogens such as influenza and pneumonia, which are significant disease problems among humans. Thus, the finding of an association of e-cigarette use with respiratory disorder in epidemiological studies is biologically plausible because respiratory disease can develop through these mechanisms, though animal models may not directly mimic human disease.

Strength of relationship. Our meta-analysis of epidemiological studies showed the unique association between e-cigarette use and respiratory disease is an adjusted odds ratio of 1.39 for asthma and 1.45 for COPD. Whether this would be characterized as a large or small effect size is somewhat arbitrary [116] but an important consideration is that a moderate effect size spread across a large publication can have substantial public health impact. We think the strength of relationship is such as to warrant concern about public health consequences.

Coherence with existing knowledge. Hill [112] argued that a cause-effect interpretation of data should not seriously conflict with generally known facts about the natural history and biology of the disease. Based on the evidence presented here and existing knowledge about the etiology of respiratory disease [e.g., 28, 74], the postulate of a relation between e-cigarette use

and respiratory disease does not conflict with existing knowledge.

It should be noted that a recent development is an outbreak of severe lung disease termed E-cigarette/Vaping Related Lung Injury (EVALI, 117-121]. Importantly, the e-cigarettes that these persons had been using typically contained tetrahydrocannabinol [122] and this has been accepted as a defining characteristic of the outbreak. In addition, Vitamin E acetate (VEA) has been detected in bronchoalveolar lavage fluid in almost all cases where this was available [123] and a VEA mechanism has been supported in an animal model [124]. Thus VEA is strongly suspected of being a causal factor for EVALI although a small minority of affected patients deny having vaped THC [125] and other constituents have been suggested for consideration [126]. Whether EVALI results from processes similar to or different from those discussed here, such as oxidative stress [126, 127] or from alternative mechanisms such as lipid deposition [128, 129] is unknown at present. The most recent brands of e-cigarettes have cytotoxic effects and disrupt lung functioning [71, 130, 131], suggesting that the issues we have noted may not go away. Both mechanisms should be considered, and continuing epidemiologic surveillance and laboratory research are needed to determine the social and biological effects of current electronic delivery systems.

Conclusion and Further Research

In summary, we find that Hill's criteria have been adequately satisfied and the evidence supports the conclusion of a real relationship between e-cigarettes and respiratory disorder. There are still many questions that need to be clarified, for example whether e-cigarette use is more related to onset of disease or to exacerbation of existing symptomatology, or whether there are different types of effects at different ages. However, we think the state of the evidence is sufficient to warrant concern about the population impact of e-cigarettes [132].

The research discussed here has generally used good experimental parameters but further research is needed to solidify knowledge about the health consequences of e-cigarettes. Toward

this end, we integrate the findings in a heuristic model of e-cigarettes and respiratory disorder (Figure 3). This model is testable based on methods used in prior research on behavioral consequences of e-cigarette use [10, 30]. It is not clear whether the processes we have discussed work independently or in tandem and the model aims to clarify tests of this question.

We can suggest that e-cigarette use affects susceptibility to infection indirectly through altering expression of genes involved in immune-system function and ciliary mobility, whereas effects of e-cigarettes on cytotoxicity and oxidative stress may occur through biochemical effects on lung or airway membranes. All three processes are hypothesized to increase the likelihood of asthma and/or COPD, possibly at different ages. Our model recognizes that other risk factors for respiratory disease (e.g., cigarette smoking and obesity) have their own effects on outcomes and need to be included as covariates in research on e-cigarettes. Direct effects from e-cigarette use to asthma or COPD, not mediated through the specified biological processes, are possible in principle and are testable in appropriately designed studies. Whether direct or indirect effects are found, more would be learned about how e-cigarette use is related to respiratory outcomes.

Epidemiological studies have consistently noted that dual users have significantly more respiratory symptomatology compared with exclusive e-cigarette users or exclusive smokers. While e-cigarette use tends to be correlated with smoking, they are not interchangeable and they produce additive effects. Laboratory studies of genetic expression also show that effects of e-cigarettes occur in part through different biological pathways than cigarettes. E-cigarette use does not merely parallel effects of smoking, but contributes independently to risk. Thus there is every reason to work actively to deter e-cigarette use among smokers as well as nonsmokers.

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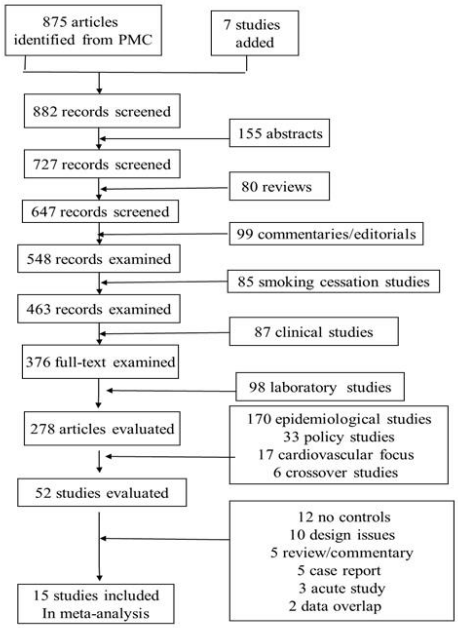
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882 records screened
155 abstracts
727 records screened
80 reviews
647 records screened
99 commentaries/editorials
548 records examined
85 smoking cessation studies
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87 clinical studies
376 full-text examined
98 laboratory studies
278 articles evaluated
170 epidemiological studies
33 policy studies
17 cardiovascular focus
6 crossover studies
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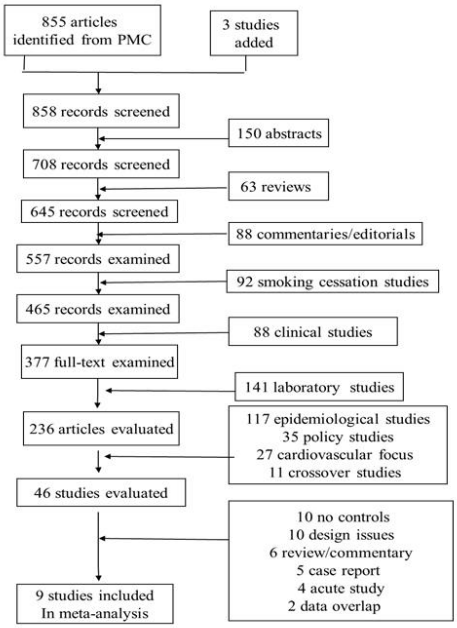
12 no controls
10 design issues
5 review/commentary
5 case report
3 acute study
2 data overlap
15 studies included
In meta-analysis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557 records examined
92 smoking cessation studies
465 records examined
88 clinical studies
377 full-text examined
141 laboratory studies
236 articles evaluated
117 epidemiological studies
35 policy studies
27 cardiovascular focus
11 crossover studies
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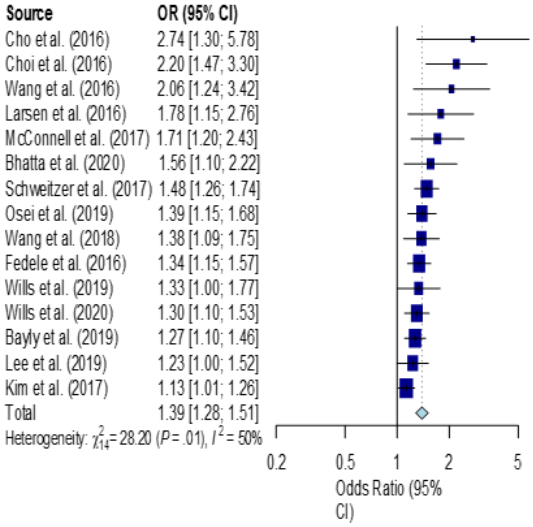
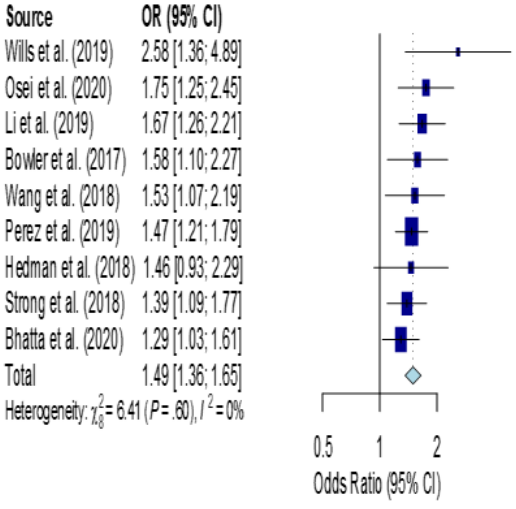
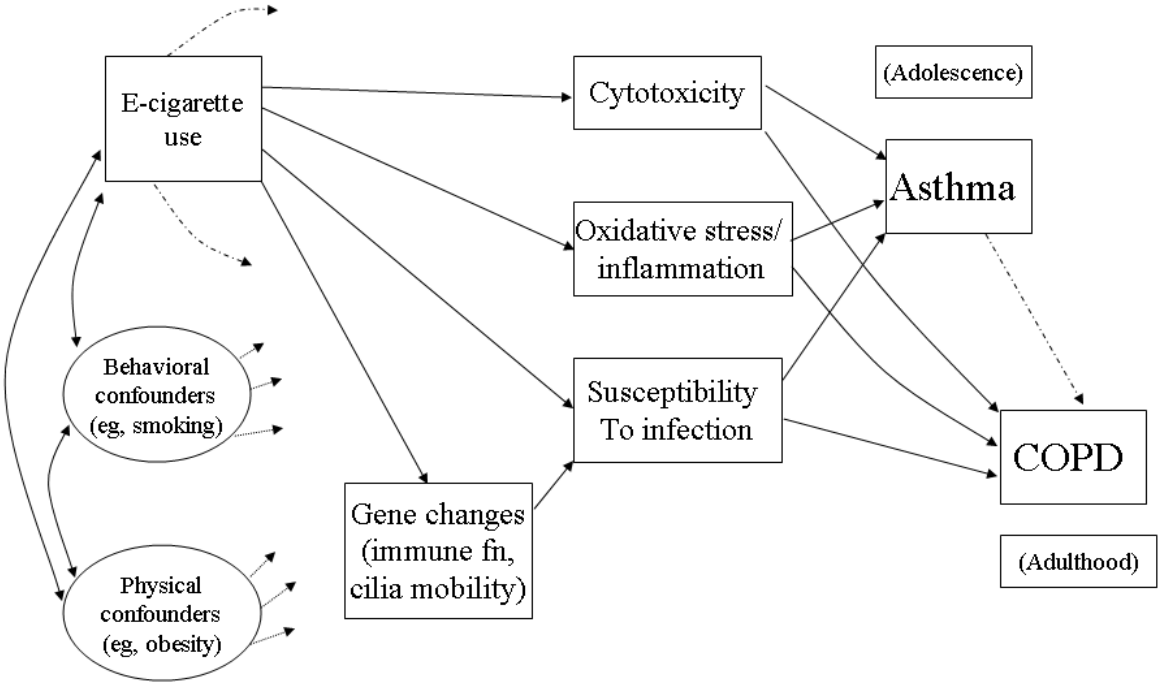
5 case report
4 acute study
2 data overlap
9 studies included
In meta-analysis


Source
OR (95% CI)
Cho et al. (2016) 2.74 [1.30; 5.78]
Choi et al. (2016) 2.20 [1.47; 3.30]
Wang et al. (2016) 2.06 [1.24; 3.42]
Larsen et al. (2016) 1.78 [1.15; 2.76]
McConnell et al. (2017) 1.71 [1.20; 2.43]
Bhatta et al. (2020) 1.56 [1.10; 2.22]
Schweitzer et al. (2017) 1.48 [1.26; 1.74]
Osei et al. (2019) 1.39 [1.15; 1.68]
Wang et al. (2018) 1.38 [1.09; 1.75]
Fedele et al. (2016) 1.34 [1.15; 1.57]
Wills et al. (2019) 1.33 [1.00; 1.77]
Wills et al. (2020) 1.30 [1.10; 1.53]
Bayly et al. (2019) 1.27 [1.10; 1.46]
Lee et al. (2019) 1.23 [1.00; 1.52]
Kim et al. (2017) 1.13 [1.01; 1.26]
Total 1.39 [1.28; 1.51]
Heterogeneity: χ^2_{14} = 28.20 (P = .01), I^2 = 50%
0.2
0.5
1
2
5
Odds Ratio (95% CI)


Source
OR (95% CI)
Wills et al. (2019) 2.58 [1.36; 4.89]
Osei et al. (2020) 1.75 [1.25; 2.45]
Li et al. (2019) 1.67 [1.26; 2.21]
Bowler et al. (2017) 1.58 [1.10; 2.27]
Wang et al. (2018) 1.53 [1.07; 2.19]
Perez et al. (2019) 1.47 [1.21; 1.79]
Hedman et al. (2018) 1.46 [0.93; 2.29]
Strong et al. (2018) 1.39 [1.09; 1.77]
Bhatta et al. (2020) 1.29 [1.03; 1.61]
Total 1.49 [1.36; 1.65]
Heterogeneity: χ^2_8 = 6.41 (P = .60), I^2 = 0%
0.5
1
2
Odds Ratio (95% CI)


E-cigarette use
Cytotoxicity
(Adolescence)
Asthma
Oxidative stress/ inflammation
Behavioral confounders (eg, smoking)
Susceptibility To infection
COPD
Gene changes (immune fn, cilia mobility)
(Adulthood)
Physical confounders (eg, obesity)

Supplementary Material. Selection Bias: Copas Selection Modeling

We conducted a sensitivity analysis to assess the impact of selection bias on the pooled AOR for e-cigarette use and asthma in adolescents by fitting a Copas selection model (Supplementary Tables 1, 2). Adjusting for selection bias, the Copas model estimated the pooled AOR of e-cigarette use and asthma as 1.22 (95% CI 1.15- 1.29) compared to the random effects model estimate of 1.39. In a similar analysis for adults (Supplementary Tables 3, 4), the Copas model estimated the pooled AOR of e-cigarette use and COPD as 1.36 (CI 1.08-1.70) compared to the random effects estimate of 1.45.

One of the nine adolescent-based studies on e-cigarette use and asthma fell outside the 95% confidence intervals denoted by the diagonal dashed lines shown in the funnel plot (Supplementary Figure 1, Panel A), which suggests possible heterogeneity and publication bias. We then assessed the sensitivity of the meta-analysis to selection mechanisms of varying strength.[1,2] Specifically, γ_0 is approximately equal to the probit of the probability that a study with a large standard error is published and γ_1 is approximately equal to the probit of the probability that a study with precision equal to the inverse of its standard error is published. The contour plot (Supplementary Figure 1, Panel B) suggests that the estimated adjusted pooled odds ratio from the meta-analysis may be sensitive (i.e., varies between 1.11 [$e^{0.10}$] and 1.38 [$e^{0.32}$]) to the range of (γ_0, γ_1) values. We further explore this sensitivity in Supplementary Figure 1, Panels C and D. As the probability of publishing the study with the largest standard error decreases from 100% to 39%, the estimated adjusted pooled odds ratio deceases from 1.40 ($e^{0.33}$) to 1.22 ($e^{0.20}$; Supplementary Figure 1, Panel C). Notably, the confidence interval of the adjusted pooled odds ratio remains above 1 (i.e., confidence interval of log odds ratio remains above 0) across the range of probabilities of publishing the study with the largest standard error. For each of the selection probabilities shown in Supplementary Figure 1, Panel C, the Copas selection model calculates a p-value for the test of any remaining selection bias. Selection mechanisms for which this p-value is not statistically significant (i.e., p-value $\geq$ 5%) correspond to more plausible estimates of the pooled adjusted odds ratio under the Copas selection model.[1] The model indicates statistically significant residual publication bias (i.e., p-value < 5%) until the probability of publishing the study with the largest standard error falls below 40% (Supplementary Table 1). In other words, estimated pooled adjusted odds ratios corresponding to probabilities of publishing the study with the largest standard error below 40% are the most plausible under the model. Overall, adjusting for selection bias, the estimated adjusted pooled odds ratio equaled 1.22 (95% CI: 1.15, 1.29) compared to 1.40 (95% CI: 1.23, 1.59) under the baseline random effects model (Supplementary Table 2).

One of the nine adult-based studies on e-cigarette use and COPD fell outside the 95% confidence intervals denoted by the diagonal dashed lines shown in the funnel plot (Supplementary Figure 2, Panel A), which suggests possible heterogeneity and publication bias. We then assessed the sensitivity of the meta-analysis to selection mechanisms of varying strength.[1,2] Specifically, γ_0 is approximately equal to the probit of the probability that a study with a large standard error is published and γ_1 is approximately equal to the probit of the probability that a study with precision equal to

the inverse of its standard error is published. The contour plot (Supplementary Figure 2, Panel B) suggests that the estimated adjusted pooled odds ratio from the meta-analysis may be sensitive (i.e., varies between 1.11 [$e^{0.10}$] and 1.38 [$e^{0.32}$]) to the range of (γ_0, γ_1) values. We further explore this sensitivity in Supplementary Figure 2, Panels C and D. As the probability of publishing the study with the largest standard error decreases from 100% to 34%, the estimated adjusted pooled odds ratio deceases from 1.46 ($e^{0.38}$) to 1.27 ($e^{0.24}$; Supplementary Figure 2, Panel C). Notably, the confidence interval of the adjusted pooled odds ratio remains above 1 (i.e., confidence interval of log odds ratio remains above 0) across the range of probabilities of publishing the study with the largest standard error. For each of the selection probabilities shown in Supplementary Figure 2, Panel C, the Copas selection model calculates a p-value for the test of any remaining selection bias. Selection mechanisms for which this p-value is not statistically significant (i.e., p-value $\geq$ 5%) correspond to more plausible estimates of the pooled adjusted odds ratio under the Copas selection model.[1] The model indicates statistically significant residual publication bias (i.e., p-value < 5%) until the probability of publishing the study with the largest standard error falls below 85% (Supplementary Table 3). In other words, estimated pooled adjusted odds ratios corresponding to probabilities of publishing the study with the largest standard error below 85% are the most plausible under the model. Overall, adjusting for selection bias, the estimated adjusted pooled odds ratio equaled 1.28 (95% CI: 1.18, 1.38) compared to 1.46 (95% CI: 1.35, 1.57) under the baseline random effects model (Supplementary Table 4).

Supplementary Table 1. Pooled Adj. Odds Ratio Varying Prob. of Publishing Study with Largest Standard Error

Probability of publishing study with largest standard error	OR [95% CI]	P-value for hypothesis of overall treatment effect	P-value for hypothesis that no selection remains unexplained
1.00	1.40 (1.23-1.59)	<0.001	0.000
0.97	1.38 (1.24-1.52)	<0.001	0.000
0.90	1.35 (1.26-1.45)	<0.001	0.000
0.79	1.32 (1.23-1.42)	<0.001	0.000
0.66	1.30 (1.21-1.39)	<0.001	0.001
0.56	1.27 (1.21-1.34)	<0.001	0.001
0.47	1.25 (1.17-1.32)	<0.001	0.002
0.39	1.22 (1.15-1.29)	<0.001	0.045

Note: Adj.=Adjusted; Prob.=Probability; OR=odds ratio; CI=confidence interval

Supplementary Table 2**.** Pooled Adj. Odds Ratio: Copas Selection Model and Random Effects Model

Model	OR [95% CI]	P-value for hypothesis of overall treatment effect	P-value for hypothesis that no selection remains unexplained
Copas Selection	1.22 (1.15-1.29)	<0.001	0.045
Random Effects	1.40 (1.23-1.59)	<0.001	—

Note: Adj.=Adjusted; OR=odds ratio; CI=confidence interval

Supplementary Table 3. Pooled Adj. Odds Ratio Varying Prob. of Publishing Study with Largest Standard Error

Probability of publishing study with largest standard error	OR [95% CI]	P-value for hypothesis of overall treatment effect	P-value for hypothesis that no selection remains unexplained
1.00	1.46 (1.35-1.57)	<0.001	0.058
0.52	1.42 (1.32-1.53)	<0.001	0.311
0.41	1.36 (1.26-1.47)	<0.001	0.611
0.34	1.28 (1.18-1.38)	<0.001	0.314

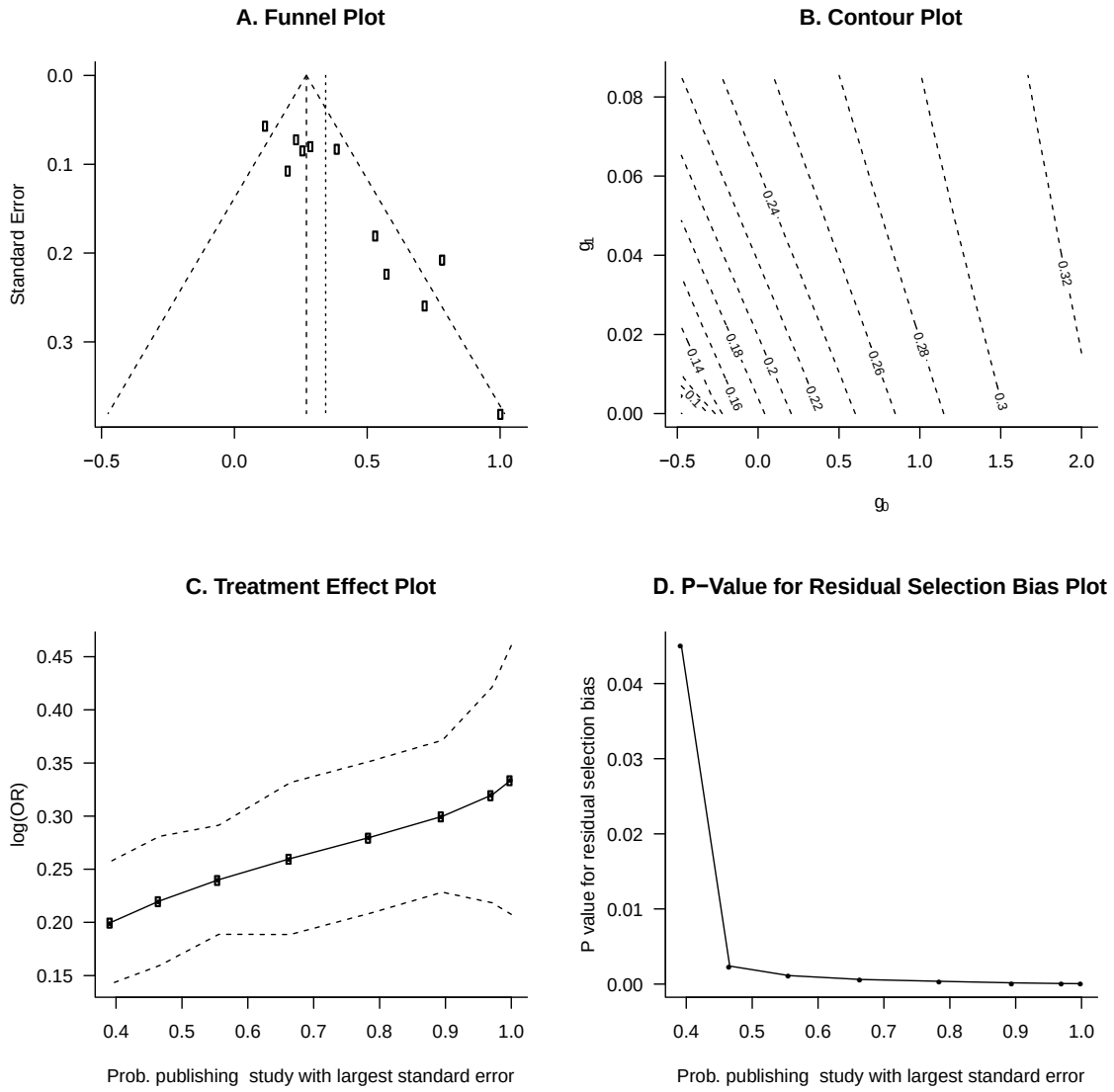
Note: Adj.=Adjusted; Prob.=Probability; OR=odds ratio; CI=confidence interval

Supplementary Table 4**.** Pooled Adj. Odds Ratio: Copas Selection Model and Random Effects Model

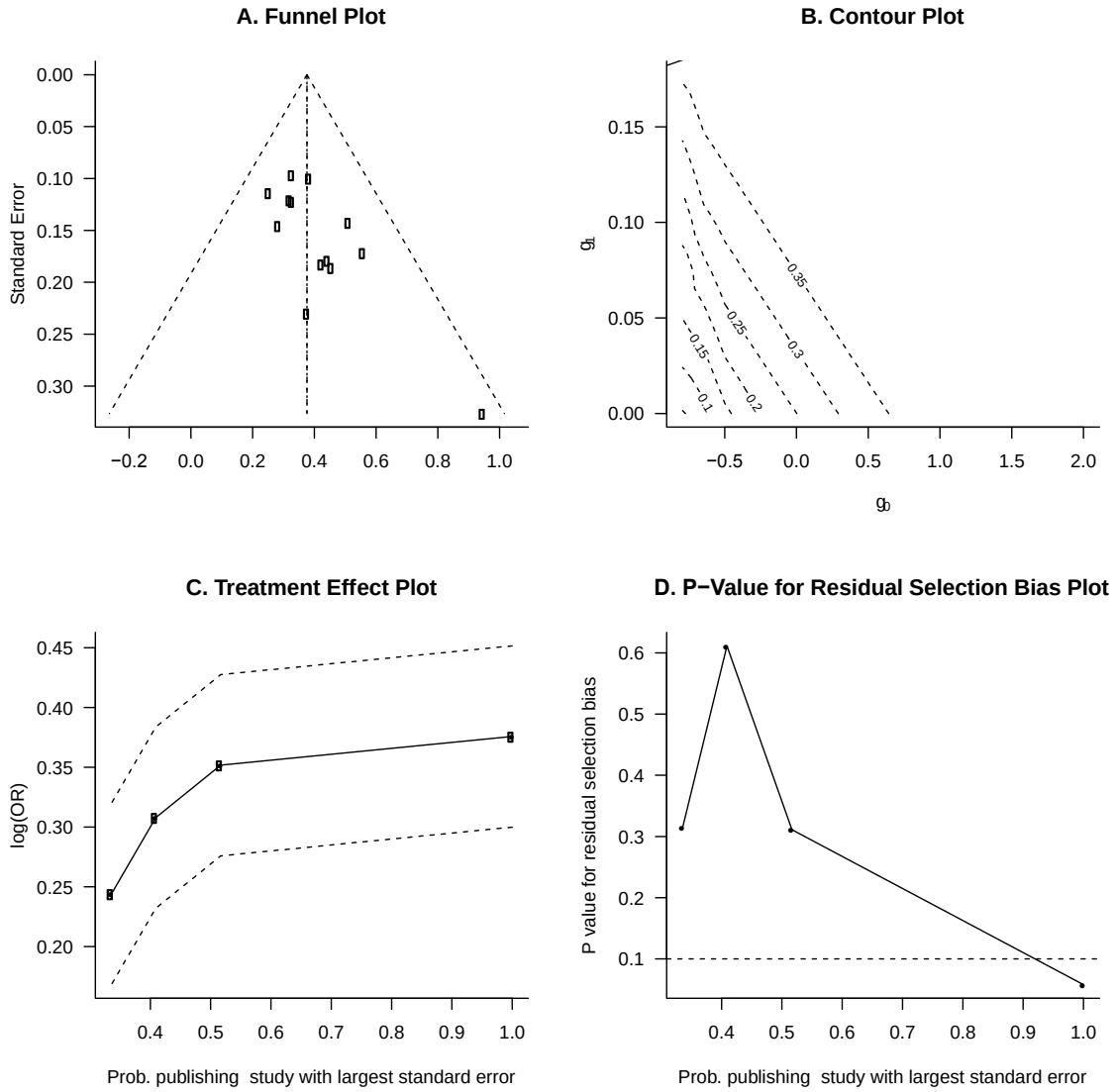
Model	OR [95% CI]	P-value for hypothesis of overall treatment effect	P-value for hypothesis that no selection remains unexplained
Copas Selection	1.28 (1.18-1.38)	<0.001	0.314
Random Effects	1.46 (1.35-1.57)	<0.001	—

Note: Adj.=Adjusted; OR=odds ratio; CI=confidence interval

Supplementary Figure 1. Copas Selection Modelling, Adolescent Studies



Supplementary Figure 2. Copas Selection Modelling, Adult Studies



Supplementary Table 5

Laboratory Studies on Cytotoxic Effects of E-cigarettes (E-cigs)

Ref	Cell type	E-cig liquid/ aerosol	Results	Assays	E-cigarette comparison with control	E-cigarette comparison with cigarette
[67]	Umbilical vein endothelial	Aerosol	Cytotoxicity found for 5 of 11 aerosols tested. Reduced cell proliferation also observed for aerosol from these products. Results independent of nicotine. Little effect for reactive oxygen species.	Cell death Prolif. inhibition ROS Morphology	5>ctrl, 6=ctrl 5>ctrl, 6=ctrl 1>ctrl, 10=ctrl 3> ctrl, 0=ctrl	1< cig, 0=cig 9< cig, 0=cig 10<cig, 1=cig 1< cig, 2=cig
[68]	Bronchial Epithelial	Aerosol	6 products tested. Exposure to e-cig aerosol decreased metabolic activity and cell viability compared to air control. Also significant release of inflammatory cytokines (IL-6, IL-10, CXCL1,2). Effects not related to nicotine concentration.	Metab. activity Cell viability Cytokines	3<ctrl, 3=ctrl 3<ctrl, 3=ctrl 4>ctrl, 2=ctrl	3<cig, 3=cig 3<cig, 3=cig 3>cig, 3=cig
[69]	Umbilical vein epithelial	Aerosol	4 products tested. E-cig aerosol caused cell death and DNA damage, generated significant levels of reactive oxygen species. Dose-dependent effects. Representative products tested for DNA, cell death. Antioxidant Tx reduced cell death.	Cell viability ROS DNA damage Cell apoptosis Cell necrosis	3<ctrl, 2=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	5<cig, 0=cig 1<cig, 1=cig 1<cig, 0=cig n.a. n.a.
[70]	Airway epithelial	Liquid, aerosol	13 e-cig liquids screened. Found decreases in cell viability, proliferation, and metabolism. Dose-dependent effects in all assays. Only 3-4 products tested for subsidiary analyses. Similar effects for e-liquid and aerosol.	Cell proliferation Cell viability Cytotoxicity	9<ctrl, 0=ctrl 6<ctrl, 1=ctrl 3>ctrl, 4=ctrl	n.a. n.a. n.a.
[71]	Bronchial epithelial	Liquid, aerosol	8 JUUL pods tested. Cytotoxicity found for all flavors tested. Nicotine also was cytotoxic. Aerosols were more cytotoxic than pod fluids.	Toxicity (MTT) Toxicity (NRU) Cell lysis (LDH)	8>ctrl, 0=ctrl 8>ctrl, 0=ctrl 0>ctrl, 8=ctrl	n.a. n.a. n.a.
[72]	Bronchial epithelial	Liquid	20 popular products screened. Most showed significant cytotoxicity (30% cell death or below). Four products reached 50% or below. In tests of 10 isolated flavoring chemicals, 3	Cytotoxicity (MTT)	16>ctrl, 4=ctrl	n.a.

			showed toxicity only at highest concentration and 5 showed toxicity at several concentrations.			
[73]	Bronchial epithelial	Aerosol condens.	3 humectant products tested. Evidence found for increases in cytokine release (15 tests) and cellular stress (3 tests). Cytotoxicity found for aerosolized but not liquid humectants.	Cytokines Cellular stress Cytotoxicity (LDH)	8>ctrl, 7=ctrl 3>ctrl, 0=ctrl 1>ctrl, 2=ctrl	n.a. n.a. n.a.

Note: Cell lines are human unless otherwise noted. E-cig = e-cigarette; prolif. = cell proliferation; ROS = reactive oxygen species; morphol. = morphological alterations; metab. = metabolic; Tx = treatment; condens. = condensate. MTT = dimethylthiazol-diphenyltetrazolium; NRU = neutral red (dye) uptake; LDH = lactate dehydrogenase. n.a. = data not available or analysis not performed. Control conditions included clean air in [68, 69, 71, 73], medium control in [67, 72], positive cell control in [69], untreated control in [69, 70, 71, 72, 73]. **For columns:** First column at right indicates level of a given assay in the e-cig group compared with the level in the control group. For example, 4 > ctrl, 2 = ctrl indicates that of 6 tests conducted, level of the assay was significantly higher in the e-cig condition than in the control condition for four tests and not significantly different from the control for two tests. Second column indicates level of the assay in the e-cig condition compared with the cigarette condition; for example, 2<cig, 2=cig indicates that of 4 tests conducted there were two cases where level of the assay was lower in the e-cig condition than in the cigarette condition and two cases where levels did not differ not significantly for the e-cig condition and the cigarette condition.

Supplementary Table 6

Laboratory Studies of Oxidative Stress/Inflammation Effects for E-cigarettes (E-cigs)

Ref	Cell type	E-cig liquid/ aerosol	Results	Assays	E-cigarette comparison with control	E-cigarette comparison with cigarette
[75]	Bronchial Epithelial	Aerosol	1 product tested, 2 cell lines. E-cig exposure produced decreased cell viability and increased oxidative stress. Differing effects for rat and human cells. Also effect for PG humectant. Some effects independent of nicotine.	Cell viability Oxidative stress	4<ctrl, 0=ctrl 4>ctrl, 0=ctrl	4<cig, 0=cig 4<cig, 0=cig
[76]	Lung endothelial (rat, mouse, human)	Aerosol condens.	2 products tested. E-cigarettes disrupted lung endothelial barrier function. Evidence of oxidative stress from e-cig exposure also observed. Effects independent of nicotine. Similar results for cells, animal models.	Lung barrier fn. Cell prolif. Oxidative stress (8-OHdG)	4<ctrl, 1=ctrl 1<ctrl, 0=ctrl 2>ctrl, 0=ctrl	0>cig, 1=cig n.a. n.a.
[77]	Bronchial epithelial, whole body (mice)	Liquid, aerosol	Exposure to inhaled e-cig vapor decreased lung barrier function (mice), increased chemokine secretion (cells). Increase in renal fibrosis also observed. Results independent of flavorings.	Lung barrier fn. IL-8 Fibrosis	2<ctrl, 0=ctrl 1>ctrl, 0=ctrl 2>ctrl, 0=ctrl	n.a. n.a. n.a.
[78]	Bronchial epithelial lung fibroblasts, whole body (mice)	Liquid, aerosol	22 flavors screened. All e-cigs generated reactive oxygen species. E-cig exposure reduced cell viability, increased indices of oxidative stress. Morphological changes to cells also noted in e-cig conditions. Evidence of changes in inflammatory mediators (IL-6, IL-8) with dose-dependent effects from nicotine. Acute e-cig exposure increased levels of proinflammatory mediators (MCP-1, IL-1, IL-6, IL-13).	ROS Cell number Cell viability Interleukins Macrophage # Cyt/chemokines Oxidative stress	5>ctrl, 0=ctrl 4<ctrl, 0=ctrl 3<ctrl, 1=ctrl 3>ctrl, 3=ctrl 0>ctrl, 1=ctrl 6>ctrl, 5=ctrl 3>ctrl, 1=ctrl	n.a. 2<cig, 2=cig 3<cig, 0=cig 3<cig, 1>cig n.a. n.a. n.a.
[79]	Oral Keratinocytes	Aerosol	2 products tested. Substantial # of nanoparticles observed. E-cigs produced oxidative stress, dose-dependent. Evidence of cytotoxicity also observed.	Cytotoxicity Oxidative stress	2>ctrl, 0=ctrl 2>ctrl, 0=ctrl	n.a. n.a.
[80]	Whole body	Aerosol	4 products tested. Exposure to e-cig aerosol	Airway resist.	1>ctrl, 3=ctrl	1>cig, 3=cig

	(mice)		produced impairments in lung function, independent of nicotine. No effects observed for inflammatory mediators (KC, IL-1, IL-12). Effects independent of nicotine.	Tissue damping Tissue elastance Cytokines Inflammation	4>ctrl, 0=ctrl 4>ctrl, 0=ctrl 0>ctrl, 3=ctrl 1>ctrl, 7=ctrl	2>cig, 2=cig 2>cig, 2=cig 3<cig, 0=cig 8<cig, 0=cig
[81]	Pleural tissue	Liquid	18 products, 3 cell lines screened. Several flavorings and e-liquids had effects on cell viability. Evidence of increased reactive oxygen species and inflammatory mediators observed. Differing effects for different cell lines.	Cytotoxicity ROS Interleukin-8	4>ctrl, 4=ctrl 7>ctrl, 1=ctrl 1<ctrl, 7>ctrl	n.a. n.a. n.a.
[82]	Pulmonary microvascular endothelial	Aerosol	1 e-cig product tested, 5 repeated measures of outcomes. Exposure of pulmonary cells to post-vaping (human) blood serum produced increases in markers for inflammation and oxidative stress 30-120 min after e-cig inhalation.	CRP Nitric oxide-x sICAM ICAM express. ROS	4>ctrl, 1=ctrl 3<ctrl, 2=ctrl 3>ctrl, 2=ctrl 1>ctrl, 2=ctrl 5>ctrl, 0=ctrl	n.a. n.a. n.a. n.a. n.a.
[83]	Alveolar macrophages	Aerosol conden., e-liquid	6 e-cig products tested. Dose-dependent reduction in cell viability, increase in production of reactive oxygen species and pro-inflammatory cyto/chemokines (IL-6, IL-8, TNF, MCP-1, MMP-9), reduced phagocytosis.	Cell viability Cytotoxicity ROS Cyt/chemokines Phagocytosis	4<ctrl, 2=ctrl 5>ctrl, 1=ctrl 2>ctrl, 0=ctrl 9>ctrl, 1=ctrl 4<ctrl, 0=ctrl	n.a. n.a. n.a. n.a. n.a.

Note: Cell lines are human unless otherwise noted. E-cig = e-cigarette; PG = propylene glycol; fn = function; prolif. = proliferation; 8-OHdG = 8-hydroxydeoxyguanosine; ROS = reactive oxygen species; MCP = monocyte chemoattractant protein; CRP = C-reactive protein; resist. = resistance; ICAM = intracellular adhesion molecule; sICAM = soluble ICAM; expr. = expression; MMP = matrix metalloproteinase; TNF = tumor necrosis factor. Control conditions were clean air in [75, 76, 77, 78, 80], medium or incubator control in [75, 81], untreated or saline control in [76, 78, 81, 83], positive control in [79]. For other notes, see footnote for Supplementary Table 5.

Supplementary Table 7

Laboratory Studies for E-cigarette (E-cig) Effects on Immune Function and Disease Susceptibility

Ref	Cell type	E-cig liquid/ aerosol	Results	Assays	E-cigarette comparison with control	E-cigarette comparison with cigarette
[84]	Tracheo-bronchial	Liquid	1 e-cig product tested. Exposed cells showed dose-dependent effects for markers of inflammation, higher levels of HRV viral load, reduced levels of host defense molecule SPLUNC-1. Results independent of nicotine.	Cytotoxicity IL-6 HRV-16 SPLUNC-1	0>ctrl, 6=ctrl 6>ctrl, 0=ctrl 4>ctrl, 0=ctrl 2<ctrl, 0=ctrl	n.a. n.a. n.a. n.a.
[85]	Alveolar epithelial, keratinocytes; whole body (mice)	Liquid, aerosol	8 products tested. Cells exposed to e-cig aerosol showed increased cell death in a dose-dependent manner, increased # of infected MRSA bacteria. Exposed macrophages and neutrophils showed reduced anti-microbial activity. Aerosol inhalation didn't affect lung histology but increased levels of inflammatory cytokines (KC, IL-1, and TREM-1), decreased levels of protective ones (IL-3 and GM-CSF). E-cig exposed MRSA bacteria had more resistance to antimicrobial peptide L-37. In infection study, mice exposed to e-cigs had higher bacterial burden and higher mortality.	Cytotoxicity Toxicity (LDH) MRSA # Antimicrobial act. Bacterial burden Mortality	4>ctrl, 2=ctrl 4>ctrl, 0=ctrl 2>ctrl, 0=ctrl 4<ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	n.a. n.a. n.a. n.a. n.a. n.a.
[86]	Alveolar macrophages, whole body (mice)	Liquid, aerosol	2 products tested. Aerosol-exposed mice had more oxidative stress (TBARS). No risk effects for cytokines (IL-6, MCP-1, MIP-2). Exposed pneumonia-infected mice showed greater bacterial burden and impaired anti-bacterial defense. In controlled-infection study with influenza virus, mice in e-cigarette condition had higher morbidity and mortality.	Oxidative stress Cytokines Bacterial burden Phagocytosis Viral titer (H1N1) Mortality	1>ctrl, 0=ctrl 1<ctrl, 2=ctrl 3>ctrl, 0=ctrl 2<ctrl, 0=ctrl 1>ctrl, 0=ctrl 2>ctrl, 0=ctrl	n.a. n.a. n.a. n.a. n.a. n.a.
[87]	Bacteria (influenza, pneumonia,	Aerosol	E-cigarette exposure produced increased biofilm formation. Bacterial virulence was increased for all cell types. Generally similar	Biofilm formation Bacterial virulence Inflammation pot.	1>ctrl, 2=ctrl 4>ctrl, 0=ctrl 7>ctrl, 1=ctrl	1>cig, 3=cig 3<cig, 1=cig 2>cig, 6=cig

	staph)		results for e-cigs, cigarettes.			
[88]	Macrophages	Aerosol extract	4 products tested. Macrophages were exposed to e-cig extract and then infected with tuberculosis. Exposure reduced phagocytosis. Cytokine response (IL-1, IL-8, TNF-alpha) was greater for e-cigs than for cigarettes.	Phagocytosis Cytokines	1<ctrl, 3=ctrl 2>ctrl, 2=ctrl	3<cig, 1=cig 3>cig, 0=cig
[89]	Whole body (mice)	Aerosol	1 product tested. Mice were exposed to e-cig aerosol or cig smoke for 4 mo. No e-cig effect found for inflammation but macrophages of e-cig exposed mice showed pathogenic changes in lipid content and host defense interferon. Influenza-infected mice exposed to e-cigs showed increased morbidity and mortality. Effects independent of nicotine.	Lung inflammation Cytokines Macrophage lipids Interferon Morbidity Mortality	0>ctrl, 2=ctrl 0>ctrl, 6=ctrl 3>ctrl, 1=ctrl 2<ctrl, 2=ctrl 2>ctrl, 0=ctrl 1>ctrl, 1=ctrl	2<cig, 0=cig 6<cig, 0=cig n.a. n.a. n.a. n.a.
[90]	Lung, bronchial epithelial, whole body (mice)	Liquid, aerosol	2 products tested. Aerosol-exposed mice had reduced lung function. Cell studies indicated e-cig exposure increased macrophages; no effect for neutrophils or lymphocytes. E-cig exposure produced increased cell death, increased cytokines (IL-1, IL-6, CXCL, MMP), reduced ciliary beat frequency, expression of ciliogenesis gene FOXJ1. Effects mostly nicotine dependent.	Airway resistance Cell type, number Apoptosis Cytokines Ciliary function FOXJ1	1>ctrl, 1=ctrl 1>ctrl, 2=ctrl 2>ctrl, 0=ctrl 5>ctrl, 1=ctrl 1<ctrl, 0=ctrl 1<ctrl, 0=ctrl	n.a. n.a. n.a. n.a. n.a. n.a.
[91]	Sputum	Aerosol	7 (est.). Human study with 44 participants. E-cig users had similarities and differences in mucus protein composition compared with smokers and nonusers. E-cig users were more susceptible to NET formation. Mucus type ratio was elevated comparably in e-cig users and smokers. Evidence of increased oxidative stress and inflammatory mediators.	Smoking proteins Defense proteins Neutrophil protein NET-rel. proteins NET formation Mucins ratio	3>ctrl, 2=ctrl 2<ctrl, 2=ctrl 5>ctrl, 0=ctrl 4>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	3<cig, 2=cig 2<cig. 2=cig 3>cig, 2=cig 2>cig, 2=cig 1>cig, 0=cig 0<cig, 1=cig
[92]	Bronchial epithelial (vapers and smokers);	Aerosol	5 (est.) Human study with 34 participants. E-cig users (vapers) had more irritable airway mucosa. Vapers and smokers had considerably different protein profiles, with some overlap.	MUC4 (human) STIM1 (human) MUC5A (human) CYP1B1 (human)	1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	1<cig, 0=cig 0>cig, 1=cig 0>cig, 1=cig 0>cig, 1=cig

	whole body (mice)		Proteins related to mucin production and virus infection defense were particularly altered in vapers. Similar results in humans, mice, and cell cultures. Much of effect was attributable to aerosolized PG/VG humectant.	MUC5AC (mice) STIM1 (mice)	1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	n.a. n.a.
[93]	Bronchial epithelial	Liquid, aerosol	3 products tested. E-cig exposure reduced ciliary beat frequency and cilia motility, mostly at higher doses of cinnamaldehyde, and reduced membrane permeability. Similar effects for e-liquid and aerosol.	Cilia beat freq. % cilia in motion Mitochondrial membrane perm.	1<ctrl, 2=ctrl 1<ctrl, 2=ctrl 2<ctrl, 1=ctrl	n.a. n.a. n.a.
[94]	Neutrophils	Liquid	Two flavoring chemicals impaired neutrophil function in a dose-dependent manner, for all concentrations. Benzaldehyde acetal had a particularly potent effect.	Oxidative burst Phagocytosis	4<ctrl, 1=ctrl 3<ctrl, 1=ctrl	n.a. n.a.
[95]	Neutrophils, whole body (mice)	Aerosol Extract	Studied neutrophil function in relation to two types of infectious bacteria. E-cig exposure impaired several indices of neutrophil function, independent of nicotine. Controlled-infection study found aerosol exposure decreased # of leukocytes at peritoneal site and increased bacterial count at site.	Chemotaxis Membrane fluidity ROS production NET suppression Phagocytosis Leukocytes # bacteria	1<ctrl, 0=ctrl 1>ctrl, 0=ctrl 1<ctrl, 0=ctrl 1<ctrl, 0=ctrl 2<ctrl, 0=ctrl 1<ctrl, 0=ctrl 1>ctrl, 0=ctrl	n.a. n.a. n.a. n.a. n.a. n.a. n.a.
[96]	Bronchial epithelial (e-cig users, smokers, nonsmokers)	Aerosol	Cells obtained from bronchoscopies. Protease levels were significantly elevated among e-cig users, comparable to smokers. Levels of protease inhibitors (A1AT, SLP1, TIMP-1 TIMP-2) were not significantly different.	Neutrophil elastase MMP-2 MMP-9 Antiproteases	1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 0<ctrl, 4=ctrl	0<cig, 1=cig 0<cig, 1=cig 0<cig, 1=cig 0<cig, 4=cig

Note: Cells are human unless otherwise noted. E-cig = e-cigarette; est. = estimated; HRV = human rhinovirus; MRSA = methicillin-resistant staphylococcus aureus; MCP = monocyte chemoattractant protein; TNF = tumor necrosis factor; NET = neutrophil extracellular traps; rel. = related; STIM = stromal interaction molecule; permeab = membrane permeability; MMP = matrixmetalloprotease. Control conditions were clean air in [75, 86, 89, 92, 94], medium control in [84, 85, 87, 90, 91, 93], untreated or littermate control in [84, 88, 95]. For other notes, see footnote for Supplementary Table 5.

Supplementary Table 8

Studies on Effects of E-cigarettes on Genetic Damage and Gene Expression

Ref	Cell type	E-cig liquid/ aerosol	Results	Assays	E-cigarette comparison with control	E-cigarette comparison with cigarette
[99]	Epithelial cell lines (normal, cancerous)	Aerosol	2 products tested. E-cig exposure resulted in significant DNA damage on neutral comet assay and greater double-strand breaks on H2AX assay. Also observed increased cell death through apoptosis and necrosis. Effects independent of nicotine.	Comet assay H2AX Cytotoxicity Annexin	5>ctrl, 0=ctrl 5>ctrl, 0=ctrl 3>ctrl, 2=ctrl 5>ctrl, 0=ctrl	n.a. 3<cig, 2=cig 5<cig, 0=cig 5<cig, 0=cig
[100]	Nasal lavage (e-cig users, smokers, nonusers)	Aerosol	13 (est.) Human study with 39 participants. Of 543 genes assayed, 53 were differentially expressed comparing smokers with nonusers and 358 differentially expressed when comparing e-cigarette users with nonusers. The magnitude of suppression of genes involved in host defense responses against bacterial and viral infections was consistently larger for e-cigarette users.	CSF-1 CCL26	1< ctrl, 0 =ctrl 0<ctrl, 1=ctrl	0>cig, 1=cig 1<cig, 0=cig
[101]	Bronchial epithelial; whole lung (human)	Aerosol	1 product tested. 546 genes were differentially expressed across 5 conditions for smoking/e-cigarette use. Patterns of gene expression had both similarities and differences for cigarettes and e-cigarettes. Genes that were downregulated involved ciliary function; upregulated genes involved oxidative stress and DNA damage.[A]	DNAH10A FOXJ1 CYP1A1 CYP1B1 8-isoprostane	8<ctrl, 4>ctrl 8<ctrl, 4>ctrl 4>ctrl, 6<ctrl 8<ctrl, 4>ctrl 6>ctrl, 0=ctrl	5<cig, 1>cig 5<cig, 1>cig 4>cig, 1<cig 5<cig, 1>cig 4<cig, 1>cig
[102]	Bronchial epithelial; normal, dysplastic and cancer	Aerosol	2 products tested. E-cig exposure produced DNA damage, dose-dependent, independent of nicotine. Also significant increases in oxidative stress and reactive oxygen species, decrease in total antioxidant capacity and expression of DNA excision repair proteins	q-PADDA[B] 9-oxo-dG DNA damage ROS Antioxidant cap. OGG1	19>ctrl, 5=ctrl 5>ctrl, 0=ctrl 4>ctrl, 0=ctrl 2>ctrl, 0 =ctrl 2<ctrl, 0=ctrl 4<ctrl, 0=ctrl	12<cig12=cig[B] 2<cig, 2>cig 4<cig, 0=cig 0<cig, 2=cig 0>cig, 2=cig 1<cig, 3=cig

			OGG1 and ERCC1. Though most short-term effects of e-cigs were lower than for cigarettes, long-term exposures showed comparable or greater effects in some assays.			
[103]	Bronchial epithelial	Aerosol	7 products tested. JUUL pod constituents exposed to e-cig aerosol showed increased ROS generation, reduced barrier function, increased cytokines. Results dependent on cell lines. 3 flavors produced significant DNA damage.	ROS IL-8 Prostaglandin Cytokines Barrier function	2>ctrl, 4=ctrl 3>ctrl, 1=ctrl 2>ctrl, 2=ctrl 13>ctrl 11=ctrl 1<ctrl, 0=ctrl	6<cig, 0=cig n.a. n.a. n.a. n.a.
[104]	Epithelial—Lung, heart, bladder (mouse, human)	Aerosol	1 product tested. E-cig exposure caused significant levels of two harmful deoxyguanosine adducts. E-cig exposure also produced significant decrements in DNA repair mechanisms for lung cells, both nucleotide excision repair (NER)and base excision repair (BER). Effects observed in both mouse and human cells.	O6-medG PdG NER BER	3>ctrl, 1=ctrl 3>ctrl, 1=ctrl 1<ctrl, 0=ctrl 1<ctrl, 0=ctrl	n.a. n.a. n.a. n.a.
[105]	Whole lung (human)	Aerosol	15 (est.) Human study with 93 participants (smokers, e-cig users, nonusers). Large number of differentially expressed transcripts in both e-cig users and smokers, but little overlap. A majority of the deregulated genes for e-cig users were related to tumorigenesis. Specific downregulation for two tumor suppressor genes, NOTCH1 and HERC2.	NOTCH1 HERC2	1<ctrl, 0=ctrl 1<ctrl, 0=ctrl	0<cig,1=cig 0<cig,1=cig
[106]	Bronchial epithelial (e-cig users, smokers, nonsmokers)	Aerosol	Large number of differentially expressed transcripts for e-cig users and smokers. E-cig users' gene expressions were intermediate between nonsmokers and smokers for almost all genes studied. Cytokine levels for e-cig users tended to be intermediate between nonsmokers and smokers but most tests were nonsignificant.	Cytokines	3>ctrl, 7=ctrl	1<cig, 9=cig

Note: Cell lines are human unless otherwise noted. E-cig = e-cigarette; est. = estimated; CSF = Colony stimulating factor; CCL26 = C-C chemokine ligand 26; q-PADDA = primer-anchored DNA damage detection assay; 8-oxo-dG = 8-hydroxy-deoxyguanosine; ROS = reactive oxygen species; O6-medG = O6-methyl-deoxyguanosine; PdG= N2-propano-deoxyguanosine. Control conditions were clean air in [101, 104], medium control in [101, 102, 103], untreated cells [99, 102], positive control in [100], nonsmokers in [100, 105, 106].

[A] Notation shows fold change for e-cigarettes and cigarettes, respectively, compared with air control.
[B] By comparison with previous study.