

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 S1, S2). 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 S3, S4), 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 S1, 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 S1, 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 S1, 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 decreases from 1.40 ($e^{0.33}$) to 1.22 ($e^{0.20}$; Supplementary Figure S1, 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 S1, 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 S1). 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 S2).

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 S2, 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 S2, 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 S2, 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 decreases from 1.46 ($e^{0.38}$) to 1.27 ($e^{0.24}$; Supplementary Figure S2, 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 S2, 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 S3). 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 S4).

Supplementary Table S1. 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 S2. 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 S3. 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

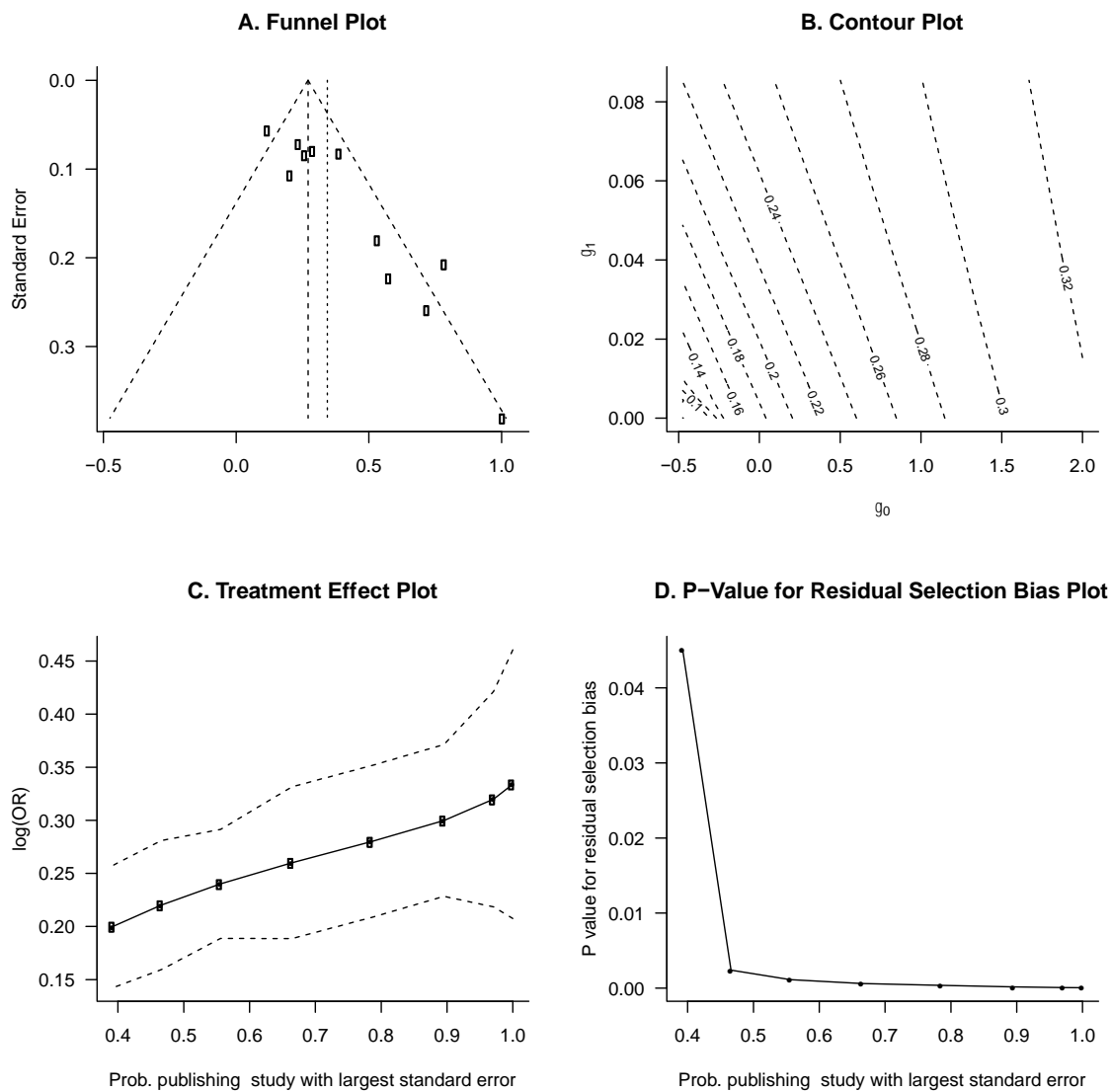
Note: Adj.=Adjusted; Prob.=Probability; OR=odds ratio; CI=confidence interval

Supplementary Table S4. Pooled Adj. Odds Ratio: Copas Selection Model and Random Effects Model

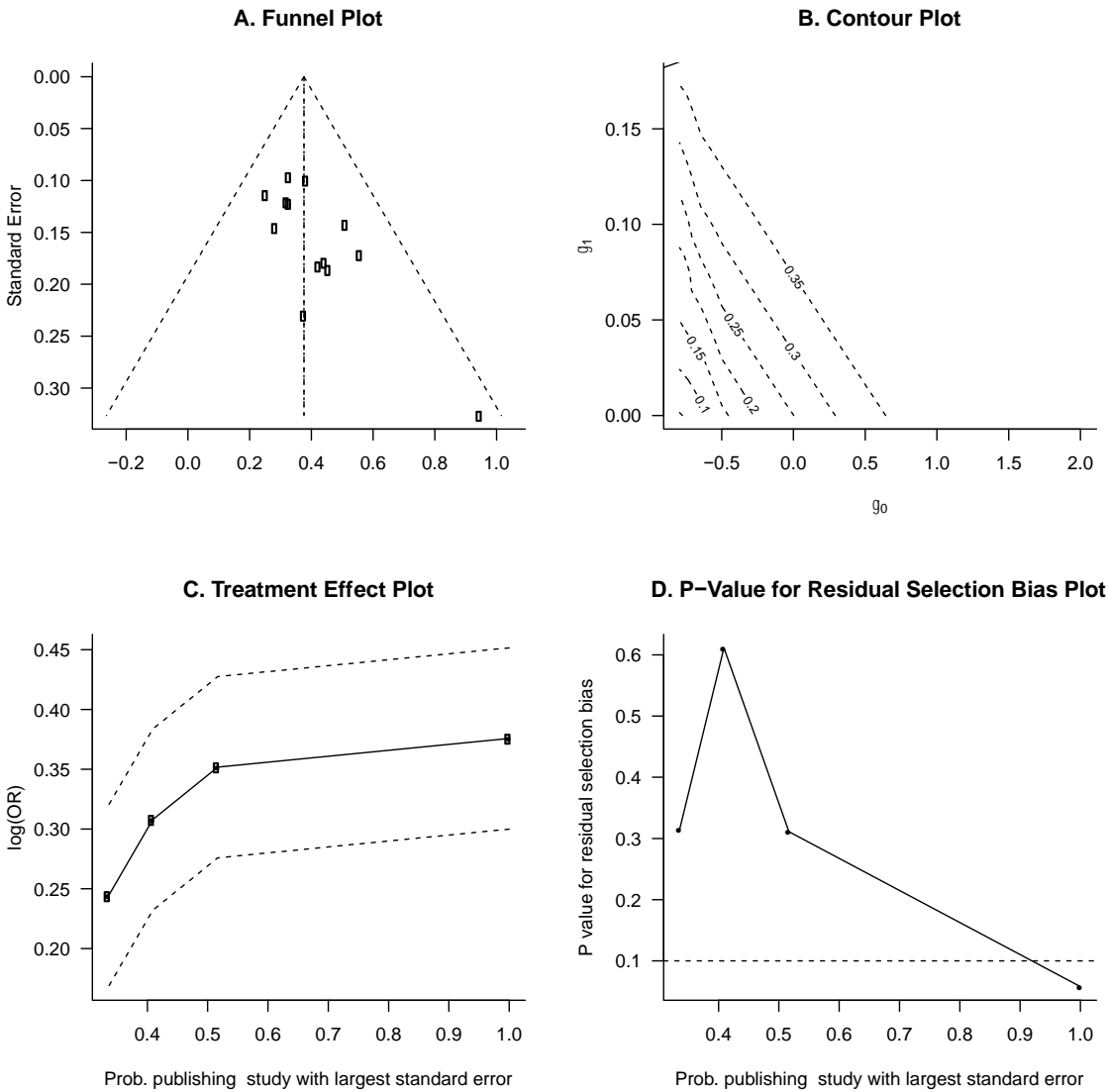
Model	OR [95% CI]	P-value for hypothesis of overall treatment effect	P-value for hypothesis that no selection remains unexplained
Copas Selection	1.28 (1.18-1.38)	<0.001	0.314
Random Effects	1.46 (1.35-1.57)	<0.001	—

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

Supplementary Figure S1. Copas Selection Modelling, Adolescent Studies



Supplementary Figure S2. Copas Selection Modelling, Adult Studies



Supplementary Table S5

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 S6

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 prolifer. 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 (mice)	Aerosol	4 products tested. Exposure to e-cig aerosol produced impairments in lung function, independent of nicotine. No effects observed for inflammatory mediators (KC, IL-1, IL-12). Effects independent of nicotine.	Airway resist. Tissue damping Tissue elastance Cytokines Inflammation	1>ctrl, 3=ctrl 4>ctrl, 0=ctrl 4>ctrl, 0=ctrl 0>ctrl, 3=ctrl 1>ctrl, 7=ctrl	1>cig, 3=cig 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 condens., 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 S7

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,	Aerosol	E-cigarette exposure produced increased biofilm formation. Bacterial virulence was	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

	pneumonia, staph)		increased for all cell types. Generally similar 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	Aerosol	5 (est.) Human study with 34 participants. E-cig users (vapers) had more irritable airway mucosa. Vapers and smokers had considerably	MUC4 (human) STIM1 (human) MUC5A (human)	1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	1<cig, 0=cig 0>cig, 1=cig 0>cig, 1=cig

	smokers); whole body (mice)		different protein profiles, with some overlap. 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.	CYP1B1 (human) MUC5AC (mice) STIM1 (mice)	1>ctrl, 0=ctrl 1>ctrl, 0=ctrl 1>ctrl, 0=ctrl	0>cig, 1=cig 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 S8

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,	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,	q-PADDA ^B 9-oxo-dG DNA damage ROS	19>ctrl, 5=ctrl 5>ctrl, 0=ctrl 4>ctrl, 0=ctrl 2>ctrl, 0 =ctrl	12<cig12=cig ^B 2<cig, 2>cig 4<cig, 0=cig 0<cig, 2=cig

	dysplastic and cancer		decrease in total antioxidant capacity and expression of DNA excision repair proteins 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.	Antioxidant cap. OGG1	2<ctrl, 0=ctrl 4<ctrl, 0=ctrl	0>cig, 2=cig 1<cig, 3=cig
[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	Cytokines	3>ctrl, 7=ctrl	1<cig, 9=cig

			nonsmokers and smokers but most tests were nonsignificant.			
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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.