

**Inflammation and Oxidative Stress from E-cigarette Exposure: Implications for COPD and Asthma****Abstract**

Currently, little is known about the effects of e-cigarette use on chronic respiratory diseases, due to their relative novelty. This review compiles data on the cellular effects of e-cigarette use with population data on disease incidence to determine potential risk for COPD and asthma development, two of the most prevalent respiratory diseases. We searched the Google Scholar database for studies on e-cigarette exposure and levels of inflammation and oxidative stress in human cells and e-cigarette users, as well a population studies analyzing e-cigarette use and respiratory disease incidence. All reviewed studies found significant increases in inflammatory biomarkers, as well as pro-inflammatory cytokines, demonstrating a correlation between e-cigarette use and a pro-inflammatory affect. Our findings suggest e-cigarette vapor contains reactive oxygen species, and that exposure increases cellular oxidation and lowers antioxidant power. Every population study we reviewed found significant correlations between COPD and e-cigarette use, and asthma and e-cigarette use. These population studies cannot provide causational data, though the basic cellular data provides support for causative effects. Further research should investigate the link between the cellular and population data to identify causation and understand the impact of e-cigarette use on disease rates.

**Keywords:** Electronic Cigarettes, Inflammation, Oxidative Stress, Reactive Oxidative Species, COPD, Asthma

<b>Abbreviations</b>	
8-OHdG	8-oxo-2'-deoxyguanosine
A549	Adenocarcinoma Human Alveolar Basal Epithelial Cells
ACT	Asthma Control Test
AOR	Adjusted Odd's Ratio
BAL	Bronchio-alveolar lavage
BEAS-2B	Bronchial Epithelial Cell Line
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-Reactive Protein
CXCL1	C-X-C motif ligand 1
CYP1A1/2	Cytochrome P450 Family 1 Subfamily A Member 1 and Member 2
CYP1B1	Cytochrome P450 Family 1 Subfamily B Member 1
CYP2B1/2	Cytochrome P450 Family 1 Subfamily B Member 1 and Member 2
CYP3A	Cytochrome P450 Family 3 Subfamily A
ECG	Electrocardiogram
EVALI	E-cigarette and Vaping Associated Lung Injury
FGF	Fibroblast growth factor
GM-CSF	Granulocyte macrophage colony stimulating factor
H292	Influenza A virus subtype H2N2
HaCaT	Human Epidermal Keratinocyte Line
HFL1	Human fetal lung fibroblasts
HMOX1	Heme Oxygenase 1
IFN $\gamma$	Interferon Gamma
IL-	Interleukin -
LDH	Lactate dehydrogenase
MAPK	mitogen-activated protein kinase
MIP-1 $\beta$	Macrophage Inflammatory Protein - 1 $\beta$
MMP-9	Matrix metalloproteinase-1
NQO1	NAD(P)H Quinone Dehydrogenase 1
OR	Odds Ratio
OX/ROS	Oxidants/Reactive Oxygen Species
PG	Propylene Glycol
RAGE	Receptor for Advanced Glycation End Products
TNF $\alpha$	Tumor Necrosis Factor $\alpha$

## **Introduction**

The Global Burden of Disease Study states that respiratory disease makes up 30 of the most common causes of death around the world, with COPD as the 3<sup>rd</sup> most common cause, lower

respiratory tract infection, tracheal, chronic, and lung cancers as 6<sup>th</sup>, Tuberculosis as 12<sup>th</sup>, and asthma as 28<sup>th</sup><sup>1</sup>. For the purposes of this review, we focus on COPD and Asthma to understand how long-term use of e-cigarettes could affect risk for these common respiratory diseases<sup>2</sup>.

E-cigarettes are devices that vaporize e-liquids (which are typically a mixture of propylene, glycol, glycerin, nicotine, and flavorings<sup>3</sup>) for inhalation. These e-cigarettes can contain nicotine and/or tetrahydrocannabinol and come in a variety of sweet and fruit flavors<sup>4</sup>. These devices were invented as a smoking cessation aid, though they are also used for nicotine and THC delivery, for convenience (if smoking is not an option), and for their flavors<sup>5</sup>.

Currently, due to the lack of data we have on real-world asthma and COPD rates in e-cigarette users, the only way to understand disease risk is through existing data on cellular effects and correlational population surveys. Though this cellular data often comes from animal lungs, human explant tissue, or *in vitro* cells which can't provide concrete evidence for *in vivo* exposure, while population surveys can only provide correlation data.

In this review we present current data on the inflammation and oxidative stress effects of e-liquids and e-vapors, to provide insight into mechanisms important in COPD and asthma development and disease course. We then compile population data on COPD and asthma incidence in e-cigarette users, to provide human-level data on the potential link between e-cigarette use and COPD and asthma incidence.

## **Methods**

This review had two parts, the first was a search for articles on inflammation and oxidative stress biomarkers after e-cigarette exposure, the second was a search for population studies of e-cigarette use and COPD and asthma incidence. The Google Scholar database was used for

searches, as it provided the largest breadth of results and is open access. The following search terms were used to gather papers that met our search criteria.

<b>Table 1: Search terms for human, cellular, and Maurine studies on inflammatory and oxidative effects of e-cigarette exposure</b>		
e-cig*	AND	inflammation
Electronic cigarette	AND	inflammation
e-cig*	AND	Oxid*
e-cig*	AND	Oxidative stress
-----	NOT	EVALI
-----	NOT	Oral effects

<b>Table 2: Search terms for population studies on COPD/asthma and e-cigarette use correlations</b>		
e-cig*	AND	Chronic obstructive
Electronic cigarettes	AND	COPD
e-cigarettes	AND	COPD
Electronic nicotine delivery system*	AND	Chronic Obstructive
e-cig*	AND	asthma
Electronic cigarettes	AND	asthma

\*- used to truncate search terms

For this portion of the study, our search criteria included being published in English, published after 2000, and no grey literature or literature reviews were used. Our search terms yielded results in Google Scholar that were title reviewed for inclusion. Included titles underwent abstract screening before undergoing full text screening and data extraction.

Data extraction was accomplished using the table shown below, slight modifications for the population-study portion of this review.

<b>Table 3: Data extraction table with sample study</b>			
<b>Study</b>	<b>Exposed Material</b>	<b>Vapor Type/Device</b>	<b>Cellular Changes/Biomarkers</b>
Smith et al	Lung adenocarcinoma cell line A549	0mg- 20mg/mL nicotine, 3 <sup>rd</sup> Generation Device	No Viability change (A549 only)

	Human Lung epithelial BEAS-2B cells		Lowered Viability (BEAS-2B) No LDH Change Increased DNA strand breaks (only with nicotine)
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(example study, not valid data)

After extraction, data was written up and presented in the report shown below.

## **Results**

### **Inflammation**

Inflammation is an important part of the immune response, but chronic inflammation has been shown to have serious adverse effects. Combustion cigarette use has been linked to increased respiratory inflammation and this contributes to the increased disease risk associated with cigarette smoking.

To understand whether e-cigarettes would have similar effects we reviewed cell and human studies on the effects of e-cigarettes on inflammatory biomarkers.

**Table 4:** Inflammatory changes and exposure scenarios across reviewed studies

Study	Cell Type	Exposure Scenario	Liquid Type	Inflammatory Change
Higham et al (2016) <sup>6</sup>	Neutrophils from healthy non-smokers	24h intubation in 0.1-0.3 optical density solutions	VIP brand, "USA Tobacco" flavor, 24mg/mL nicotine	+ CD11b +CD66b + MMP-9 + Neutrophil Elastase + IL8
Wu et al <sup>7</sup>	Lung Epithelial Cells	Exposure to liquid extracts for 24 or 48h.	Treated with "medium", tobacco flavored e-liquid, 0 or 18 mg/mL nicotine, InnoVapor LLC brand	+IL-6
Higham et al (2018) <sup>8</sup>	Bronchial Epithelial & Calu-3 Cells	24h incubation in 0.1-0.3 optical density solutions	VIP brand, "USA Tobacco" flavor, 24mg/mL nicotine	+LDH O IL-6/CXCL8 (epithelial) + IL-6/CXCL8 (Calu-3) + p38 MAPK Phosphorylation
Cervellati et al <sup>9</sup>	A549 HaCaT	50min exposure via a vacuum pump	e-CIG Mini Touch T-Fumo T-TEX	- Viability + LDH + Shape Change +IL-8 +IL-6 +IL-10 +IL-12 + FGF + MCP-1 + GM-CSF + MIP-1 $\beta$
Lerner et al <sup>10</sup>	H292 BAL fluid from mice	Cells were exposed to 4sec puffs every 30secs for durations of 5, 10, and 15min. Mice had 5h exposures over 3d	Refillable pen-style & Blue e-cig with disposable cartomizer. Used tobacco, menthol, and fruit/sweet flavors. Levels of nicotine varied	+ Shape Change O Viability +IL-8 (H292) +IL-6 (H292) +IL-6 (BAL) O Macrophage (BAL)

+ = Increase - = decrease in O = no change sec- seconds min – minutes d – days h - hour

Cellular studies have identified several different markers of inflammation, namely Interleukins (IL-8, IL-6, etc.) and lactate dehydrogenase<sup>11</sup>. There also were increases in inflammatory pathways, such as p38 MAPK. Signs of neutrophil activation also were increased, such as IL-8 (a key neutrophil attractant<sup>12,13</sup>) CD11b and CD66B (biomarkers for neutrophil

attraction and are key for neutrophil recruitment and granulation)<sup>14,15,16</sup>. Neutrophils play an important role in the inflammatory response and development of COPD.

One study also found an upregulation of MMP-9 and Neutrophil Elastase activity which are released from neutrophils in COPD, correlate to disease severity, and have the potential to damage the lung parenchyma<sup>17</sup>.

These cellular studies show the possibility for e-cigarette exposure to instigate pulmonary inflammation. Studies have since been done on humans to test the impact of e-cigarette use on inflammatory biomarkers and pulmonary functioning.

**Table 5: Pulmonary Functioning and Inflammatory Biomarkers in Human E-Cigarette Users**

Study	Exposed Population	Inflammatory Changes
Song et al (2018) <sup>18</sup>	13 e-cigarette users	+cell count +IL-8 +IL-6 +IL-1 $\beta$
Vakali et al <sup>19</sup>	53 subjects, 10min e-cigarette challenge	-FeNO (no nicotine) +airway temperature (nicotine) +exhaled CO (both)
Song et al (2020) <sup>20</sup>	30 never smoker 4-week e-cigarette challenge	O cell counts O cytokines at follow up +cells correlating with urinary PG +IL-8, -13 correlating with urinary PG + TNF $\alpha$ correlating with urinary PG O mRNA changes
Chatterjee et al <sup>21</sup>	10 participant e-cigarette challenge, 2sec puffs, 16-17 inhalations	+ Serum CRP (peak 60-120mins post-exposure) did not return to baseline at 360mins
Brozek et al <sup>22</sup>	120 adults (control, tobacco, 30 e-cigarette, 30 dual) Multi-fruit flavor e-cigarette challenge, 12mg/mL nicotine average use for 5 min	O O <sub>2</sub> -FeNO -CO (dual users) -Peak expiratory flow -Maximal expiratory flow (75% FVC) (dual users) +Air temp
Song, Freudenheim, et al (2020) <sup>23</sup>	742 never smokers, 15 e-cigarette users, 16 smokers BAL/lung brushings	+inflammatory cytokines +cell counts +IL-1 $\beta$ , -6, -8,
Kameshwar	Collected plasma, urine, saliva,	+IL-6, IL-8, IL-13, IFN $\gamma$ (plasma)

et al <sup>24</sup>	exhaled breath condensate, and measured pulmonary function and vaping characteristics from e-cigarette and never smokers	+ MMP-9 (plasma) -CXCL1 -RAGE - pro-resolving resolvin D1/D2 (plasma) + IFN $\gamma$ (urine) -IL-8, IL-10, IL-13 (urine)
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+ = Increase - = decrease in O = no change sec- seconds min – minutes d – days h - hour

We consistently found increases in biomarkers of inflammation, including interleukins (specifically IL-8 and IL-6), exhaled air temperature, which is another biomarker for inflammation.

Cervellati et al<sup>25</sup> found that liquids with flavorings and nicotine had a greater effect than flavorings alone and reported no change in humectants alone. This was verified by the Lerner et al study that found IL-8 increase only with flavorings/nicotine. The 2020 Song et al study<sup>20</sup> found similar results, with non-significant cell count and cytokine increases in their participants. The increases became significant when correlated to the levels of urinary PG (used as a biomarker for e-cigarette exposure). This is potentially due to the fact that their study utilized e-liquid that only contained humectants, without flavorings or nicotine.

### **Oxidative Stress**

Oxidative stress has been linked to a variety of chronic diseases. Oxidative stress occurs when the production of free radicals and reactive metabolites (often called oxidants and reactive oxidation species (OX/ROS)) are not cleared by the body's protective antioxidant mechanisms. Evidence has also demonstrated a link between inflammation and oxidative stress. As an increase in inflammatory cells leads to an increase in oxidation and decrease in antioxidant power.

Oxidative stress also can cause cellular and DNA damage that can contribute to the development

of age-related diseases; like COPD. In COPD markers of oxidative stress are significantly higher<sup>26</sup>. The link between oxidative stress and asthma is less clear, though there appear to be correlations between the two and evidence to suggest that OX/ROS plays a role in asthma development<sup>27</sup>. Thus, several studies have tested the levels of OX/ROS in vapor and the potential oxidative effects of vapor on users.

**Table 6:** Oxidative changes and exposure scenarios across reviewed studies

Study	Cell Type	Exposure Scenario	Liquid Type	Oxidative Change
Lerner et al <sup>10</sup>	H292 & HFL1  Mouse Model	Cells were exposed to 4sec puffs every 30secs for 5, 10, or 15 min. Mice exposed 5h/3d	Refillable pen-style & Blue e-cig with disposable cartomizer. Tobacco, menthol, and fruit/sweet flavors, varying nicotine levels	+ OX/ROS (vapor alone) + OX/ROS (cells) + Shape Change - Lung glutathione (mice)
Canistro et al <sup>28</sup>	Rat Model	1ml/day of e-liquid delivered at 17s puffs and 20min rest periods. 11 cycles/d, 5ds per week, 4w	18mg/mL nicotine, Essential cloud brand, red fruit flavor	+CYP1A1/2 + CYP2B1/2 + CYP3A -Ferric antioxidant power + 8-OHdG
Dusautoir et al <sup>29</sup>	BEAS-2B	Cells were incubated with 0.1-0.3 optical density solutions with liquid for 24h.	VIP brand, "USA Tobacco" flavor, 24mg/mL nicotine	+ CYP1A1 + CYP1B1 + Nuclear erythroid 2-related factor 2 + NQO1 + HMOX1
Song et al <sup>23</sup>	E-cigarette Users BAL	E-cigarette users	E-cigarette users	+ Inflammatory biomarkers + Oxidative stress
Kameshwar et al <sup>24</sup>	Collected plasma, urine, saliva, exhaled breath condensate	E-cigarette users	E-cigarette users	+ 8-OHdG (urine) +8-isopostane

+ = Increase - = decrease in O = no change sec- seconds min – minutes d – days h - hours

The reviewed studies identified an increase in oxidative stress and reactive oxygen species in both mouse and cellular models, which is believed to be caused by several factors.

One source of OX/ROS is believed to be the vapor itself. The Lerner et al study<sup>10</sup> detected OX/ROS in vapor alone. Finding that both nicotine and non-nicotine liquids contained elevated levels of OX/ROS. Indicating that even without flavorings or nicotine the aerosolization of propylene glycol and glycerin produced OX/ROS. Levels of reactive oxygen species also correlate to levels of carbonyls in vapor. Carbonyls like formaldehyde and acetaldehyde are consistently found in e-cigarettes<sup>30</sup> and can contribute to antioxidative imbalance<sup>31</sup>.

NQO1 (an OX/ROS protective enzyme)<sup>32</sup>, Nuclear erythroid 2-related factor 2 (upregulates antioxidant response enzymes and is protective against COPD, asthma, etc.)<sup>33</sup>, and HMOX1 (which increases in response to oxidative stress and is associated with an increased risk for COPD)<sup>34, 35</sup> were also upregulated in exposed cells, and are involved in the body's response to oxidants and can be used as biomarkers for increased oxidation.

Cells exposed to e-cigarette vapor also saw increases in cellular OX/ROS production. Exposure to vapor led to an increase in Cytochrome P50 genes, like CYP1A1 and 2, CYP1B1 and 2, and CYP3A, which affect OX/ROS production<sup>36, 37</sup>. In humans, this would correlate to an increase in CYP monooxygenases that could similarly elevate levels of OX/ROS, as CYP monooxygenases are involved in metabolizing xenobiotic compounds<sup>38, 39</sup>. Mice also saw lowered levels of lung glutathione (a thiol important to maintaining a cellular redox balance)<sup>37</sup>.

In tandem with increasing OX/ROS production, studies also found a decrease in antioxidant capacity. Finding a reduction in antioxidant enzymes catalase, DT-diaphorase, and

superoxide dismutase were also found to be reduced in the rat's lungs<sup>40</sup>. As was the conjugating phase 2 glutathione s-transferase enzyme which is involved in the detoxification of foreign substances<sup>10</sup>. The Lerner study also found significantly reduced antioxidant power in exposed rats, using ferric antioxidant power testing<sup>10</sup>. Meanwhile, there was an increase in antioxidant power in control rats, which demonstrates that the healthy rats were able to respond to changes with an increase in antioxidant systems while exposed rats were less able to clear OX/ROS<sup>40</sup>. This downregulation of antioxidant enzymes would contribute to the number of free radicals.

Oxidative imbalance can contribute to the development of COPD, asthma, and other respiratory disease. It also can damage DNA and increase risk for cancer. Oxidative changes led to an increase in the guanosine oxidation of 8-OHdG<sup>40</sup>, a common free radical induced DNA oxidative lesion that is correlated to increased mutagenicity in mammalian cells<sup>41</sup>. It is used as a biomarker for carcinogenesis and oxidative stress<sup>42</sup> and was found to be significantly increased in exposed rats by Canistro et al<sup>28</sup>. Many studies also identified cellular shape changes and increases in inflammatory cytokines. Further corroborating a link between OX/ROS, inflammation, and cellular damage.

Overall, there is considerable evidence that e-cigarette exposure affects the oxidative balance of the lungs. Though the extent to which this will contribute to disease risk is still not understood given the lack of human evidence.

### **Chronic Obstructive Pulmonary Disease**

Inhalation of particles (such as cigarette smoke) is a major risk factor for COPD, as inhalation can create chronic inflammation and tissue remodeling that increase risk for COPD.

Given the link between smoking, particle inhalation, and COPD, studies have investigated the potential effects of e-cigarette use on COPD rates. Currently, most of the data on COPD and e-cigarette use is correlational data from population studies. Here, we aimed to combine *in vitro* laboratory data with population studies to better understand the potential link between e-cigarette use and COPD.

<b>Data Set</b>	<b>Data Collection</b>	<b>E-cigarette use Definition</b>	<b>COPD Definition</b>	<b>OR</b>
PATH Wave 1 <sup>43</sup>	Longitudinal survey of tobacco use in U.S. youth and adults	E-cigarette use = every day or occasional, current or former	COPD = emphysema, chronic bronchitis, or COPD	1.86
Path Wave 1, 2, 3 <sup>44</sup>	Longitudinal survey of tobacco use in U.S. youth and adults	E-cigarette use = every day or occasional, current or former	Respiratory Disease = COPD, chronic bronchitis, emphysema, asthma	1.29 (Current) 1.32 (former)
PATH Wave 4 <sup>45</sup>	Longitudinal survey of tobacco use in U.S. youth and adults	E-cigarette use = every day or occasional, current or former	Respiratory Disease = COPD, chronic bronchitis, emphysema, asthma	0.63-2.43
BRFSS <sup>46</sup>	Cross-sectional random-dial telephone survey	E-cigarette use = ever use (former or current use), some days or every day use	Respiratory Disease = been diagnosed by a professional with COPD, emphysema, chronic bronchitis, and/or asthma	2.58
BRFSS <sup>47</sup>	Cross-sectional random-dial telephone survey	E-cigarette use = ever use (former or current use), some days or everyday use	Respiratory disease = ever use (former or current use), some days or everyday use	1.65 (some days) 2.77 (daily)
Southern California Children's Health Study <sup>48</sup>	Survey of grade 11 and 12 southern California students	e-cigarette use = never-users, former users (not in past 30 days), current users (used in past 30 days)	Chronic Bronchitis Symptoms = daily cough for 3 months in a row, congestion, or phlegm not with a cold or bronchitis	1.41 (current) 1.70 (former)

Several analysis of the Population Assessment of Tobacco and Health (PATH) study identified a correlation between e-cigarette use and COPD diagnosis<sup>43</sup>. While the PATH study provides a significant bolus of data on tobacco use, it is only correlational. To better determine a timeline of risk, a longitudinal analysis from Bhatta et al analyzed three waves of the PATH study to provide more longitudinal data<sup>44</sup>. They conducted a reverse-causality analysis of the data using a combined analysis of those with respiratory disease at Wave 1 who were never users and estimating the odds of beginning e-cigarette use by Wave 2 or 3. Having a respiratory disease at wave 1 significantly predicted e-cigarette use at Wave 2 or 3. This may prove a correlative and not causative relationship found in this study. Potentially showing that after diagnosis, patients began using e-cigarettes with the belief that they will be therapeutic.

Flavorings and nicotine have been identified as the most harmful part of e-cigarette vapor. So to identify the impacts of different liquid types on COPD prevalence Wave 4 of the PATH study was then analyzed by Shi et al.<sup>45</sup> Specifically, this study tested for associations between use of different e-liquid flavors and COPD. Finding that COPD prevalence differed across flavor categories. Compared to other flavors, tobacco flavor had a higher AOR for self-reporting COPD (AOR=2.43) among current combustion smokers. Fruit flavors had a lower AOR= 0.63, which provides an interesting venue for research. Other flavors had an AOR=1.89. Tobacco flavor having an association with COPD also held true for former smokers. This association could be due to the fact that recent research has identified that tobacco flavorings may provide an independent source of oxidants and reaction oxygen species.

Two analysis of the Hawaiian Behavior Risk Factor Surveillance System (BRFSS) found positive associations between respiratory diseases and e-cigarette use, and this is corroborated by other studies<sup>49</sup>.

These studies can only provide correlational data. Though they form a consensus on a correlation between COPD and e-cigarette use. This provides interesting patient data, given the evidence on cellular effects that may increase risk on a cellular level.

E-cigarettes were originally intended as tobacco cessation devices, and questions still remain as to whether e-cigarettes provide a harm reduction or smoking cessation strategy for smokers with COPD. A retrospective study<sup>50</sup> matched 24 COPD patients who reported using e-cigarettes daily (in addition to cigarette use) to 24 regular-smoking COPD. Patients that were selected had a pack year smoking history  $\geq 30$ . They analyzed cigarettes/day, number of exacerbations in 12 months, and change in exacerbations data over the baseline and 2 follow ups that took place over the 24-month period of study.

They found a significant reduction in combustion cigarette use in the e-cigarette user group. Cigarettes/day went from 21.8 at baseline, to 1.8 at follow up 1 and 1.58 at follow up 2, with no change seen in the combustion cigarette control group. There were significant reductions in COPD exacerbations in the EC-using group, from 2.3 at baseline to 1.8 and 1.4 at follow up 1 and 2 respectively and no change in the control group. No medications were significantly changed or improved during this time.

Data on this is mixed, with studies supporting a reduction in COPD exacerbations in former smokers<sup>51,52</sup>, with others stating otherwise<sup>53, 54</sup>.

## Asthma

Vaping is most prevalent among 15-24 year olds<sup>55</sup> and Asthma is the most common chronic health condition in young people. This has risen concerns about the effects of e-cigarette use on asthma rates and exacerbations. Preliminary data on the vapor profile and cellular effects of vaping spurred population studies on e-cigarette use and asthma prevalence.

Our review identified significant correlations between e-cigarette use and asthma diagnosis from several studies and analysis (as outlined below).

**Table 8:** Odds Ratios of asthma and E-cigarette use from population surveys

<b>Data Set</b>	<b>Data Collection</b>	<b>Asthma/ Respiratory Definition</b>	<b>E-cigarette Use</b>	<b>OR (Total)</b>
2016 BRFSS <sup>46</sup>	Cross-sectional random-dial telephone survey of adults 18+ (mean age 55 years)	Diagnosed with asthma and still have asthma	E-cigarette use = ever use, (former use), current some days, every day, or no use (current or former)	1.33
2016 BRFSS <sup>56</sup>	Cross-sectional random-dial telephone survey of adults never smokers	Diagnosed with asthma and still have asthma	Current e-cigarette use (daily or some days use)	1.56 (total) 1.90 daily use 1.48 some days use)
2016-2017 BRFSS <sup>57</sup>	Cross-sectional random-dial telephone survey of adults never smokers	Diagnosed with asthma and still have asthma	Current use = (daily or some days use)	1.36 (daily) 1.11 (sometimes, non-significant)
Hawaii Youth Risk Behavior Survey <sup>58</sup>	9-12 grade students	Ever diagnosed with asthma, Current asthma	Current (last 30 days), former, or never users	1.48 (current asthma) 1.22 (former asthma)
South Korean Student Survey <sup>59</sup>	Cross-sectional survey of high school students	Asthma diagnosis in past 12 months	Current, former, or never use	2.74 (current vs. never)
2012 Florida Youth Survey <sup>60</sup>	Survey of high school students	Asthma attack in past 12 months	Last 30 day use of e-cigarettes	1.78 (of having attack in past 12 months)
Hong Kong	Grade 7 -12	Cough or	Past 30 days e-	1.39 (ever-smokers)

Student Survey <sup>61</sup>	students	phlegm for 3 months in past 12months	cigarette use	1.40 (ex-smokers), 2.06 (never smokers)
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Data from the Behavioral Risk Factor Surveillance System (BRFSS) study was analyzed by several teams<sup>46, 56, 59</sup> and identified significant correlations between e-cigarette use and asthma diagnosis. With differences between daily and some days use when frequency was considered. Though one of the teams did not identify a significance in former e-cigarette uses and asthma diagnosis. Analysis of the Hawaii Youth Risk Behavior Survey (HYRBS)<sup>58</sup> found similar results. Their associations between e-cigarette use and asthma were significant independent of cigarette or marijuana use (which have significant effects on asthma).

To understand e-cigarette use and asthma severity, a survey of Florida youth<sup>60</sup> found significant associations between e-cigarette use in past 30 days and reported asthma attacks in the last 12 months (AOR=1.78). This provides evidence that e-cigarette use may exacerbate existing asthma. Though the timelines may confound this data, as students could have had asthma attacks prior to the 30-day period for e-cigarette use. Though the Hong Kong student survey also asked students about number of days absent from school due to asthma attacks. Finding that in never-smoking/current e-cigarette using students had a 13.21-18.59 OR for 4 days absent and 5.04-6.81 for 1-3 days absent. This was used as a marker of disease severity. of absence with asthma were also more likely to have tried E-cigarettes in the past 30 days than their non-asthmatic counterparts. This study also found significant associations between e-cigarette use and respiratory symptoms (cough or phlegm for 3 consecutive months, having not asked about asthma) for all tobacco use types except for current dual users.

While e-cigarettes have health concerns, from the given evidence they appear to be less harmful than combustion cigarettes. There is conflicting data on whether or not e-cigarettes provide an effective option for smoking cessation. Evidence for the effects of e-cigarette use on abstinence and reduction in asthma symptoms is mixed. Polosa et al<sup>62</sup> conducted a small retrospective review of pulmonary function in smoking asthmatics who switched to e-cigarettes. They analyzed spirometry data, airway hyper-responsiveness, asthma exacerbations, and subjective asthma control taken at baseline and in 2 follow ups. Of the 18 participants whose records were reviewed, 10 were sole e-cigarette users and 8 were dual users. Both single and dual users saw a significant improvement in asthma control, spirometry data, and airway hyper-responsiveness. There was a reduction in asthma exacerbations but it didn't reach statistical significance.

Another study included 130 asthmatic patients who underwent the clinical testing. Users consisted of 42 smokers, 48 non-smokers, and 41 e-cigarette users underwent spirometry, differential cell counts in induced sputum, a clinical exam, Asthma Control Test questionnaire, chest radiograph, O<sub>2</sub> Saturation, ECG, and eosinophil blood counts. E-cigarette users had no differences between cigarette users in asthma control and pulmonary function. There also was a significant reduction in pulmonary function in spirometry parameters like FVC/FEV<sub>1</sub>/FEV<sub>1</sub>/FVC ratio, maximal mid expiratory flow, and ACT score in e-cigarette users compared to non-smokers.

Forced sputum found some interesting results. Eosinophil sputum is the most common type for non-smokers and neutrophil sputum the most common for smokers. E-cigarette users had a mixed sputum type, indicating that e-cigarettes increased inflammation and mucus hypersecretion; two things that predispose a patient to more asthma exacerbations<sup>63</sup>. There also

was a significant reduction in lung functioning as sputum type moved more from eosinophil to neutrophil, with the worst lung functions being associated with a higher neutrophil count.

Finally, a study<sup>64</sup> used a web survey to collect data on the effects of e-cigarette use on asthma symptoms. 631 asthmatics responded to the survey. 90% of users reported no change in symptoms after switching to e-cigarettes and would recommend other asthmatic smokers switch to e-cigarettes. A group of asthmatics using e-cigarettes for smoking cessation then underwent clinical testing at an outpatient clinic. Outpatient visits included physical exam, asthma severity questionnaires, and pulmonary function tests. 55 volunteers underwent a clinical evaluation including 15 asthmatics. Of the 10 asthmatics who underwent testing, there was a significant improvement in scores from the Asthma Control Questionnaire, Asthma Control Test, and the 36-Item Short Form Survey. Over the study course, there was no change in pulmonary function tests. Though the questionnaires improved.

## **Discussion**

### ***Inflammatory and Oxidative Effects***

Our review identified that in both cells and humans, exposure to e-cigarette vapor led to an increase in a variety of inflammatory cytokines and biomarkers. There was significant heterogeneity in the liquids, devices, exposure scenarios, or inflammatory biomarkers used in these studies, making direct comparison difficult. Despite these differences each study found increases in their inflammatory measures, providing strong evidence for *in vitro* pro-inflammatory effects. The only study that didn't find such inflammatory increases was the 2020 Song et al study<sup>20</sup>. They only found significant inflammatory marker increases when correlated

with urinary PG levels (which was used as a biomarker for level of e-cigarette use), though it should be noted that participants only used unflavored, non-nicotine liquids. Studies have found that nicotine and flavorings are the most harmful part of e-liquids, and that humectants alone pose little risk. Thus, our conclusion that e-cigarettes increase lung inflammation stands.

However, it does appear that OX/ROS levels can increase with humectants alone, as found by the Lerner study when testing humectants alone for OX/ROS in vapor<sup>37</sup>. Further research should focus on the effects of flavorings, nicotine, and humectants on inflammation and OX/ROS levels to identify avenues for harm reduction.

We also found that OX/ROS levels increased significantly in cells and mice. This increase is believed to originate from two sources with the first being the vapor itself, mostly likely from the combustion of carbonyls like formaldehyde and acetaldehyde commonly found in e-vapor<sup>37</sup>. Furthermore, there was an increase in OX/ROS production. This is likely caused by the chemical constituents of vapor entering the body and metabolizing into reactive oxygen species, as is seen with other chemicals and medications<sup>65 66 67 68 69 70 71 72 73 74</sup>. Heavy metals are also a known cause of OX/ROS increase and have been found in e-cigarette vapor by several studies<sup>75,76</sup>. The antioxidant decrease is still not understood, and further research should endeavor to understand how vapor lowers the antioxidant response. Further research should focus on the human levels of oxidative stress to understand how the *in vitro* effects seen here will translate into humans. This oxidative stress also induced 8-OHdG adducts, which have been proposed as a biomarker of oxidative stress<sup>77,78,79</sup>. These adducts also have mutagenic and deleterious effects on DNA, contributing to cancer risk.

Inflammation and oxidative stress also induce each other, and this feedback may contribute to chronic stress and damage to lung tissues. Further research should investigate the

chronicity of this inflammation and oxidative stress, as the data currently only demonstrates short term effects ranging from several hours to weeks. Diseases like COPD and cancer rely on persistent inflammation and oxidative stress to develop, and the chronicity of e-cigarette-induced inflammation and oxidative stress is unknown.

Inflammation and oxidative stress also play a significant role in the etiology of many respiratory diseases. To understand how these cellular changes may affect disease rates we reviewed population studies on tobacco use and disease prevalence. The major complication of the reviewed COPD and Asthma population studies is their lack of causal power due to their cross-sectional nature. There is a possibility that those who develop respiratory diseases begin using e-cigarettes with therapeutic or tobacco cessation intentions, thus representing a reverse causality. In their longitudinal analysis of PATH data Bhatta et al found a significant reverse causality association between COPD and e-cigarette use<sup>44</sup>. Given that this was the only study to conduct such an analysis, it does cast doubt on the correlations. It is possible that in these population surveys, participant recall is incomplete. E-cigarette users may also be exposed to other forms of inhalational substances, including marijuana and second-hand smoke. Given that e-cigarettes are marketed as therapeutics, it is entirely possible that those with respiratory diseases begin using after receiving a diagnosis with the notion that it will improve their condition.

The basic evidence regarding contents and cellular effects of e-cigarettes provides some evidence of a causal relationship to the established correlations as activation of certain proteins and pathways identified in studies can contribute to tissue destruction and are upregulated in cigarette smokers, who have the highest risk for COPD development. Asthma can also be exacerbated by persistent respiratory inflammation. Compared to the COPD population

data, asthma population studies may provide weaker causative data. As the asthma definitions used in most of the surveys were vague and less time dependent (and thus could have developed prior to e-cigarette use). Also, many asked about former, current, and/or official diagnosis of asthma, which leaves room for significant recall bias; especially in youth. Evidence for e-cigarettes as asthma harm reduction is also mixed, though one small study found improvement in asthma status and symptoms in smokers switching to e-cigarettes.

Despite being marketed as such, the efficacy of e-cigarettes as tobacco cessation is contentious and undetermined. There are mixed results on whether e-cigarettes provide harm reduction options for cigarette smokers with respiratory diseases. Some studies found decreases in COPD exacerbations and improvement in asthma symptoms upon switching to e-cigarette use while others found only subjective improvements and not laboratory changes in patients.

Further research and reviews should gather data on the effectiveness of e-cigarettes as tobacco cessation and harm reduction strategies. From our review we can state that e-cigarettes appear to be less harmful than combustion cigarettes, but still pose health risks and potentially increase rates of COPD and asthma. At this point, next steps should focus on providing more longitudinal data, through prospective or retrospective studies of e-cigarette use and respiratory diseases to understand risk to users.

### **Conclusion**

E-cigarette exposure caused a significant increase in inflammatory biomarkers, specifically IL-8 and IL-6 production, LDH loss, and MMP9 changes in cells. Human studies found consistent increases in interleukins, exhaled air temperature, and other inflammatory biomarkers. Showing that e-cigarettes induce lung inflammation in users.

Elevated levels of reaction oxygen species were identified. Exposure to e-cigarettes increased OX/ROS production and decreased antioxidant power. OX/ROS present in vapor itself may also contribute.

Reviewed population studies found significant correlations between e-cigarette use and COPD or asthma, though these cross-sectional and self-reported surveys cannot prove causation and may be affected by participant error.

There is no consensus on the effects of e-cigarettes as a smoking cessation or harm reduction strategy for smokers with COPD or Asthma. Given the collected data, there could be benefits to e-cigarettes as harm-reduction, given that they appear to be less toxic than cigarettes. But from the data presented here, they do pose risks for respiratory disease by inducing cellular and physiological changes that contribute to disease risk.

#### **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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