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

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Abstract

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

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Keywords

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INTRODUCTION

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

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

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

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

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

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

Smoking, Inflammation, and the Human Lung

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

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

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

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

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

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

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

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

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

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

E-Cig Toxicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Summary and Research Gaps

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

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

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

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

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

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

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

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

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

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Summary of Human Biomarker Studies

Table 1

Author, et al year (reference)	Study design	Population	Criteria for each group of tobacco user (baseline)	Duration	Products tested	Markers assessed*	Results
Cravo et. al., 2016 (209)	To evaluate the safety profile of an e-vapor product (EVP; 2.0% nicotine) in smokers of conventional cigarettes (CCs) switching to use the EVP	Healthy subjects (n=408) in UK EVP group (n=306): - Mean age: 34 - Mean BMI: 26 - 55% Males CC group (n=102): - Mean age: 35 - Mean BMI: 25 - 57% Males	EVP group (n=306): - CPD 5-10 CPD: 36% 11-20 CPD: 56% 21-30 CPD: 8% - FTND Mild: 30% Moderate: 57% Severe: 13% CC group (n=102): - CPD 5-10 CPD: 31% 11-20 CPD: 62% 21-30 CPD: 7% - FTND* Mild: 29% Moderate: 54% Severe: 17%	12 weeks	EVP prototype developed by Fontem Ventures B.V. A rechargeable battery (voltage range of 3.0-4.2 V), an atomizer and a capsule (small cartridge) containing e-liquid The base components of the e-liquids: PG (70-75% w/w), glycerol (18-20% w/w) and water (5% w/w)	Urine biomarkers: NEQ, SPMA, 3HPMA and total NNAL, PG	% change in week 12 from baseline: EVP vs. CC - NEQ: -25% vs. -6% - 3HPMA: -29% vs. 6% - SPMA: -35% vs. 1% - Total NNAL: -31% vs. 3% - PG: 119% vs. -3%
Goniewicz et. al. 2016 (216)	To evaluate effects of e-cigarettes (e-cigs) on nicotine delivery and exposure to selected carcinogens and toxicants in a longitudinal study within subjects observational study	Healthy subjects (n=20) in Poland - Aged 18 or older - 100% Caucasian - 40% Males - Mean age: 31	- Current daily cigarette smokers (>5 CPD within the last 12 months) - Years of smoked: 12	2 weeks	An e-cig (M201 Mild, Poland) with 20 tobacco-flavored cartridges per week containing 11 mg of nicotine in a mixture of PG* and Gly* (50:50)	Urine biomarkers: NEQ, NNAL, Volatile organics: HEMA, MHBMA, HPMA, 3HPMA, SPMA, AAMA, CNEMA, and 2HPMA Metabolites of PAHs (free plus conjugated): 2-naphthol, 1-hydroxyfluorene, 2-	Baseline/Week 1/Week 2, P-value - NEQ(μmol/g): 50/45/43, NS - NNAL (ng/g): 165/60/69, <0.001 - HEMA(ng/g): 3120/864/1573, 0.001 - MHBMA(ng/g): 1283/478/887, <0.001 - HPMA(μg/g): 1379/387/575, <0.001 - 3HPMA(μg/g): 700/455/465, 0.001 - SPMA(ng/g): 674/193/481, <0.001

Author, et al year (reference)	Study design	Population	Criteria for each group of tobacco user (baseline)	Duration	Products tested	Markers assessed*	Results
McRobbie et. al., 2015 (217)	To investigate exposure to nicotine and to acrolein before and after e-cigs use	Adult smokers (n=40) in UK E-cigs use only (n=16) - Mean age: 45 - 63% White - 50% Males Dual users (n=17) - Mean age: 48 - 53% White - 52.9% Males	E-cigs use only - Mean CPD: 16 - Mean FTCD: 3.9 Dual users - Mean CPD: 21 - Mean FTCD: 4.7	4 weeks	A Green Smoke EC (labeled 2.4% nicotine)	hydroxyfluorene, 3-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3+4-hydroxyphenanthrene; 1-hydroxypyrene	<ul style="list-style-type: none"> - AAMA(µg/g): 148/188/97, 0.005 - CNEMA(µg/g): 178/58/66, <.001 - 2HPMA(µg/g): 24/18/15, <.001 - 1-Hydroxyfluorene(ng/g): 864/492/833, <.001 - 3-,4-Hydroxyphenanthrenes(ng/g): 669/544/1262, NS - 2-Hydroxyfluorene(ng/g): 463/315/495, 0.048 - 1-Hydroxypyrene(ng/g): 338/279/627, NS - 3-Hydroxyfluorene(ng/g): 312/192/349, 0.001 - 2-Hydroxyphenanthrene(ng/g): 333/492/800, NS – 1-Hydroxyphenanthrene(ng/g): 211/196/415, NS - 2-Naphthol(µg/g): 13/8/14, NS
Pulvers et. al., 2016 (218)	Assess nicotine consumption and toxicant exposure of cigarette smokers switching to e-cigs; observational study of smokers provided the e-cig independent of quitting intention	Adult US smokers (n=40)	Male (73%) Mean age: 30.08 (SD = 8.82) White 50% Hispanic 25%	4 weeks	e-Go C non-variable battery and refillable atomizers and choice of eight flavors in 12 or 24 mg nicotine dosage	Urine biomarkers: cotinine, NNAL, VOCs	<ul style="list-style-type: none"> - % reduction in week 4 from baseline: E-cigs use only vs. Dual users - Cotinine (ng/mg creatinine): 17% vs. 44%, P =0.010 - 3-HPMA (ng/mg creatinine): 79% vs. 60% P <.001
						Urine biomarkers: cotinine, NNAL, VOCs	<ul style="list-style-type: none"> - Reductions (p value): - Cigs/day 50% (<.001) - CO 37% (<.001) - Cotinine 23% 0.(90) - NNAL 46% (<.001) - PMA 17% (0.01)

Author, et al year (reference)	Study design	Population	Criteria for each group of tobacco user (baseline)	Duration	Products tested	Markers assessed*	Results
O'Connell et. al., 2016 (219) D'Ruiz et. al., 2016 (220)	To compare changes in biomarkers among different user groups from usual brand conventional tobacco cigarettes to e-cigs and dual uses E-cigs use only <ul style="list-style-type: none"> - Group A1: Tobacco flavor rechargeable bluTM e-cigs - Group A2: Cherry flavor rechargeable bluTM e-cigs - Group A3: Cherry flavor disposable bluTM e-cigs Dual Use <ul style="list-style-type: none"> - Group B1, B2, and B3: Usual brand combustible tobacco cigarette plus products from Group A1, A2, or A3, respectively. 	Healthy adult male or female smokers (n=105) in USA E-cigs use (n=15 for each) Group: A1/A2/A3 <ul style="list-style-type: none"> - Mean age: 37, 40, 33 - 87%, 60%, 93% White - 60%, 80%, 40% Males Dual use (n=15 for each) Group: B1/B2/B3 <ul style="list-style-type: none"> - Mean age: 36, 39 - 87%, 73%, 87% White - 60%, 80%, 53% Males 	E-cigs use only Group: A1/A2/A3 <ul style="list-style-type: none"> - CPD: 18/17/15 - Years smoked: 19/20/15 - FTND score: 5.3/5.1/5.3 Dual use Group: B1/B2/B3 <ul style="list-style-type: none"> - CPD: 18/20/21 - Years smoked: 19/14/21 - FTND score: 5.5/5.7/5.2 	5 days	BluTM e-cigs All e-cigs contained 24 mg/mL (2.4%) nicotine, vegetable glycerol (~50% in cherry flavor and ~80% in tobacco flavor), PG (45% in cherry flavor and ~10% in tobacco flavor), and distilled water, and flavorings.	Urine biomarkers: NEQ, NNN, NNAL, IOHP, 3HPMA, SPMA, MHBMA, HMPMA, CEMA	<p>HEMA 14% (0.85)</p> <p>MMA increased 11% (0.27)</p> <p>CNEMA 52% (<0.01)</p> <p>3-HPMA 21% (0.16)</p> <p>2-HPMA 12% (0.96)</p> <p>AAMA 12% (0.67)</p> <p>HPMMA 14% (0.99)</p> <p>E-cigs use only groups A1/A2/A3, Day -1 vs. Day 5 days</p> <ul style="list-style-type: none"> - NNAL (ng/24 h): 423/384/299 vs. 174/150/111 - 3HPMA (lg/24 h): 1522/1903/1354 vs. 214/263/247 - HMPMA (lg/24 h): 523/657/533 vs. 71/83/78 - CEMA (lg/24 h): 220/266/201 vs. 33/41/26 - IOHP (ng/24 h): 317/ 302/261 vs. 94/86/91 - NNN (ng/24 h): 19/14/14 vs. 1/0.7/1 - MHBMA (lg/24 h): 5/6/5 vs. 0.3/0.3/0.3 - SPMA (lg/24 h): 6.3/8.1/6.3 vs. 0.3/0.3/0.4 - NEQ (mg/24 h): 17/18/15 vs. 11/13/11 <p>Dual use groups B1/B2/B3, Day -1 vs. Day 5 days</p> <ul style="list-style-type: none"> - NNAL (ng/24 h): 431/422/343 vs. 329/321/269 - 3HPMA (lg/24 h): 1644/1475/1490 vs. 1046/1071/1155 - HMPMA (lg/24 h): 591/598/505 vs. 392/395/387 - CEMA (lg/24 h): 256/246/223 vs. 172/168/173

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							<ul style="list-style-type: none"> - IOHP (ng/24 h): 364/295/304 vs. 235/206/224 - NNN (ng/24 h): 14/12/11 vs. 9/8/7 - MHBMA (lg/24 h): 5/3/5 vs. 4/3/4* - SPMA (lg/24 h): 7/5/7 vs. 5/4/6 - NEQ (mg/24 h): 17/16/16 vs. 18*/16*/16* <p>Note: All levels in Day 5 from three groups were statistically different compared to the levels in Day-1, * not significant.</p>
Campagna et. al., 2016 (221)	To investigate long-term changes in exhaled breath measurements and respiratory symptoms in smokers invited to quit or reduce their cigarette consumption by switching to e-cigs Group A: 12 weeks of Original either 2.4 mg/ml or 1.8 mg/ml nicotine Group B: 6 weeks of Original either 2.4 mg/ml and a further 6 weeks of Categoria 1.8 mg/ml nicotine or no nicotine Group C: 12 weeks of Original 0% without nicotine(sweet tobacco aroma)	Regular smokers not intending to quit (n=134) in Italy Group A (N = 49) - Mean age: 45 - 26 Males Group B (N = 49) - Mean age: 42 - 28 Males Group C (N = 40) - Mean age: 40 - 25 Males	Group A - Packs/year: 25 - CPD: 20 - FTND: 5.5 - eCO: 18 - FeNO: 5.8 Group B - Packs/year: 24 - CPD: 18 - FTND: 5.6 - eCO: 21 - FeNO: 5.9 Group C - Packs/year: 24 - CPD: 20 - FTND: 5.8 - eCO: 19 - FeNO: 6.4	Baseline and at week 12, week 24 and week 52	E-cig model '401' with a rechargeable three-piece design		<ul style="list-style-type: none"> - There was no difference of baseline characteristics between failures, reducers, and quitters. - A significant effect of quitting classification was found on FeNo and eCO at all time points (P < 0.0001) - Among quitters, FeNO rose from 5.5 ppb to 17.7 ppb by week 52. - Baseline eCO decreased from 17 ppm to 3 ppm by week 52. - No significant changes in FeNO and eCO levels were observed in failures and reducers.
Jorenby et. al., 2017 (225)	To evaluate, nicotine levels and smoking reduction success for cigarette smokers	Regular smokers or dual users in USA Smokers	Smoker - Years smoked: 25 - Mean FTCD: 4.9	26 days	- Disposable: 34% - Replaceable cartridge: 16%	Urine biomarkers: Nicotine, CO	<ul style="list-style-type: none"> - Compared to smokers, dual users did not smoke significantly fewer cigarettes during either periods of ab libitum use or during

Author, et al year (reference)	Study design	Population	Criteria for each group of tobacco user (baseline)	Duration	Products tested	Markers assessed*	Results
	and dual users of cigarettes and e-cigs Cigarettes only (n=74) Dual users: cigarettes + e-cigs (n=74) Ad libitum period Days 1-8 and 16-21 75% reduction period: Days 9-14 100% cessation period: Days 24-25	- Mean age: 43 - 42% Males - 80% White Dual users - Mean age: 33 - 41% Males - 91% White	Dual users - Years smoked: 17 - Mean FTCD: 4.5		- Tank system: 14% - Unknown: 37% Nicotine concentration 0.1-0.3% : 4% 0.4-0.6% : 8% 0.7-1.2% : 7% 1.3-1.8% : 8% 1.9+% : 1% Unspecified: 72%		periods of smoking restriction, nor did they produce lower CO levels. Dual users increased vapes/day from 1.3 and 1.9 during Ad Libitum use to 6.3 and 4.4 during 75% Reduction for women and men, respectively.
Vardavas et. al., 2012 (225)	To assess an impact of using an e-cigarette for 5 min on the pulmonary function tests and FeNO of healthy adult smokers Experimental group were instructed to use the e-cigs <i>ad lib</i> for 5 min as they would usually smoke. The control group subjects were asked to use the e-cigarette with similar frequency, but without the e-cigs cartridge included	Regular healthy smokers (n=40) in Greece Experimental group (n=30) and control group (n=10)	A minimum pack-year: 5	5 min	NOBACCO MLB-MED filter, 11 mg of nicotine, PG>60%, linalool <5%, nicotine <10%, tobacco essence <5%, and methyl vanillin <1%; no polyaromatic hydrocarbons were detected.	FeNO	FeNO, ppb - Experimental group Pre usage: 13 vs. Post usage: 11, P=0.005 - Control group Pre usage: 9 vs. Post usage: 9, NS
Ferrari et. al., 2015 (226)	To compare the effects of ad libitum use of a nicotine free e-cigs or/and a cigarette for 5 min in healthy adult smokers (n= 10) and non-smokers (n=10)	Healthy subjects (n=20) in Italy Smokers (n=30) - Mean age: 42 - 40% Males Non-smokers (n=10) - Mean age: 36 - 30% Males	Smokers - Packs/year: 19	5 min.	ELIPS C Series	FeNO and FeCO	FeNO Smokers: no difference, NS Non-smokers: no difference, NS CO Smokers: Decreased FeCO after e-cig use, P < 0.001 Non-smokers: Decreased FeCO after e-cig use, P = 0.048
Schober et. al., 2014 (227)	To measure indoor air quality and FeNO levels of e-cig consumers	9 healthy e-cig users in Germany - 100% Males	All subjects were occasional smokers with a cigarette consumption of <10 cigarettes per week (no e-cigarettes)	2 hours	Liquids (with and without nicotine, all with tobacco flavor) and rechargeable e-	CO and FeNO	- FeNO increased in 7 of 9 individuals after vaping a nicotine e-cigs at P = 0.030, but the effect was not significant when nicotine-free liquids were used.

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	In six vaping sessions, nine volunteers consumed e-cigs with and without nicotine	- Mean age: 25			cigs from Red Kiwi, Seevetal, Germany nicotine: 18mg/ml		- eCO levels were not significantly influenced by e-cig consumption.

* CO: carbon monoxide; FeNO: fractional excretion of nitric oxide, FTND: Fagerstrom Test for Nicotine Dependence, Gly: glycerine, NEQ: nicotine equivalents, PG: propylene glycol tobacco specific nitrosamines; NNAL = volatile organic compounds; SPMA, 3HPMA
HEMA, MHBMA, HPMA, AAMA, CNEMA, and 2HPMA