

Emerging Research on Potential Health Effects of Electronic Cigarettes

The proposed Orange County Board of Health rule to prohibit use in enclosed areas of restaurants and bars is primarily concerned with preventing secondhand exposure and not with the role of e-cigarettes on smoking promotion vs. cessation. Therefore, the research cited below focuses on the chemical composition of e-liquids and aerosol, toxicology studies, the potential for secondhand exposure, and documented health effects. There are additional bodies of research on the effectiveness and safety of e-cigarettes for smoking cessation that are not listed here. The emerging body of research on e-cigarettes suggests that emitted aerosol may contain potentially harmful chemicals in addition to nicotine or other drugs.

Secondhand Exposure

Research demonstrates the potential for secondhand exposure to e-cigarette aerosol through biomarkers of nicotine exposure, as well as through studies done in controlled indoor conditions.

Ballbé M, Martínez-Sánchez JM. (2014). Cigarettes vs. E-Cigarettes: Passive Exposure at Home Measured by Means of Airborne Marker and Biomarkers. Environmental Research. 135:76–80. This study showed that non-smokers passively exposed to e-cigarettes absorb nicotine. This study characterized passive exposure to nicotine from e-cigarette vapor and conventional cigarette smoke at home among non-smokers under real-use conditions. The airborne markers were statistically higher in conventional cigarette homes than in e-cigarettes homes (5.7 times higher). However, concentrations of both biomarkers among non-smokers exposed to conventional cigarette smoke and e-cigarette vapor were statistically similar (only 2 and 1.4 times higher, respectively). The levels of airborne nicotine and cotinine concentrations in the homes with e-cigarette users were statistically higher than control homes. These results show that non-smokers passively exposed to e-cigarettes absorb nicotine.

Thornburg, J., Malloy, Q., Cho, S-H. Studabaker, W., and Lee, Y. O. (2014). Exhaled Electronic Cigarette Emissions: What's Your Secondhand Exposure? RTI Press. Available at:

https://www.rti.org/pubs/secondhand_exposure_to_electronic_cigarette_emissions.pdf

Dosimetry modeling calculated that a teenage male e-cigarette user exhales 50% of the e-cigarette emissions that he inhaled. Research also shows that a non-user may be exposed to aerosol particles smaller than 1000 nanometers, similar in size to tobacco smoke and diesel engine smoke. The exact size distribution depends on the chemical composition of the electronic cigarette liquid, the e-cigarette device operation, and user vaping preferences.

Thornburg, J. (2016). E-Cigarettes and Vapor Products: State of the Science. Presentation to Orange County Board of Health on January 27, 2016. Available at: <https://youtu.be/OHNqLcl7iBo>. Accessed March 28, 2016. Research by RTI International suggests that an e-cigarette non-user may be exposed to secondhand aerosol particles in an indoor setting. Model results predicted 47% of inhaled emissions were deposited in the lung, mostly in the deep lung, with the remaining emissions exhaled by the user and introducing the possibility of secondhand exposure. Exhaled e-cigarette vapors from a single user were detected 6 feet away; concentrations were 25 times lower than adjacent to the user.

Flavorings

Several chemicals commonly used as flavorings in e-cigarettes, such as diacetyl and cinnamaldehyde, have known associations with respiratory disease. Studies demonstrate that the level of exposure through e-cigarettes can be of toxicological concern.

Allen, J. G., Flanigan, S. S., Leblanc, M., Vallarino, J., Macnaughton, P., Stewart, J. H., & Christiani, D. C. (2015). Flavoring Chemicals in E-Cigarettes: Diacetyl, 2,3-Pentanedione, and Acetoin in a Sample of 51 Products, Including Fruit-, Candy-, and Cocktail-Flavored E-Cigarettes. *Environmental Health Perspectives*.

This study analyzed 51 types of flavored e-cigarettes sold by leading e-cigarette brands and flavors deemed appealing to youth. E-cigarette contents were fully discharged and the air stream was captured and analyzed for total mass of diacetyl, 2,3-pentanedione, and acetoin. At least one flavoring chemical was detected in 47 of 51 unique flavors tested. Diacetyl was detected above the laboratory limit of detection 39 of the 51 flavors tested, ranging from < limit of qualification (LOQ) to 239 µg/e-cigarette. 2,3-pentanedione and acetoin were detected in 23 and 46 of the 51 flavors tested at concentrations up to 64 and 529 µg/e-cigarette, respectively. Due to the associations between diacetyl, bronchiolitis obliterans and other severe respiratory diseases observed in workers, urgent action is recommended to further evaluate this potentially widespread exposure via flavored e-cigarettes.

Behar RZ, Davis B, Wang Y, Bahl V, Lin S, Talbot P. (2014). Identification of toxicants in cinnamon-flavored electronic cigarette refill fluids. *Toxicol In Vitro* 28: 198–208. In a prior study on electronic cigarette refill fluids, Cinnamon Ceylon was the most cytotoxic of 36 products tested. The purpose of the current study was to determine if high cytotoxicity is a general feature of cinnamon-flavored EC refill fluids and to identify the toxicant(s) in Cinnamon Ceylon. The majority of the eight cinnamon-flavored refill fluids were cytotoxic.

Human embryonic stem cells were generally more sensitive than human adult pulmonary fibroblasts. Most products were highly volatile and produced vapors that impaired survival of cells in adjacent wells. In most products, CAD (cinnamaldehyde), which was identified as the most cytotoxic, was the dominant flavorant with 2MOCA (2-methoxycinnamaldehyde) and vanillin sometimes being present in lesser amounts. All of the products had different amounts of these chemicals demonstrating that users cannot assume to know their content even when purchasing duplicate bottles.

Farsalinos, K. E., Kistler, K. A., Gillman, G., & Voudris, V. (2014). Evaluation of Electronic Cigarette Liquids and Aerosol for the Presence of Selected Inhalation Toxins. *Nicotine & Tobacco Research*, 17(2), 168-174.

The purpose of this study was to evaluate sweet-flavored electronic cigarette (EC) liquids for the presence of diacetyl (DA) and acetyl propionyl (AP), which are chemicals approved for food use but are associated with respiratory disease when inhaled. DA and AP were found in 74% of sweet-flavored EC liquids, with many of them exposing users to higher than safety levels. Their presence in EC liquids represents an avoidable risk.

Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip DJ, McIntosh S, Robinson R, Rahman I. (2015). Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PLoS One* 10:e0116732.

Oxidative stress and inflammatory response are the key events in the pathogenesis of chronic airway diseases. Exposure to e-cig aerosols/juices in this study incurred measurable oxidative and inflammatory responses in mouse lung cells and tissues, reflecting potential unrealized health consequences in humans exposed to these toxicants. Unvaporized e-liquids were oxidative in a manner dependent on flavor additives, while flavors containing sweet or fruit flavors were stronger oxidizers than tobacco flavors.

Exposure of human airway epithelial cells to electronic nicotine delivery systems (ENDS) resulted in increased secretion of inflammatory cytokines, such as IL-6 and IL-8. Furthermore, human lung fibroblasts exhibited stress and morphological change in response to treatment with ENDS/e-liquids. These cells also secreted increased IL-8 in response to a cinnamon flavored e-liquid and are susceptible to loss of cell viability by ENDS e-liquids. Finally, exposure of mice to aerosols produced from a popular e-cig increased pro-inflammatory cytokines and diminished lung glutathione levels, which are critical in maintaining cellular redox balance.

Tierney, P. A., Karpinski, C. D., Brown, J. E., Luo, W., & Pankow, J. F. (2015). Flavour chemicals in electronic cigarette fluids. Tobacco Control. TC Online First. 10.1136/tobaccocontrol-2014-052175. Two brands of single-use e-cigarettes were selected and their fluids in multiple flavor types analyzed by gas chromatography/mass spectrometry. For the same flavor types and for selected confectionary flavors (e.g., bubble gum and cotton candy), convenience samples of e-cigarette fluids in refill bottles from local 'vape' shops and online retailers were also analyzed. A significant number of the flavor chemicals were aldehydes, a compound class recognized as 'primary irritants' of mucosal tissue of the respiratory tract. Many of the products contained the same flavor chemicals: vanillin and/or ethyl vanillin was found in 17 of the liquids as one of the top three flavor chemicals, and/or at ≥ 0.5 mg/mL. Based on recommended occupational exposure limits, the concentrations of some flavor chemicals in e-cigarette fluids are sufficiently high for inhalation exposure by vaping to be of toxicological concern.

Rowell, T. R., Lee, S., and Tarran, R. (2015). Select E-Cigarette Flavors Alter Calcium Signaling Cell Viability And Proliferation in Lung Epithelia. American Thoracic Society International Conference Abstracts. Available online at: http://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2015.191.1_MeetingAbstracts.A2896. Thirteen representative flavors of e-cigarette liquid were tested for their effects on airway epithelial calcium signaling, cell viability, and cell proliferation. Flavors such as Hot Cinnamon Candies, Banana Pudding (Southern Style), and Menthol Tobacco evoked a strong calcium response and cytotoxicity at higher doses during the 30 minute exposure. These same flavors also decreased cell proliferation and the ability of cells to respond to a pharmacological agent that releases internal calcium stores in a dose-dependent manner after the 24 hour exposure. Moreover, these effects were not reprised by nicotine or the e-liquid vehicle for chemical constituents (propylene glycol and vegetable glycerin).

Sussan TE, Gajghate S, Thimmulappa RK, Ma J, Kim JH, Sudini K, Consolini N, Cormier SA, Lomnicki S, Hasan F, Pekosz A, Biswal S. (2015). Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model. PLoS One 10: e0116861. This study demonstrates that e-cigarette compounds elicit impaired pulmonary anti-microbial defenses in mice. Mice were exposed in an inhalation chamber to menthol-flavored e-cig vapor had serum cotinine (nicotine biomarker) concentrations comparable to human e-cigarette users. E-cigarette aerosol exposure for 2 weeks produced a significant increase in oxidative stress and moderate macrophage-mediated inflammation. Mice exposed to e-cigarette aerosol showed significantly impaired pulmonary bacterial clearance, compared to air-exposed mice, following an intranasal infection with *Streptococcus pneumoniae*. This defective bacterial clearance was partially due to reduced phagocytosis by alveolar macrophages from e-cigarette exposed mice. In response to Influenza A virus infection, e-cigarette exposed mice displayed increased lung viral titers and enhanced virus-induced illness and mortality.

Vulnerable Populations

For ethical reasons, there is limited research on the human health effects of secondhand aerosol exposure among vulnerable populations. Research in mice, however, suggests that chemicals in e-cigarettes may exacerbate asthma and that there is potential for e-cigarette aerosols to affect development of neonates.

Lim HB, Kim SH. (2014). Inhalation of e-cigarette cartridge solution aggravates allergen-induced airway inflammation and hyper-responsiveness in mice. Toxicol Res 30: 13–18. This study suggests that the inhalation of e-cigarette solutions can function as an important trigger for allergy-induced asthma symptoms. Long-term e-cigarette inhalation elicited no remarkable changes in the activities of murine

(mouse) alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase enzymes in serum; however, increased infiltration of inflammatory cells including eosinophils, into airways from blood, aggravated the asthmatic airway inflammation and airway hyper-responsiveness, and stimulated the production of cytokines such as interleukin (IL-4, IL-5 and IL-13), and OVA-specific IgE production. These results indicate that the inhalation of cartridge nicotine solution in e-cigarettes is likely to exacerbate asthmatic symptoms by elevating infiltration of inflammatory cells including eosinophils into airways.

McGrath-Morrow SA, et al. (2015). The effects of electronic cigarette emissions on systemic cotinine levels, weight and postnatal lung growth in neonatal mice. *PLoS One* 10: e0118344.

Neonatal mice were exposed to e-cigarettes for the first 10 days of life. Mice exposed to 1.8% nicotine/propylene glycol had a 13.3% decrease in total body weight and elevated plasma cotinine levels compared to room air controls. After adjusting for sex and weight, the nicotine exposed mice were found to have modestly impaired lung growth by mean linear intercept compared to room air control mice. These studies indicate that exposure to e-cigarette emissions during the neonatal period can adversely impact weight gain. In addition, exposure to nicotine containing e-cigarettes can cause detectable levels of systemic cotinine, diminished alveolar cell proliferation and a modest impairment in postnatal lung growth.

Wu Q, Jiang D, Minor M, Chu HW. (2014). Electronic cigarette liquid increases inflammation and virus infection in primary human airway epithelial cells. *PLoS One* 9: e108342.

The purpose of this study was to determine if e-cigarette use alters functions such as inflammatory response, innate immune defense against respiratory viral (i.e., human rhinovirus, HRV) infection, and the expression of host defense molecules (e.g., short palate, lung, and nasal epithelium clone 1, SPLUNC1) in primary human airway epithelial cells from young healthy non-smokers. Nicotine-free e-liquid promoted inflammatory response and HRV infection. Addition of nicotine into e-liquid further amplified the effects of nicotine-free e-liquid. Moreover, host defense molecules deficiency in mice significantly increased lung HRV loads. E-liquid inhibited SPLUNC1 expression in primary human airway epithelial cells. This current study provides strong data suggesting the harmful health effects of e-cigarettes on the lung, with a particular focus on airway epithelial inflammation and the effects on innate immunity in young people.

Acute Health Effects: Propylene Glycol

Propylene glycol is generally considered a low toxicity compound and is widely used in many products. However, occupational exposures have led to acute ocular and upper respiratory irritation, cough, reduced lung function, and chronic work-related wheezing and chest tightness. Employees in venues where e-cigarette use not prohibited, such as restaurants and bars, may experience involuntary, long-term, occupational exposure to propylene glycol and other components of e-cigarette emissions.

Wieslander G, Norback D, Lindgren T. (2001). Experimental exposure to propylene glycol mist in aviation emergency training: acute ocular and respiratory effects. *Occup Environ Med* 58: 649–655.

Non-asthmatic volunteers (n=27) were exposed in an aircraft simulator to propylene glycol (PG) mist over 1 minute, during realistic training conditions. Short exposure to PG mist from artificial smoke generators was found to cause acute ocular and upper airway irritation in non-asthmatic subjects. A few also reacted with cough and slight airway obstruction.

Varughese, S., Teschke, K., Brauer, M., Chow, Y., van Netten, C., & Kennedy, S. M. (2005). Effects of theatrical smokes and fogs on respiratory health in the entertainment industry. *American Journal of Industrial Medicine*, 47, 411–418.

Theatrical fogs (glycol or mineral oil aerosols) are widely used in the entertainment industry to create special effects and make lighting visible. One hundred one employees at 19

sites were studied using measured personal fog exposures, across work shift lung function, and acute and chronic symptoms were assessed. Results were also compared to a previously studied external control population. Chronic work-related wheezing and chest tightness were significantly associated with increased cumulative exposure to fogs (mineral oil and glycols) over the previous 2 years. Acute cough and dry throat were associated with acute exposure to glycol-based fogs; increased acute upper airway symptoms were associated with increased fog aerosol overall. Lung function was significantly lower among those working closest to the fog source. Mineral oil- and glycol-based fogs are associated with acute and chronic adverse effects on respiratory health among employees. Reducing exposure, through controls, substitution, and elimination where possible, is likely to reduce these effects.

Product Safety Assessment: DOW™ Propylene Glycol. The DOW Chemical Company. October 3, 2013.

Available at:

http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_08ea/0901b803808eabba.pdf?filepath=productsafety/pdfs/noreg/233-00248&fromPage=GetDoc. Eye contact with propylene glycol may cause slight, temporary irritation. Contact with heated vapor or mist during manufacturing may cause eye irritation. Mist may cause irritation of upper respiratory tract (nose and throat). In rare cases, repeated excessive exposure to propylene glycol may cause central nervous system effects.

Acute Health Effects: General

Limited research on adverse short-term effects include increased airways resistance and respiratory irritation and cough, particularly among individuals with asthma.

Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis PK. (2012). Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. *Chest* 141: 1400–1406. This study found that using an e-cigarette for 5 min has an impact on pulmonary function tests and fraction of exhaled nitric oxide (FENO) of healthy adult smokers. The study indicated a statistically significant decrease in FENO and an increase in impedance, respiratory resistance, and overall peripheral airway resistance, after using an e-cigarette. E-cigarettes assessed in the context of this study were found to have immediate adverse physiologic effects after short-term use that are similar to some of the effects seen with tobacco smoking; however, the long-term health effects of e-cigarette use are unknown but potentially adverse and worthy of further investigation.

McConnell R, et al. (2015). Electronic-Cigarette Use and Respiratory Symptoms in Adolescents. *Society for Research on Nicotine and Tobacco* 2015, PA6-4 (Presentation). Among 2,100 11–12th graders, 502 students reported ever use of e-cigarettes, among whom 212 reported no history of combustible tobacco use. 196 students reported current use of e-cigarettes, among whom 78 reported no history of combustible tobacco use. Ever users had 2-fold increased risk for bronchitis symptoms (i.e., chronic cough). The more often current users used e-cigarettes, the greater their risk for bronchitis symptoms. Even for e-cigarette users who never smoked, there was 1.6 increased risk for bronchitis symptoms.

Centers for Disease Control and Prevention. National Institute for Occupational Safety and Health. (2015). Comments of the National Institute for Occupational Safety and Health to the Food and Drug Administration (FDA) in response to Establishment of a Public Docket; Electronic Cigarettes and the Public Health Workshop Docket No. FDA—2014—N—1936 Cincinnati, Ohio. Available at: http://www.cdc.gov/niosh/topics/flavorings/pdfs/comment_from_the_national_institute_for_occupational_safety_and_health_niosh.pdf

Sufficient data are currently unavailable to accurately predict the risk associated with use of e-cigarettes or secondhand exposure to the particulate aerosols and gases they emit. Nonetheless, the FDA and others have noted that e-cigarette cartridges and aerosols may contain harmful chemicals and substances such as diethylene glycol, nicotine, formaldehyde, acetaldehyde, toxic metals, and others [FDA 2014; Kosmider et al. 2014; Jensen et al. 2015]. In addition, several organizations have expressed concern about potential long-term health effects of using or secondhand exposure to ENDS, such as e-cigarettes, while recognizing that current data are insufficient to quantify the risks [Wagener et al. 2012; BMA 2013; Kamerow 2013; AIHA 2014; Bhatnagar et al. 2014; Drummond and Upson 2014; Schraufnagel et al. 2014]. Thus, evaluating potential long-term health effects of ENDS use is an important research priority [Andrade and Hastings 2013; AIHA 2014].

Preliminary, limited reports are available about adverse short-term effects, such as significantly increased airways resistance [Gennimata et al. 2012] and respiratory irritation and cough, particularly among individuals with asthma [Tsikrika et al. 2014].

Product Variability

Variability in e-cigarette emissions makes it very difficult to assess risk. In addition, users sometimes mix and use their own e-liquid, rather than one commercially available, which is challenging to simulate in toxicological studies and other research.

Cheng T. (2014) Chemical Evaluation of Electronic Cigarettes. Nicotine & Tobacco Research. 23:ii11–7. The levels of nicotine, tobacco-specific nitrosamines (TSNAs), aldehydes, metals, volatile organic compounds (VOCs), flavours, solvent carriers and tobacco alkaloids in e-cigarette refill solutions, cartridges, aerosols and environmental emissions vary considerably. The delivery of nicotine and the release of TSNAs, aldehydes and metals are not consistent across products. Furthermore, the nicotine level listed on the labels of e-cigarette cartridges and refill solutions is often significantly different from measured values. Phenolic compounds, polycyclic aromatic hydrocarbons and drugs have also been reported in e-cigarette refill solutions, cartridges and aerosols. Varying results in particle size distributions of particulate matter emissions from e-cigarettes across studies have been observed. Methods applied for the generation and chemical analyses of aerosols differ across studies. Performance characteristics of e-cigarette devices also vary across and within brands.