

pubs.acs.org/est Article

# Adverse Biophysical Impact of e-Cigarette Flavors on Pulmonary Surfactant

Ria A. Goros, Xiaojie Xu, Guangle Li, and Yi Y. Zuo\*



Cite This: Environ. Sci. Technol. 2023, 57, 15882-15891



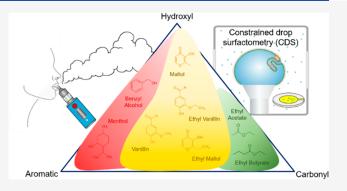
**ACCESS** I

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: The attractiveness and abundance of flavors are primary factors eliciting youth to use e-cigarettes. Emerging studies in recent years revealed the adverse health impact of e-cigarette flavoring chemicals, including disruption of the biophysical function of pulmonary surfactants in the lung. Nevertheless, a comprehensive understanding of the biophysical impact of various flavoring chemicals is still lacking. We used constrained drop surfactometry as a new alternative method to study the biophysical impact of flavored e-cigarette aerosols on an animal-derived natural pulmonary surfactant. The dose of exposure to e-cigarette aerosols was quantified with a quartz crystal microbalance, and alterations to the ultrastructure of the surfactant film were visualized using atomic force microscopy. We have systematically studied eight



representative flavoring chemicals (benzyl alcohol, menthol, maltol, ethyl maltol, vanillin, ethyl vanillin, ethyl acetate, and ethyl butyrate) and six popular recombinant flavors (coffee, vanilla, tobacco, cotton candy, menthol/mint, and chocolate). Our results suggested a flavor-dependent inhibitory effect of e-cigarette aerosols on the biophysical properties of the pulmonary surfactant. A qualitative phase diagram was proposed to predict the hazardous potential of various flavoring chemicals. These results provide novel implications in understanding the environmental, health, and safety impacts of e-cigarette aerosols and may contribute to better regulation of e-cigarette products.

KEYWORDS: pulmonary surfactant, electronic cigarette, flavor, menthol, constrained drop surfactometry

#### ■ INTRODUCTION

Electronic cigarettes (e-cigarettes) are nicotine-delivery devices that employ a battery-powered heating element to vaporize a liquid and produce inhalable aerosols. The liquid, known as an "e-liquid" or "e-juice", is held in a cartridge where it is soaked up by a wicking material, such as a stainless steel mesh, to come into contact with a heating coil.<sup>2</sup> The e-liquid generally contains propylene glycol (PG), vegetable glycerin (VG), various levels of nicotine, and a variety of flavoring chemicals.<sup>3,4</sup> The vaporization of e-liquids is smoke-free, thus reducing the production of tobacco-specific combustion products, such as carbon monoxide.<sup>5</sup> Accordingly, when introduced in the mid-2000s, e-cigarettes were marketed as a healthier nicotine source than traditional cigarettes, and intended to be used as a tobacco cigarette cessation method. The flavoring component was used to promote the use of e-cigarettes as it masks the harsh taste of nicotine; however, the enticing nature of the flavored aerosols has led to many adolescents and young adults initiating their use of nicotine via e-cigarettes. Numerous studies have reported the attractiveness of flavors to be one of the primary motivations for usage of e-cigarettes among youth.<sup>7–10</sup>

Reportedly, there are  $\sim 17,000$  flavored e-liquid products available on the market from over 450 retail brands. These

flavors can be grouped into 12 categories, i.e., tobacco, menthol/ mint, nuts, spices, coffee/tea, alcohol, other beverages, fruit, dessert, candy, other sweets, and others. 14 Although most of the flavoring chemicals used in e-liquids are considered safe for ingestion by the Flavors and Extract Manufacturers Association (FEMA), this assessment is not necessarily valid for inhalation. 15-17 In accordance with flavor variety and attractiveness among youth, there has been a vast emergence of research on the pulmonary toxicity of e-cigarette flavors. 18-23 A collection of in vitro studies have identified a dose-dependent toxicological effect of common flavoring ingredients used in commercial e-liquids, including maltol, ethyl maltol, vanillin, and cinnamaldehyde, on human respiratory cells. 24-26 Likewise, benzaldehyde is a primary ingredient in many cherry flavored eliquids, yet it is a known respiratory irritant with potential to cause acute lung injury. 17,27 Studies have also found flavored e-

Received: July 23, 2023 Revised: September 26, 2023

Accepted: September 27, 2023 Published: October 12, 2023





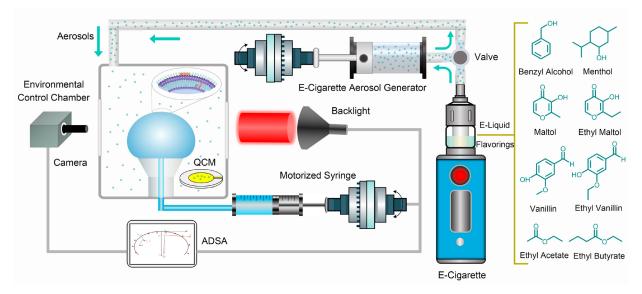


Figure 1. Constrained drop surfactometry (CDS) is a new alternative method to evaluate the adverse biophysical impact of e-cigarette aerosols on pulmonary surfactant. CDS uses the air—water surface of a sessile droplet, with an adsorbed pulmonary surfactant film and constrained on a 3 mm pedestal with a knife-sharp edge, to simulate the air—water surface of the lung. The adsorbed surfactant film can be periodically compressed and expanded by precisely controlling liquid flow into and out of the droplet using a motorized syringe. The surface tension and surface area of the surfactant film are simultaneously determined from the shape of the droplet using closed-loop axisymmetric drop shape analysis (CL-ADSA). The droplet-pedestal assembly is enclosed in an environmental control chamber that simulates intra-alveolar conditions. An e-cigarette aerosol generator with a refillable cartridge is connected to the environmental control chamber via a three-way valve. e-Cigarette aerosols, with various flavoring chemicals, are generated by simulating mainstream puff profiles. The dose of exposure is quantified with a quartz crystal microbalance (QCM).

liquids to produce reactive oxygen species (ROS) when vaporized, <sup>28</sup> which can impair blood vessels, thus increasing the risk of developing cardiovascular diseases. <sup>29</sup> The nanoscale size of e-cigarette aerosols likely permits penetration across the blood—brain barrier, raising concern for neurovascular health, regardless of nicotine content. <sup>30,31</sup> To address these health concerns, the U.S. Food and Drug Administration (FDA) placed a ban on flavored e-liquid cartridges, not including menthol or tobacco flavors. Although restrictions were enforced, non-cartridge-based products such as JUUL and Puff Bar are exempt from the ban, and thus numerous flavored e-cigarettes are still available on the market.

The median size of aerosols produced from e-cigarette devices ranges from 11 to 175 nm, which is within the size limits to bypass airway clearance barriers and enter the alveolar region of the lungs. 32-34 Once deposited onto alveoli, the e-cigarette aerosols must first interact with the thin film lining of the alveolar surface, known as pulmonary surfactant.<sup>35</sup> It consists of ~80 wt % phospholipids, 5-10% neutral lipids, and 5-10% proteins. Primarily, pulmonary surfactant plays a critical role in lung physiology to reduce alveolar surface tension to near-zero values upon exhalation for effective inhalation, minimizing the energy expended, and thus maintaining tidal breathing.<sup>36</sup> Disrupted function of the pulmonary surfactant has life-threatening health impacts including acute lung injury and acute respiratory distress syndrome, which can lead to fatal consequences. 36,37 To date, very limited research has been conducted on the effect of ecigarette aerosols on the pulmonary surfactant film under physiologically relevant conditions, while all these studies suggested that e-cigarette flavors play a predominant role in affecting the biophysical function of pulmonary surfactant.<sup>38,39</sup> Nevertheless, a comprehensive study of the biophysical impact of various e-cigarette flavors on pulmonary surfactants is still lacking.

Here, we used constrained drop surfactometry (CDS) to study the biophysical impact of flavored e-cigarette aerosols on Infasurf, an animal-derived natural pulmonary surfactant. CDS has been used as a new alternative method to simulate nanobio interactions in the lung <sup>40</sup> and to evaluate the acute lung toxicity of particulate matters <sup>41</sup> and e-cigarette aerosols. <sup>38,39</sup> The dose of exposure to e-cigarette aerosols was quantified with a quartz crystal microbalance (QCM), 42 and alterations to the ultrastructure and topography of the surfactant film were visualized using atomic force microscopy (AFM).<sup>43</sup> We systematically studied eight representative flavoring chemicals (benzyl alcohol, menthol, maltol, ethyl maltol, vanillin, ethyl vanillin, ethyl acetate, and ethyl butyrate) and six popular recombinant ecigarette flavors (coffee, vanilla, tobacco, cotton candy, menthol/mint, and chocolate). Our results suggested a flavordependent inhibitory effect of e-cigarette aerosols on the biophysical properties of pulmonary surfactant. Based on these experimental data, a qualitative phase diagram has been proposed to predict the hazardous potential and acute lung toxicity of flavoring chemicals in various classes (alcohols, heterocycles, aldehydes, and esters). These results provide novel implications in understanding the environmental, health, and safety impacts of e-cigarette aerosols and may contribute to better regulation of e-cigarette products.

## MATERIALS AND METHODS

**Pulmonary Surfactant.** A natural pulmonary surfactant, Infasurf, was purchased from ONY Biotech (Amherst, NY). Through centrifugation and extraction, Infasurf was produced from the lung lavage of the newborn calves. This preparation process removed the hydrophilic surfactant proteins (SP-A and SP-D), yet preserved most of the hydrophobic components, including SP-B and SP-C. ⁴⁴ Infasurf was stored in sterilized vials at −20 °C. The phospholipid concentration of Infasurf was diluted on the day of the experiment from 35 to 1 mg/mL using a

Table 1. Summary of Flavoring Chemicals Used in This Study and Their Concentration Levels Found in Commercial e-Cigarette Products

Flavor chemicals	Benzyl alcohol	Menthol	Maltol	Ethyl maltol	Vanillin	Ethyl vanillin	Ethyl acetate	Ethyl butyrate
Chemical structure	ОН	H <sub>3</sub> C CH <sub>3</sub> OH	O CH <sub>3</sub>	OH CH <sub>3</sub>	HO OCH3	OH O CH3	H <sub>3</sub> C CH <sub>3</sub>	H <sub>3</sub> C CH <sub>3</sub>
Chemical formula	C <sub>7</sub> H <sub>8</sub> O	$C_{10}H_{20}O$	$C_6H_6O_3$	$C_7H_8O_3$	$C_8H_8O_3$	$C_9H_{10}O_3$	$C_4H_8O_2$	$C_6H_{12}O_2$
CAS number	100-51-6	89-78-1	118-71-8	4940-11-8	121-33-5	121-32-4	141-78-6	105-54-4
Primary chemical class	Alcohol	Alcohol	Alcohol	Alcohol	Alcohol	Alcohol	Ester	Ester
Secondary chemical class	Alcohol	Alcohol	Heterocycle	Heterocycle	Aldehyde	Aldehyde	Ester	Ester
Molar mass (g/mol)	108.14	156.26	126.11	140.14	152.15	166.17	88.11	116.16
Prevalence ranking 12	16	19	5	2	1	6	4	3
Mean conc. as the primary ingredient (mg/mL) <sup>3, 12, 48</sup>	2.44	15.88	5.45	13.83	11.66	5.30	7.20	11.10
Conc. range (mg/mL) <sup>3, 12, 48</sup>	0.1-39	2.04-84	0.13-6.2	0.05-27.1	0.1-33	0.2-8.4	0.04-7.2	0.1-11.1

saline buffer (0.9% NaCl, 1.5 mM CaCl<sub>2</sub>, and 2.5 mM HEPES, at pH 7.0).

**e-Liquid Chemicals.** The e-liquid base was composed of 50/50 (v/v) propylene glycol (PG) and vegetable glycerin (VG), with/without nicotine. Eight flavoring chemicals, including benzyl alcohol, menthol, maltol, ethyl maltol, vanillin, ethyl vanillin, ethyl acetate, and ethyl butyrate, were purchased from Sigma-Aldrich and used without further purification.

Constrained Drop Surfactometry (CDS). The biophysical effect of e-cigarette aerosols on pulmonary surfactant was studied using CDS; an advanced droplet-based tensiometry technique developed in our laboratory. 40 As shown in Figure 1, a 7 μL sessile droplet of 1 mg/mL natural pulmonary surfactant suspension is constrained on a 3 mm pedestal. The pedestal has a knife-sharp edge to minimize leakage of the adsorbed surfactant film from the air-water surface. Formation of the adsorbed surfactant film was identified upon reaching an equilibrium surface tension of 22–25 mN/m.<sup>35</sup> To simulate tidal breathing, the pulmonary surfactant film was compressed and expanded by using a motorized syringe that regulated the fluid flow into and out of the droplet. During the compression-expansion cycles, instantaneous surface tension and surface area measurements of the droplet were calculated using an in-lab developed algorithm known as closed-loop axisymmetric drop shape analysis (CL-ADSA).45 The pedestal-droplet assembly was enclosed in an environmental control chamber that mimics the intralveolar environment by maintaining a temperature of 37 °C and relative humidity of 100%.

An in-lab developed negative-pressure e-cigarette aerosol generator was modified as an extension to the CDS setup.  $^{38,46}$  It consists of a third-generation modifiable e-cigarette device (Geekvape T200 Kit) with a refillable cartridge and a stainless steel mesh 0.38  $\Omega$  coil (HorizonTech Falcon M-Dual), connected to the CDS chamber via a three-way valve. The

power of the e-cigarette device can be adjusted between 5 and 200 W; for consistency it was operated at 80 W.<sup>47</sup> To simulate an average vape session, the puff duration was controlled at 3 s per puff with a 25 s interval between puffs, consistent with mainstream vaping behaviors, 48-50 and following standards recommended by the Corporation Center for Scientific Research Relative to Tobacco (CORESTA).<sup>51</sup> For the purpose of studying the immediate effects of a short vaping period, 3 puffs<sup>52</sup> were delivered into the CDS chamber where the ecigarette aerosols were exposed to the adsorbed surfactant film. The surfactant film exposed to the e-cigarette aerosols was compressed and expanded at a physiologically relevant rate of 3 s per cycle with a compression ratio of 20% of the initial surface area to simulate normal tidal breathing.<sup>53</sup> To quantify the biophysical impact of e-cigarette aerosols on the pulmonary surfactant film, both the minimum surface tension  $(\gamma_{\min})$  and the film compressibility  $(\kappa = \frac{1}{A} \frac{\partial A}{\partial_{\gamma}})$  of the 10th compression expansion cycle were analyzed.

**Quartz Crystal Microbalance (QCM).** The dosimetry of ecigarette aerosol exposure was determined with a 5 MHz QCM (Stanford Research Systems, Sunnyvale, CA). The dose of ecigarette aerosol exposure was recorded as deposited mass per surface area ( $\mu$ g/cm²) divided by the total puff number to present dosimetry on a mean per-puff basis ( $\mu$ g/cm²/puff). As shown in Figure S1, the e-cigarette aerosols delivered to the CDS exposure chamber was approximately 10  $\mu$ g/cm²/puff, which falls into the range of exposure doses used in literature, i.e.,  $0.2-182 \mu$ g/cm²/puff.  $0.2-182 \mu$ g/cm²/puff.

Subphase Replacement and *In Situ* Langmuir—Blodgett (LB) Transfer. To visualize the impact of e-cigarette aerosols on pulmonary surfactant film, a novel subphase replacement technique<sup>43</sup> was executed prior to performing *in situ* Langmuir—Blodgett (LB) transfer. To replace the surfactant

vesicles in the aqueous subphase, a coaxial pedestal was used to simultaneously inject buffer solution while withdrawing equal amounts of the surfactant suspension. Successful subphase replacement removes the nonadsorbing phospholipid vesicles but does not disturb the adsorbed surfactant film at the air—water surface. After subphase replacement, the surfactant film was LB transferred onto a freshly peeled mica sheet across the air—water surface at a speed of 1 mm/min.

**Atomic Force Microscopy (AFM).** The immobilized Infasurf film was imaged with an Innova AFM (Bruker, Santa Barbara, CA), in tapping mode, using a silicon cantilever with a resonance frequency of 300 kHz and a spring constant of 42 N/m. Multiple locations on each sample were scanned for reproducibility purposes. The topographical images were then analyzed using Nanoscope Analysis software (version 1.5).

**Statistical Analysis.** Each result is shown as mean  $\pm$  standard deviation (n = 5). The one-way ANOVA with the Tukey means comparison test (OriginPro, Northampton, MA) was used to establish differences between groups. Results were considered statistically significant when p < 0.05.

### RESULTS AND DISCUSSION

Effect of Flavored e-Cigarette Aerosols on Biophysics of Pulmonary Surfactant Film. Table 1 summarizes the molecular characteristics of eight representative flavoring chemicals selected for this study: benzyl alcohol, menthol, maltol, ethyl maltol, vanillin, ethyl vanillin, ethyl acetate, and ethyl butyrate. These chemicals were among the top 25 most frequently added flavoring ingredients in e-cigarette products, with vanillin, ethyl maltol, ethyl butyrate, ethyl acetate, maltol, and ethyl vanillin comprising, in order, the top 6, and benzyl alcohol and menthol being ranked 16 and 19, respectively. 12 In terms of their chemical structures, the first six chemicals feature a hydroxyl group (-OH) and thus are all primarily classified as an alcohol, while ethyl acetate and ethyl butyrate are categorized as esters since they possess a carbonyl group (C=O) bonded to an additional oxygen atom. Further categorization of these chemicals separates them into four distinct classes: alcohols, heterocycles, aldehydes, and esters. Maltol and ethyl maltol are heterocycles, as they contain an additional element (oxygen) in their ring structures. Vanillin and ethyl vanillin can also be more specifically identified as aldehydes, as they each have a carbonyl group bonded to a hydrogen atom. With respect to the mean concentration of each chemical when added as the primary ingredient, menthol is the most predominant. 12 The range of chemical concentration identified in commercial e-liquid products also highlights menthol to have the highest detected concentration up to 84 mg/mL.3,12,48,57

We first studied the effect of individual flavoring chemicals, all at the same concentration of 10 mg/mL, which is close to the average concentration of these flavoring chemicals used in ecigarette products (Table 1). Figure 2 shows the biophysical properties of the pulmonary surfactant film when exposed to 3-puffs of e-cigarette aerosols for each individual flavoring chemical, in which the Infasurf film adsorbed at the air—water surface without exposure to e-cigarette aerosols serves as a negative control. (The puff-dependent measurements can be found in Figure S2.) Also included in Figure 2 are the biophysical properties of the surfactant film exposed to aerosols of the vehicle solvent alone (PG/VG) and aerosols with only nicotine (also at 10 mg/mL). As depicted in Figure 2A, the dynamic compression—expansion experiments show that exposure to aerosols of PG/VG, nicotine, and all flavoring

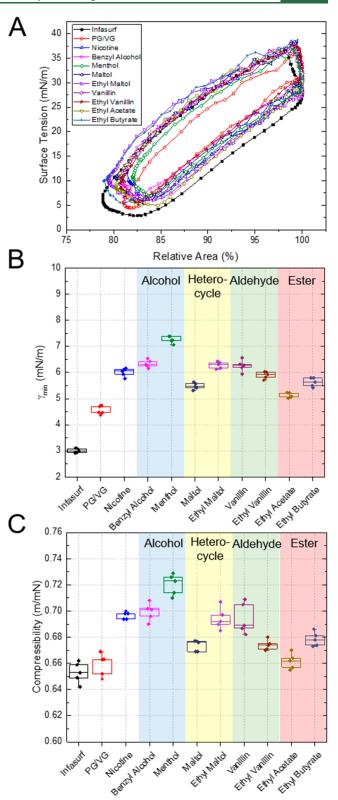


Figure 2. Effect of individual e-cigarette flavoring chemicals on the biophysical properties of a natural pulmonary surfactant, Infasurf. For the purpose of comparison, all flavoring chemicals were studied at 10 mg/mL in e-liquid, close to the average concentration of these flavoring chemicals used in commercial e-cigarette products. (A) Dynamic compression—expansion cycles, i.e., simulation of normal tidal breathing, of Infasurf before and after exposure to e-cigarette aerosols. (B) and (C) Statistical analysis of the minimum surface tension ( $\gamma_{min}$ ) and film compressibility ( $\kappa$ ) of the dynamic cycles, respectively. All data (n=5) of aerosol exposure show statistically significant differences in

Figure 2. continued

comparison to the Infasurf film without exposure to aerosols (p < 0.05). Color code for the flavoring chemicals used in panels B and C: Blue represents alcohols (including benzyl alcohol and menthol); yellow represents heterocycles (maltol and ethyl maltol); green represents aldehydes (vanillin and ethyl vanillin); red represents esters (ethyl acetate and ethyl butyrate).

chemicals increases the minimum surface tension  $(\gamma_{min})$  of Infasurf at the end of compression. Among all flavoring chemicals, the alcohols appear to have the most significant adverse effect on the pulmonary surfactant, indicated by the elevation of  $\gamma_{\min}$  (Figure 2B) and film compressibility  $\kappa$  (Figure 2C). Specifically, menthol induces the highest  $\gamma_{\min}$  increase from 3 to 7.3 mN/m. Benzyl alcohol and menthol also increase  $\kappa$  from 0.653 to 0.699 and 0.720 m/mN, respectively, indicating that exposure to alcohols fluidizes the surfactant film. The heterocycles and aldehydes have a similar, relatively moderate inhibitory effect on the biophysical properties of the surfactant film. In comparison to other flavoring chemicals, the estergrouped flavoring ingredients have the smallest adverse effect; nevertheless, the  $\gamma_{\min}$  of the surfactant film is elevated more than that exposed to aerosols of the vehicle solvent alone. In general, these results are in good agreement with previous studies that found exposure to menthol-flavored aerosols causes the most notable biophysical inhibition on pulmonary surfactant. 38,39 Moreover, the present data show that benzyl alcohol, ethyl maltol, and vanillin also have significant inhibitory effects on the biophysical properties of the pulmonary surfactant.

Next we studied recombinant flavor chemicals analogous to six popular commercial e-cigarette flavors: coffee, vanilla, tobacco, cotton candy, menthol/mint, and chocolate.<sup>3,12</sup> As shown in Figure 3A,B, each of these flavors is made of a mixture of 3–4 primary flavoring chemicals, with the total concentration of the flavoring chemicals varying from 6.5 mg/mL (for the coffee flavor) to 42.5 mg/mL (for the chocolate flavor).<sup>3</sup> In addition, we have studied the recombinant e-liquids of these flavors, without or with 12 mg/mL nicotine, which represents the median nicotine concentration used in all e-cigarette products.<sup>3,48,58,59</sup> It was found that although the chocolate flavor has the highest total flavoring chemical concentration, the menthol/mint flavor appears to have the most significant inhibitory effect on Infasurf, indicated by the highest  $\gamma_{\rm min}$  and  $\kappa$ values, i.e., 8.2 mN/m and 0.717 m/mN, respectively (Figure 3C,D). Considering that menthol is the primary ingredient of the menthol/mint flavor (Figure 3B), these data correspond well with menthol's adverse effect on pulmonary surfactant as an individual flavoring chemical (Figure 2). It should also be noted that benzyl alcohol, ethyl maltol, and vanillin are the primary ingredients of the tobacco, cotton candy, and chocolate flavors, respectively (Figure 3B). These flavoring chemicals exhibit a moderate inhibitory effect on the biophysical properties of pulmonary surfactant, which appears to translate to the effect observed even when they are mixed with other flavoring chemicals. In comparison, the coffee and vanilla flavors do not exhibit any additional inhibitory effect on pulmonary surfactant than the vehicle solvent alone. This is likely related to the overall low concentrations of flavoring chemicals (<10~mg/mL) used in constructing these flavors. It was also found that addition of nicotine at 12 mg/mL does not alter the trends observed without nicotine but only causes a slight elevation in  $\gamma_{\min}$  and  $\kappa$  for all recombinant flavors (Figure 3E and F).

## Effect of Flavored e-Cigarette Aerosols on Ultrastructure and Topography of Pulmonary Surfactant

Film. Figure 4 shows the lateral structure and topography of the Infasurf film with/without exposure to e-cigarette aerosols of various recombinant flavors: coffee, vanilla, tobacco, cotton candy, menthol/mint, and chocolate. Reproducibility of the AFM images can be found in Figures S3-S10. As shown in Figure 4A, the *de novo* adsorbed Infasurf film without exposure to e-cigarette aerosols depicts uniformly distributed phospholipid multilayers (Figure 4A), with a mean height of 19 nm (Figure 4A1). Given the thickness of a fully hydrated phospholipid bilayer to be ~4 nm, 60 these multilayered protrusions correspond to 4-5 stacked phospholipid bilayers. After exposure to e-cigarette aerosols, the Infasurf film shows significant morphological alterations. As shown in Figure 4B, after exposure to unflavored PG/VG aerosols, the ultrastructure shows a reduced number of phospholipid multilayers as well as isolated aggregates in the surfactant film. Topological analysis reveals that the aggregates consist of an irregularly shaped group of phospholipid bilayers with a higher bilayer structure (up to ~50 nm) sitting on top of them (Figure 4B1). Likewise, the Infasurf film after exposure to vanilla (Figure 4D) or coffee (Figure 4F) flavored aerosols reveals an ultrastructure similar to that of the surfactant film exposed to PG/VG aerosols. Addition of nicotine does not significantly alter the ultrastructure of the surfactant film (Figure 4D vs Figure 4E), but reduces the maximum height of the protruding lipid bilayers (Figure 4C1).

Exposure to e-cigarette aerosols of tobacco, cotton candy, menthol/mint, and chocolate flavors (Figure 4G–J) drastically alters the ultrastructure of the pulmonary surfactant film. After exposure to aerosols of these flavors, the surfactant multilayer appears to be transformed into a monolayer conformation, indicated by height variations between 1 and 2 nm throughout the surfactant film. Such height variations indicate domain formation in the surfactant monolayer, with the solid-like lipid domains 1–2 nm higher than the surrounding fluid-like lipid phase. The area of the surfactant monolayer covered with solid-like domains appears to decrease in the order of exposure to tobacco, cotton candy, menthol/mint, and chocolate-flavored e-cigarette aerosols (Figure 4K).

Transformation of the natural surfactant film from multilayer to monolayer is a strong indication of surfactant inhibition caused by dysfunction of the hydrophobic surfactant proteins, SP-B and/or SP-C. It was found that menthol in e-cigarette aerosols is capable of penetrating into the pulmonary surfactant film, thus fluidizing the surfactant phospholipids and disturbing the secondary structure of surfactant proteins. SP-B and SP-C play a central role in maintaining the stability of surfactant film by formatting high-curvature structures, such as necks and pores, needed to stabilize the surfactant multilayers. Denaturation of these proteins, after interacting and/or adsorbing to the inhaled aerosols, therefore leads to a multilayer-to-monolayer transformation of the pulmonary surfactant film, usually associated with significant inhibition of its biophysical properties. 38,41

**Environmental and Health Implications.** Although the flavoring ingredients used in e-cigarette products are considered safe for ingestion, an increasing number of *in vitro* and *in vivo* studies have unveiled various environmental and health hazards that arise from inhaling these chemicals, such as respiratory irritation, inflammatory cell responses, and induced oxidative stress. 17-19,24-28

Results in the present study suggested a flavor-dependent inhibitory effect of e-cigarette aerosols on the biophysical

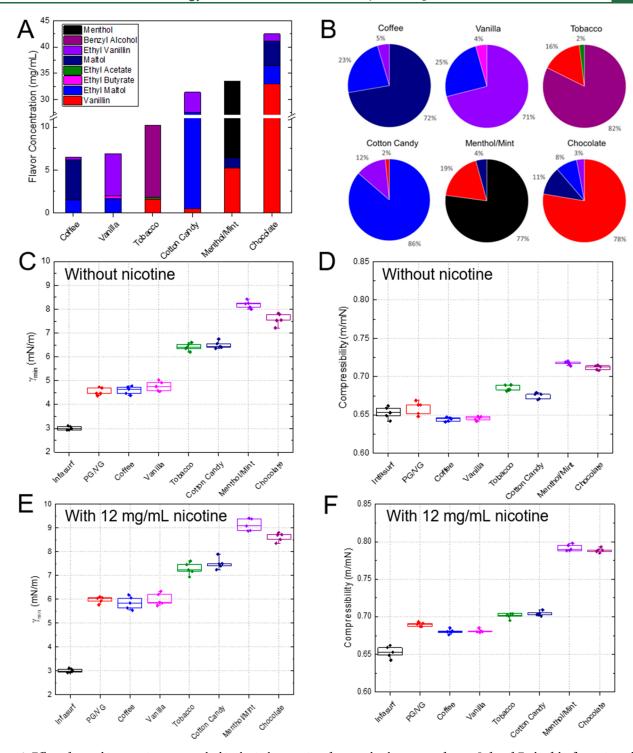


Figure 3. Effect of recombinant e-cigarettes on the biophysical properties of a natural pulmonary surfactant, Infasurf. Each of the flavors is made of a mixture of 3 to 4 primary flavoring chemicals. (A) Accumulated concentration of these flavoring chemicals. (B) Ingredient profile of these chemicals in each recombinant flavor. (C, D) Statistical analysis of the minimum surface tension ( $\gamma_{min}$ ) and film compressibility ( $\kappa$ ), respectively, for Infasurf film exposure to flavored e-cigarette aerosols without nicotine. (E, F)  $\gamma_{min}$  and  $\kappa$  of the Infasurf film exposure to flavored e-cigarette aerosols with 12 mg/mL nicotine. All data (n = 5) of aerosol exposure show statistically significant differences in comparison to the Infasurf film without exposure to aerosols (p < 0.05).

function of the pulmonary surfactant. It has reported that more than 200 ingredients were identified in commercial e-liquids, and on average, 63% of these ingredients were flavoring chemicals. Based on their chemical structures, these flavoring chemicals can be categorized into several chemical classes, such as aldehyde, alcohol, ester, ketone, and monoterpene. Reports

have made connections between the toxicity of the flavoring ingredients and their chemical structure.<sup>25,61</sup> With the comprehensive study of flavoring ingredients in various chemical classes, here, we propose a qualitative phase diagram that may be used to predict the inhibitory potential, and thus the acute lung toxicity, of the flavoring chemicals used in

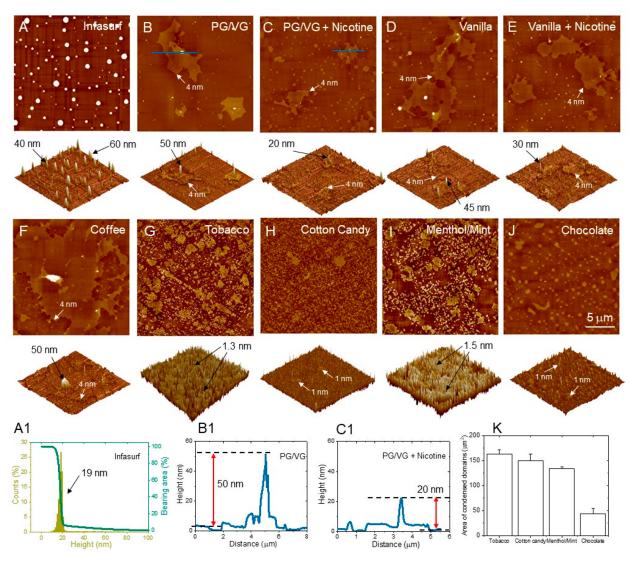


Figure 4. Effect of recombinant e-cigarette flavors on the lateral structure and topography of the Infasurf film. (A) AFM image of a *de novo* adsorbed Infasurf film. (B, C) AFM images of the Infasurf film exposed to aerosols of the vehicle solvent alone (PG/VG) and with 12 mg/mL nicotine, respectively. (D, E) AFM images of the Infasurf film exposed to the vanilla-flavored e-cigarette aerosols without/with nicotine. (F–J) AFM images of the Infasurf film exposed to e-cigarette aerosols with coffee, tobacco, cotton candy, menthol/mint, and chocolate flavors, respectively. All AFM images were obtained at the equilibrium surface tension and have the same scanning area of  $20 \times 20 \,\mu\text{m}$ . For clarity, all AFM images are shown in both 2D topography and 3D rendering. The z range is 50 nm for images in panels A–F, and 5 nm for images in panels G–J. Arrows denote the height measurements in the AFM images. (A1–C1) shows the bearing analysis and height histogram for structures shown in panels A–C. (K) Analysis of the area for the condensed domains covering the Infasurf monolayer exposed to e-cigarette aerosols with tobacco, cotton candy, menthol/mint, and chocolate flavors.

commercial e-cigarette products. As shown in Figure 5, the qualitative phase diagram suggests that esters, only possessing a carbonyl group, such as ethyl acetate and ethyl butyrate, have the least effect on the biophysical function of pulmonary surfactant. In contrast, aromatic compounds with a hydroxyl group, such as menthol and benzyl alcohol, appear to have the greatest adverse effect on the pulmonary surfactant. Aldehydes, such as maltol and ethyl maltol, and heterocycles, such as vanillin and ethyl vanillin, which are composed of aromatic, hydroxyl, and carbonyl groups, have a moderate inhibitory effect on the biophysical function of the pulmonary surfactant.

The specific molecular mechanism by which the various flavoring chemicals interact with phospholipids and hydrophobic proteins (SP-B/C) in pulmonary surfactant at the air—water surface is a subject of future study. It appears that the conformational flexibility of these molecules at the air—water

surface could play a crucial role in determining their interactions with the phospholipid monolayer. Using molecular dynamics simulations, Perkins et al. showed that the addition of a hydroxyl group altered the orientation of aromatic molecules interacting with a DPPC monolayer at the air—water surface. Our previous study also showed that menthol penetrates into the phospholipid headgroup of a DPPC monolayer by orienting its hydroxyl group toward the bulk water. Esters, which possess only a carbonyl group, such as ethyl acetate and ethyl butyrate, do not have sufficient conformational flexibility needed for effectively interacting with the phospholipid monolayer; while on the contrary, aromatic molecules with a hydroxyl group, such as menthol and benzyl alcohol, could be more energetically favorable to interact with the phospholipid monolayer in an interfacial environment.

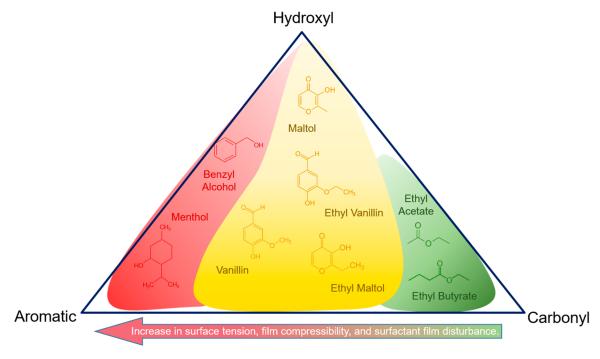


Figure 5. Qualitative phase diagram to predict the hazardous potential of the flavoring chemicals used in e-cigarette products. The three corners of the phase diagram represent carbonyl, aromatic, and hydroxyl groups of the flavoring chemicals. It suggests that esters, possessing only a carbonyl group, such as ethyl acetate and ethyl butyrate, have the least adverse biophysical impact on pulmonary surfactant. Aldehydes, such as maltol and ethyl maltol, and heterocycles, such as vanillin and ethyl vanillin, which are composed of aromatic, hydroxyl, and carbonyl groups, demonstrate a moderate inhibitory effect on pulmonary surfactant. Aromatic compounds with a hydroxyl group, such as menthol and benzyl alcohol, have the highest adverse impact. The biophysical impact and surfactant inhibition were determined by the increase in surface tension, film compressibility, and level of disturbance to the surfactant film. Color code: green represents safe; yellow represents warning; red represents hazardous in causing biophysical inhibition to the pulmonary surfactant. Color transitions from light to dark indicate an increasing severity.

Consistent with previous studies, 38,39 it was found that exposure to menthol-favored e-cigarette aerosols caused the most significant biophysical inhibition to pulmonary surfactant, indicated by elevated minimum surface tension and film compressibility (Figures 2 and 3), and disrupted multilayer structures (Figure 4). These biophysical findings are in good agreement with recent in vitro and in vivo studies that demonstrated the adverse health impact of menthol used in ecigarette products. In vitro cytotoxicity studies showed that exposure to menthol-flavored e-cigarette aerosols caused mitochondrial dysfunction in lung epithelial cells. 63,64 Chronic inhalation, i.e., three times a day for 3 months, of mentholflavored e-cigarette aerosols caused proinflammatory responses in the lung, heart, brain, and colon of mice. 65 Lerner et al. studied commercial menthol and tobacco flavored e-cigarette products, and found a dramatic increase in ROS generation, exclusively for cells exposed to the menthol-flavored e-cigarette aerosols. 18 The increased levels of ROS are potentially linked to the adverse biophysical impact observed in this study.

In conclusion, we have systematically studied eight representative flavoring chemicals (benzyl alcohol, menthol, maltol, ethyl maltol, vanillin, ethyl vanillin, ethyl acetate, and ethyl butyrate) and six popular recombinant e-cigarette flavors (coffee, vanilla, tobacco, cotton candy, menthol/mint, and chocolate). Our results suggested a flavor-dependent inhibitory effect of e-cigarette aerosols on the biophysical properties of pulmonary surfactant. Based on these experimental data, a qualitative phase diagram has been proposed to predict the hazardous potential and likely the acute lung toxicity of flavoring chemicals in various classes (alcohols, heterocycles, aldehydes, and esters). These results provide novel implications in

understanding the environmental, health, and safety impacts of e-cigarette aerosols and may contribute to better regulation of e-cigarette products.

## ASSOCIATED CONTENT

## Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c05896.

Additional experimental results. Quantification of aerosol exposure using QCM. Reproducibility of AFM measurements (PDF)

#### AUTHOR INFORMATION

## **Corresponding Author**

Yi Y. Zuo — Department of Mechanical Engineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, United States; Department of Pediatrics, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96826, United States; orcid.org/0000-0002-3992-3238; Phone: 808-956-9650; Email: yzuo@hawaii.edu; Fax: 808-956-2373

#### **Authors**

Ria A. Goros – Department of Mechanical Engineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, United States

Xiaojie Xu — Department of Mechanical Engineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, United States Guangle Li — Department of Mechanical Engineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.3c05896

## **Author Contributions**

§R.A.G. and X.X. contributed equally to this work.

#### Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

We thank Dr. Sindhu Row of ONY Biotech for the donation of Infasurf samples. This work was supported by the National Science Foundation (grant number CBET-2011317) to Y.Y.Z.

### REFERENCES

- (1) Grana, R.; Benowitz, N.; Glantz, S. A. E-cigarettes: a scientific review. Circulation 2014, 129 (19), 1972–1986.
- (2) Clapp, P. W.; Jaspers, I. Electronic Cigarettes: Their Constituents and Potential Links to Asthma. *Curr. Allergy Asthma Rep.* **2017**, *17* (11), 79.
- (3) Tierney, P. A.; Karpinski, C. D.; Brown, J. E.; Luo, W.; Pankow, J. F. Flavour chemicals in electronic cigarette fluids. *Tob. Control* **2016**, 25 (e1), e10–e15.
- (4) Li, Y.; Burns, A. E.; Tran, L. N.; Abellar, K. A.; Poindexter, M.; Li, X.; Madl, A. K.; Pinkerton, K. E.; Nguyen, T. B. Impact of e-Liquid Composition, Coil Temperature, and Puff Topography on the Aerosol Chemistry of Electronic Cigarettes. *Chemical research in toxicology* **2021**, 34 (6), 1640–1654.
- (5) Czogala, J.; Goniewicz, M. L.; Fidelus, B.; Zielinska-Danch, W.; Travers, M. J.; Sobczak, A. Secondhand exposure to vapors from electronic cigarettes. *Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco* **2014**, 16 (6), 655–62.
- (6) Fadus, M. C.; Smith, T. T.; Squeglia, L. M. The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. *Drug and alcohol dependence* **2019**, 201, 85–93.
- (7) Villanti, A. C.; Johnson, A. L.; Ambrose, B. K.; Cummings, K. M.; Stanton, C. A.; Rose, S. W.; Feirman, S. P.; Tworek, C.; Glasser, A. M.; Pearson, J. L.; Cohn, A. M.; Conway, K. P.; Niaura, R. S.; Bansal-Travers, M.; Hyland, A. Flavored Tobacco Product Use in Youth and Adults: Findings From the First Wave of the PATH Study (2013–2014). American journal of preventive medicine 2017, 53 (2), 139–151.
- (8) Tsai, J.; Walton, K.; Coleman, B. N.; Sharapova, S. R.; Johnson, S. E.; Kennedy, S. M.; Caraballo, R. S. Reasons for Electronic Cigarette Use Among Middle and High School Students National Youth Tobacco Survey, United States, 2016. MMWR. Morbidity and mortality weekly report 2018, 67 (6), 196–200.
- (9) Kong, G.; Morean, M. E.; Cavallo, D. A.; Camenga, D. R.; Krishnan-Sarin, S. Reasons for Electronic Cigarette Experimentation and Discontinuation Among Adolescents and Young Adults. *Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco* 2015, 17 (7), 847–54.
- (10) Morean, M. E.; Butler, E. R.; Bold, K. W.; Kong, G.; Camenga, D. R.; Cavallo, D. A.; Simon, P.; O'Malley, S. S.; Krishnan-Sarin, S. Preferring more e-cigarette flavors is associated with e-cigarette use frequency among adolescents but not adults. *PLoS One* **2018**, *13* (1), No. e0189015.
- (11) Hsu, G.; Sun, J. Y.; Zhu, S. H. Evolution of Electronic Cigarette Brands From 2013–2014 to 2016–2017: Analysis of Brand Websites. *J. Med. Internet Res.* **2018**, 20 (3), No. e80.
- (12) Krüsemann, E. J. Z.; Havermans, A.; Pennings, J. L. A.; de Graaf, K.; Boesveldt, S.; Talhout, R. Comprehensive overview of common eliquid ingredients and how they can be used to predict an e-liquid's flavour category. *Tobacco Control* **2021**, 30 (2), 185–191.
- (13) Alhadyan, S. K.; Sivaraman, V.; Onyenwoke, R. U. E-cigarette Flavors, Sensory Perception, and Evoked Responses. *Chemical research in toxicology* **2022**, 35 (12), 2194–2209.

- (14) Krüsemann, E. J. Z.; Boesveldt, S.; de Graaf, K.; Talhout, R. An E-Liquid Flavor Wheel: A Shared Vocabulary Based on Systematically Reviewing E-Liquid Flavor Classifications in Literature. *Nicotine Tob Res.* **2019**, *21* (10), 1310–1319.
- (15) Gordon, T.; Karey, E.; Rebuli, M. E.; Escobar, Y.-N. H.; Jaspers, I.; Chen, L. C. E-Cigarette Toxicology. *Annual Review of Pharmacology and Toxicology* **2022**, *62* (1), 301–322.
- (16) Kanwal, R.; Kullman, G.; Piacitelli, C.; Boylstein, R.; Sahakian, N.; Martin, S.; Fedan, K.; Kreiss, K. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. *Journal of occupational and environmental medicine* **2006**, 48 (2), 149–57.
- (17) Kosmider, L.; Sobczak, A.; Prokopowicz, A.; Kurek, J.; Zaciera, M.; Knysak, J.; Smith, D.; Goniewicz, M. L. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. *Thorax* **2016**, *71* (4), 376–7.
- (18) Lerner, C. A.; Sundar, I. K.; Yao, H.; Gerloff, J.; Ossip, D. J.; McIntosh, S.; Robinson, R.; Rahman, I. 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* **2015**, *10* (2), No. e0116732.
- (19) Pepper, J. K.; Ribisl, K. M.; Brewer, N. T. Adolescents' interest in trying flavoured e-cigarettes. *Tob. Control* **2016**, *25*, ii62—ii66.
- (20) Chun, L. F.; Moazed, F.; Calfee, C. S.; Matthay, M. A.; Gotts, J. E. Pulmonary toxicity of e-cigarettes. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2017**, *313* (2), L193–L206.
- (21) Klager, S.; Vallarino, J.; MacNaughton, P.; Christiani, D. C.; Lu, Q.; Allen, J. G. Flavoring chemicals and aldehydes in e-cigarette emissions. *Environ. Sci. Technol.* **2017**, *51* (18), 10806–10813.
- (22) Khlystov, A.; Samburova, V. Flavoring compounds dominate toxic aldehyde production during e-cigarette vaping. *Environ. Sci. Technol.* **2016**, 50 (23), 13080–13085.
- (23) Cao, Y.; Wu, D.; Ma, Y.; Ma, X.; Wang, S.; Li, F.; Li, M.; Zhang, T. Toxicity of electronic cigarettes: A general review of the origins, health hazards, and toxicity mechanisms. *Science of The Total Environment* **2021**, 772, No. 145475.
- (24) Clapp, P. W.; Pawlak, E. A.; Lackey, J. T.; Keating, J. E.; Reeber, S. L.; Glish, G. L.; Jaspers, I. Flavored e-cigarette liquids and cinnamaldehyde impair respiratory innate immune cell function. *American journal of physiology. Lung cellular and molecular physiology* **2017**, 313 (2), L278–L292.
- (25) Hua, M.; Omaiye, E. E.; Luo, W.; McWhirter, K. J.; Pankow, J. F.; Talbot, P. Identification of Cytotoxic Flavor Chemicals in Top-Selling Electronic Cigarette Refill Fluids. *Sci. Rep.* **2019**, *9* (1), 2782.
- (26) Gerloff, J.; Sundar, I. K.; Freter, R.; Sekera, E. R.; Friedman, A. E.; Robinson, R.; Pagano, T.; Rahman, I. Inflammatory Response and Barrier Dysfunction by Different e-Cigarette Flavoring Chemicals Identified by Gas Chromatography-Mass Spectrometry in e-Liquids and e-Vapors on Human Lung Epithelial Cells and Fibroblasts. *Applied in vitro toxicology* **2017**, 3 (1), 28–40.
- (27) Hickman, E.; Herrera, C. A.; Jaspers, I. Common E-Cigarette Flavoring Chemicals Impair Neutrophil Phagocytosis and Oxidative Burst. *Chemical research in toxicology* **2019**, 32 (6), 982–985.
- (28) Muthumalage, T.; Prinz, M.; Ansah, K. O.; Gerloff, J.; Sundar, I. K.; Rahman, I. Inflammatory and Oxidative Responses Induced by Exposure to Commonly Used e-Cigarette Flavoring Chemicals and Flavored e-Liquids without Nicotine. *Front. Physiol.* **2018**, *8*, 1130.
- (29) Lee, W. H.; Ong, S. G.; Zhou, Y.; Tian, L.; Bae, H. R.; Baker, N.; Whitlatch, A.; Mohammadi, L.; Guo, H.; Nadeau, K. C.; Springer, M. L.; Schick, S. F.; Bhatnagar, A.; Wu, J. C. Modeling Cardiovascular Risks of E-Cigarettes With Human-Induced Pluripotent Stem Cell-Derived Endothelial Cells. *Journal of the American College of Cardiology* **2019**, 73 (21), 2722–2737.
- (30) Heldt, N. A.; Seliga, A.; Winfield, M.; Gajghate, S.; Reichenbach, N.; Yu, X.; Rom, S.; Tenneti, A.; May, D.; Gregory, B. D.; Persidsky, Y. Electronic cigarette exposure disrupts blood-brain barrier integrity and promotes neuroinflammation. *Brain, behavior, and immunity* **2020**, 88, 363–380.
- (31) Ruszkiewicz, J. A.; Zhang, Z.; Goncalves, F. M.; Tizabi, Y.; Zelikoff, J. T.; Aschner, M. Neurotoxicity of e-cigarettes. Food and

- chemical toxicology: an international journal published for the British Industrial Biological Research Association 2020, 138, No. 111245.
- (32) Bennett, W. D.; Clapp, P. W.; Holbrook, L. T.; Zeman, K. L. Respiratory Tract Deposition of E-Cigarette Particles. *Comprehensive Physiology* **2022**, *12* (4), 3823–3832.
- (33) Zuo, Y. Y.; Uspal, W. E.; Wei, T. Airborne Transmission of COVID-19: Aerosol Dispersion, Lung Deposition, and Virus-Receptor Interactions. *ACS Nano* **2020**, *14* (12), 16502–16524.
- (34) Mikheev, V. B.; Brinkman, M. C.; Granville, C. A.; Gordon, S. M.; Clark, P. I. Real-Time Measurement of Electronic Cigarette Aerosol Size Distribution and Metals Content Analysis. *Nicotine Tob Res.* **2016**, *18* (9), 1895–1902.
- (35) Zuo, Y. Y.; Veldhuizen, R. A.; Neumann, A. W.; Petersen, N. O.; Possmayer, F. Current perspectives in pulmonary surfactant–inhibition, enhancement and evaluation. *Biochimica et biophysica acta* **2008**, *1778* (10), 1947–77.
- (36) Perez-Gil, J.; Weaver, T. E. Pulmonary surfactant pathophysiology: current models and open questions. *Physiology (Bethesda, Md.)* **2010**, 25 (3), 132–41.
- (37) Da Silva, E.; Vogel, U.; Hougaard, K. S.; Perez-Gil, J.; Zuo, Y. Y.; Sorli, J. B. An adverse outcome pathway for lung surfactant function inhibition leading to decreased lung function. *Current research in toxicology* **2021**, *2*, 225–236.
- (38) Xu, L.; Yang, Y.; Simien, J. M.; Kang, C.; Li, G.; Xu, X.; Haglund, E.; Sun, R.; Zuo, Y. Y. Menthol in electronic cigarettes causes biophysical inhibition of pulmonary surfactant. *Am. J. Physiol Lung Cell Mol. Physiol* **2022**, 323 (2), L165–L177.
- (39) Graham, E.; McCaig, L.; Shui-Kei Lau, G.; Tejura, A.; Cao, A.; Zuo, Y. Y.; Veldhuizen, R. E-cigarette aerosol exposure of pulmonary surfactant impairs its surface tension reducing function. *PLoS One* **2022**, *17* (11), No. e0272475.
- (40) Valle, R. P.; Wu, T.; Zuo, Y. Y. Biophysical influence of airborne carbon nanomaterials on natural pulmonary surfactant. *ACS Nano* **2015**, *9* (5), 5413–5421.
- (41) Yang, Y.; Xu, L.; Dekkers, S.; Zhang, L. G.; Cassee, F. R.; Zuo, Y. Y. Aggregation State of Metal-Based Nanomaterials at the Pulmonary Surfactant Film Determines Biophysical Inhibition. *Environ. Sci. Technol.* **2018**, 52 (15), 8920–8929.
- (42) Sørli, J. B.; Da Silva, E.; Bäckman, P.; Levin, M.; Thomsen, B. L.; Koponen, I. K.; Larsen, S. T. A Proposed In Vitro Method to Assess Effects of Inhaled Particles on Lung Surfactant Function. *American journal of respiratory cell and molecular biology* **2016**, *54* (3), 306–11.
- (43) Xu, L.; Yang, Y.; Zuo, Y. Y. Atomic Force Microscopy Imaging of Adsorbed Pulmonary Surfactant Films. *Biophys. J.* **2020**, *119* (4), 756–766.
- (44) Zhang, H.; Fan, Q.; Wang, Y. E.; Neal, C. R.; Zuo, Y. Y. Comparative study of clinical pulmonary surfactants using atomic force microscopy. *Biochim. Biophys. Acta, Biomembr.* **2011**, *1808* (7), 1832–1842.
- (45) Yu, K.; Yang, J.; Zuo, Y. Y. Automated Droplet Manipulation Using Closed-Loop Axisymmetric Drop Shape Analysis. *Langmuir: the ACS journal of surfaces and colloids* **2016**, 32 (19), 4820–6.
- (46) Brown, C. J.; Cheng, J. M. Electronic cigarettes: product characterisation and design considerations. *Tobacco control* **2014**, 23, ii4—ii10.
- (47) Escobar, Y. H.; Morrison, C. B.; Chen, Y.; Hickman, E.; Love, C. A.; Rebuli, M. E.; Surratt, J. D.; Ehre, C.; Jaspers, I. Differential responses to e-cig generated aerosols from humectants and different forms of nicotine in epithelial cells from nonsmokers and smokers. *American journal of physiology. Lung cellular and molecular physiology* **2021**, 320 (6), L1064–L1073.
- (48) Behar, R. Z.; Luo, W.; McWhirter, K. J.; Pankow, J. F.; Talbot, P. Analytical and toxicological evaluation of flavor chemicals in electronic cigarette refill fluids. *Sci. Rep.* **2018**, *8* (1), 8288.
- (49) Robinson, R. J.; Hensel, E. C.; Morabito, P. N.; Roundtree, K. A. Electronic Cigarette Topography in the Natural Environment. *PloS one* **2015**, *10* (6), No. e0129296.

- (50) Olmedo, P.; Navas-Acien, A.; Hess, C.; Jarmul, S.; Rule, A. A direct method for e-cigarette aerosol sample collection. *Environmental Research* **2016**, *149*, 151–156.
- (51) International Organization for Standardization. *Vapour products-Routine analytical vaping machine Definitions and standard conditions*; ISO Standard No. 20768:2018; International Organization for Standardization, 2018. https://www.iso.org/standard/69019.html (accessed 2023–09–26).
- (52) Kosmider, L.; Jackson, A.; Leigh, N.; O'Connor, R.; Goniewicz, M. L. Circadian Puffing Behavior and Topography Among E-cigarette Users. *Tobacco regulatory science* **2018**, *4* (5), 41–49.
- (53) Bachofen, H.; Schurch, S.; Urbinelli, M.; Weibel, E. R. Relations among alveolar surface tension, surface area, volume, and recoil pressure. *Journal of applied physiology* **1987**, 62 (5), 1878–87.
- (54) Thorne, D.; Adamson, J. A review of in vitro cigarette smoke exposure systems. *Experimental and Toxicologic Pathology* **2013**, 65 (7), 1183–1193.
- (55) Forest, V.; Mercier, C.; Pourchez, J. Considerations on dosimetry for in vitro assessment of e-cigarette toxicity. *Respir. Res.* **2022**, 23 (1), 358.
- (56) Noel, A.; Hossain, E.; Perveen, Z.; Zaman, H.; Penn, A. L. Subohm vaping increases the levels of carbonyls, is cytotoxic, and alters gene expression in human bronchial epithelial cells exposed at the airliquid interface. *Respir. Res.* **2020**, *21* (1), 305.
- (57) Omaiye, E. E.; McWhirter, K. J.; Luo, W.; Tierney, P. A.; Pankow, J. F.; Talbot, P. High concentrations of flavor chemicals are present in electronic cigarette refill fluids. *Sci. Rep.* **2019**, *9* (1), 2468.
- (58) Romberg, A. R.; Miller Lo, E. J.; Cuccia, A. F.; Willett, J. G.; Xiao, H.; Hair, E. C.; Vallone, D. M.; Marynak, K.; King, B. A. Patterns of nicotine concentrations in electronic cigarettes sold in the United States, 2013–2018. *Drug Alcohol Depend* **2019**, 203, 1–7.
- (59) Farsalinos, K. E.; Tsiapras, D.; Kyrzopoulos, S.; Savvopoulou, M.; Voudris, V. Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: comparison with the effects of regular cigarettes. *BMC Cardiovasc. Disord.* **2014**, *14*, 78.
- (60) Marsh, D. Handbook of Lipid Bilayers, 2nd ed.; CRC Press: Boca Raton, FL, 2013.
- (61) El-Hellani, A.; Soule, E. K; Daoud, M.; Salman, R.; El Hage, R.; Ardati, O.; El-Kaassamani, M.; Yassine, A.; Karaoghlanian, N.; Talih, S.; et al. Assessing toxicant emissions from e-liquids with DIY additives used in response to a potential flavour ban in e-cigarettes. *Tob. Control* **2022**, *31*, s245.
- (62) Perkins, R. J.; Kukharchuk, A.; Delcroix, P.; Shoemaker, R. K.; Roeselová, M.; Cwiklik, L.; Vaida, V. The Partitioning of Small Aromatic Molecules to Air-Water and Phospholipid Interfaces Mediated by Non-Hydrophobic Interactions. *journal of physical chemistry. B* **2016**, 120 (30), 7408–22.
- (63) Lamb, T.; Muthumalage, T.; Rahman, I. Pod-based menthol and tobacco flavored e-cigarettes cause mitochondrial dysfunction in lung epithelial cells. *Toxicol. Lett.* **2020**, *333*, 303–311.
- (64) Nair, V.; Tran, M.; Behar, R. Z.; Zhai, S.; Cui, X.; Phandthong, R.; Wang, Y.; Pan, S.; Luo, W.; Pankow, J. F.; Volz, D. C.; Talbot, P. Menthol in electronic cigarettes: A contributor to respiratory disease? *Toxicology and applied pharmacology* **2020**, 407, No. 115238.
- (65) Moshensky, A.; Brand, C. S.; Alhaddad, H.; Shin, J.; Masso-Silva, J. A.; Advani, I.; Gunge, D.; Sharma, A.; Mehta, S.; Jahan, A.; et al. Effects of mango and mint pod-based e-cigarette aerosol inhalation on inflammatory states of the brain, lung, heart, and colon in mice. *eLife* **2022**, *11*, No. e67621.