Supplement Article

Reactive Oxygen Species, Mitochondrial Membrane Potential, and Cellular Membrane Potential Are Predictors of E-Liquid Induced Cellular Toxicity

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Abstract

Introduction: The use of flavors in electronic cigarettes appeals to adults and never-smoking youth. Consumption has rapidly increased over the last decade, and in the U.S. market alone, there are over 8000 unique flavors. The U.S. Food and Drug Administration (FDA) has begun to regulate e-liquids, but many have not been tested, and their impact, both at the cellular level, and on human health remains unclear.

Methods: We tested e-liquids on the human cell line HEK293T and measured toxicity, mitochondrial membrane potential ($\Delta\Psi_m$), reactive oxygen species production (ROS), and cellular membrane potential (V_m) using high-throughput screening (HTS) approaches. Our HTS efforts included single-dose and 16-point dose–response curves, which allowed testing of ≥90 commercially available e-liquids in parallel to provide a rapid assessment of cellular effects as a proof of concept for a fast, preliminary toxicity method. We also investigated the chemical composition of the flavors via gas chromatography–mass spectrometry.

Results: We found that e-liquids caused a decrease in $\Delta\Psi_m$ and V_m and an increase in ROS production and toxicity in a dose-dependent fashion. In addition, the presence of five specific chemical components: vanillin, benzyl alcohol, acetoin, cinnamaldehyde, and methyl-cyclopentenolone, but not nicotine, were linked with the changes observed in the cellular traits studied.

Conclusion: Our data suggest that $\Delta\Psi_m$, ROS, V_m, and toxicity may be indicative of the extent of cell death upon e-liquid exposure. Further research on the effect of flavors should be prioritized to help policy makers such as the FDA to regulate e-liquid composition.

Implications: E-liquid cellular toxicity can be predicted using parameters amenable to HTS. Our data suggest that $\Delta\Psi_m$, ROS, V_m, and toxicity may be indicative of the extent of cell death upon e-liquid exposure, and this toxicity is linked to the chemical composition, that is, flavoring components. Further research on the effect of flavors should be prioritized to help policy makers such as the FDA to regulate e-liquid composition.

Introduction

Electronic cigarettes (e-cigarettes) were introduced onto the market in the early 2000s. Since then, their use has drastically increased worldwide both among adults and youth.[1] E-cigarettes comprise an electric heater that aerosolizes a liquid (e-liquid), which usually contains different concentrations of nicotine; variable ratios of propylene glycol (PG) and vegetable glycerin (VG), used as vehicle; and diverse chemicals for flavoring.[2] In the United States alone, there are over 1200 different vendors and over 8000 flavors.[3] In January 2020, after many years without proper regulatory measures, the FDA issued an enforcement policy on unauthorized flavored cartridge-based e-cigarette products, including fruit and mint flavors.[4]

We have previously shown that human embryonic kidney 293 (HEK293T) cells elicit similar cell responses to cigarette smoke as airway epithelia, including those involved in cell signaling, protein trafficking, and similar membrane stiffening when exposed to PG/VG.[5,6] Moreover, we recently demonstrated that HEK293T cells are suitable to compare the cytotoxic effects of many e-liquids in a quick and reliable way that is representative of the toxicity induced in pulmonary cell types, including air–liquid interface epithelia.[7] In this approach, we found that 123 flavors, out of 138 e-liquids tested, decreased viability over a range of concentrations from 0 to 60%.[7] Notably, high-throughput screening (HTS) approaches opens up a convenient and fast way to test market-available e-cigarettes flavors and their chemical components.[8]

Some flavored e-cigarettes contain variable amounts of chemicals that are approved for food use/ingestion, but associated with respiratory diseases when inhaled, such as the flavoring agents: benzaldehyde (fruit, bitter almond)[9,10] diacetyl (butter, pastry),[11] 2,3-pentanedione (butter and fermented diary),[9,12] and acetoin (butter, green pepper).[12–14] Chemicals that raise concerns for human health were also identified, such as coumarin, nitrosamides, aldehydes (forming toxic adducts with DNA), and other toxic carbonyl compounds found in cigarette smoke.[15] Here, we used a more diverse set of e-liquids, from different online vendors, and tested the hypothesis that the effects seen with cell death could be replicated with other germane end points, including reactive oxygen species (ROS), mitochondrial membrane potential ($\Delta\Psi_m$), and cellular membrane potential (V_m). The overall goal of this work was to screen neat e-liquids and identify potential flavors and/or chemical constituents that show other detrimental cellular effects beyond the basic live/dead assay that we previously performed.

Materials and Methods

Refer to the Supplementary Methods section for a detailed description.

E-cigarette Products

A total of 98 e-liquids were purchased from 74 different vendors (Supplementary Table S1).

Chemicals and Reagents

All reagents were ACS grade and purchased from Sigma–Aldrich unless stated otherwise.

Cell Culture

HEK293T cells were cultured as previously described.[7]

Cytotoxicity Assays

Cell Survival

The effects of the e-liquids on cell survival were evaluated as previously reported.[7]

Mitochondrial Membrane Potential ($\Delta\Psi_m$)

Briefly, HEK239T cells were exposed to e-liquids and after 22–20 hours, cells were stained using Mitotracker Red and DAPI for 30 minutes. Fluorescent images were acquired and corrected using DAPI.

ROS Production

HEK293T cells were exposed to e-liquids and later stained with 2′,7′-dichlorofluorescin diacetate for 30 minutes. Fluorescent images were acquired and corrected for cell number using bright-field images DAPI.

Membrane Potential (V_m)

HEK293T cells were exposed to e-liquids and later stained using the FLIPR Membrane Potential Blue Assay Kit according to the manufacturer's recommendations. V_m was measured as fluorescence emission before ($V_{m\text{-pre}}$) and after ($V_{m\text{-post}}$) adding KCl to induce membrane depolarization.

Gas Chromatography–Mass Spectrometry Analysis of E-liquids and Data Analysis

See Supplementary Methods section.

Results

E-Liquids Decrease Cell Viability in a Dose-Dependent Fashion

We have previously shown that e-liquids directly affect cell growth and viability.[7] However, this study was predominantly performed on locally sourced e-liquids from North Carolina. Here, we extended our study to include national vendors (see Supplementary Table S1). To perform a comparison with our previous study, we first performed HTS in HEK293T cells using the same protocol, that is, calcein-AM and propidium iodide as markers of live and dead cells respectively (Figure 1A). The HTS assay was validated according to the coefficient of variation for each 384 plate as previously described.[16] After 22–24 hours, all e-liquids caused a dose-dependent decrease in the number of live cells, an increase in dead cell number and the concomitant decrease in live/dead cell number ratio (Supplementary Table S1). The dose–response curves were then used to calculate the LC_{50} values of the e-liquids (Figure 1B–D; Supplementary Table S1). As expected, the phosphate buffer (PBS) control did not have a toxic effect, whereas PG/VG decreased cell viability in a dose-dependent manner (Figure 1B–D). PG/VG had an LC_{50} of 4.34% vol/vol, showing values within range of our previous studies.[7] The LC_{50}s of the e-liquids tested ranged from 4.53 to 0.0025% vol/vol (Figure 1E; Supplementary Table S1). Consistent with our previous study, all e-liquids caused toxicity in a dose-dependent fashion.

E-Liquids Affect $\Delta\Psi_m$

Because we consistently saw effects of e-liquids on cell viability, we tested other commercially available dyes that are suitable for HTS approaches and relevant to cell death. We investigated changes in
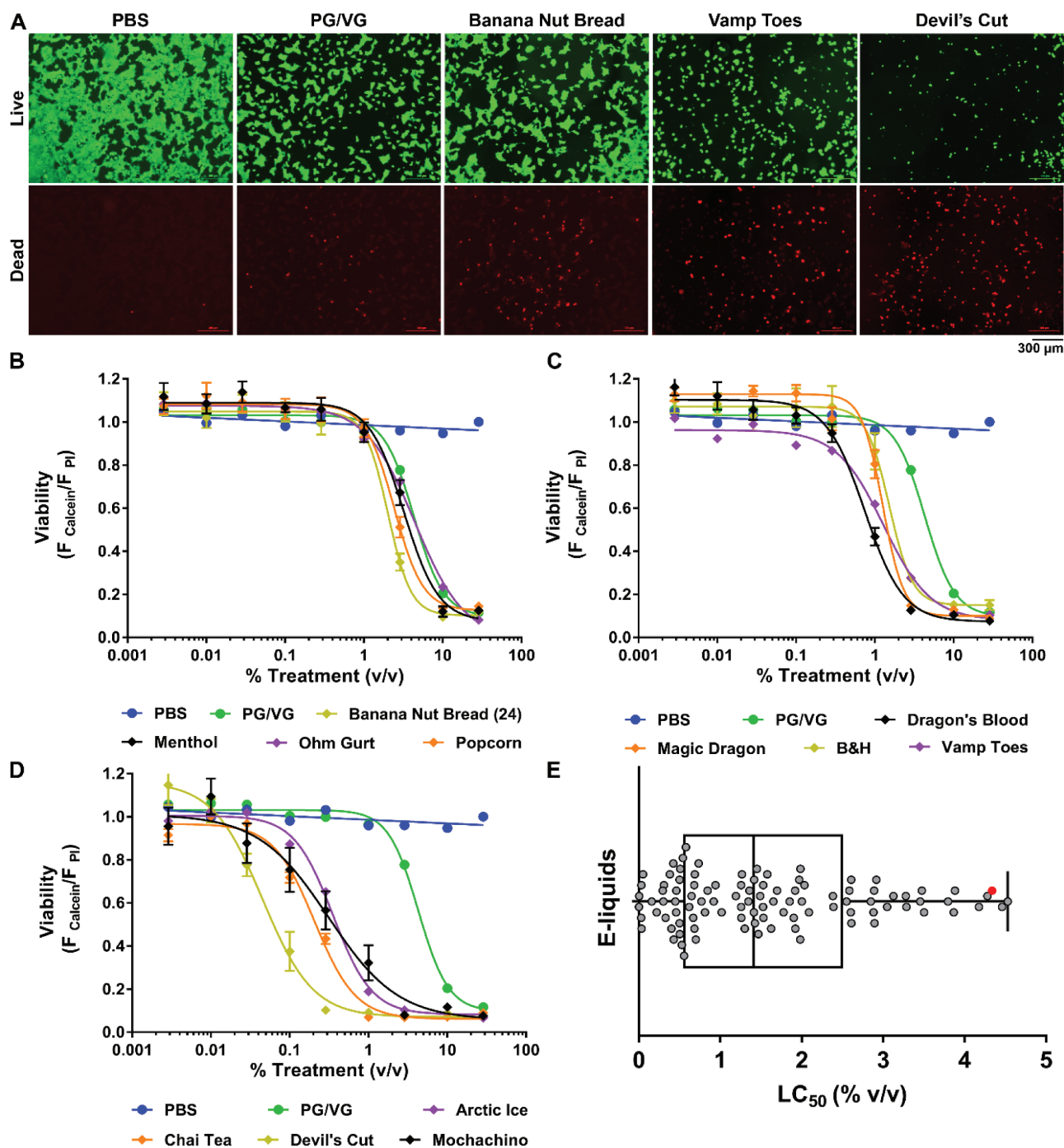
Figure 1. E-liquids affect HEK293T cell toxicity. (A) Cells were stained with calcein-AM (live, green) and propidium iodide (dead, red). PBS, PG/VG, and representative e-liquids are shown. (B–D) Dose–response curves for PBS, PG/VG, and representative e-liquids. (E) LC_{50} distribution (reported as concentration, % vol/vol) of the 98 e-liquids tested. ●= PG/VG (e-liquid vehicle). *n* = 9.

$\Delta\Psi_m$ using the Mitotracker Red stain. We also added DAPI to identify cell nuclei to normalize the signal to cell number (Figure 2A). HEK293T cells were exposed to e-liquids at 1% vol/vol for 24 hours. Out 92 e-liquids, 57 fell beyond the average ± 3 SD cutoff, including the positive control, CCCP (Figure 2B and C). These were selected to perform dose-responses. The positive control CCCP and all tested e-liquids showed a dose-dependent inhibition of $\Delta\Psi_m$, whereas PG/VG and PBS did not (Figure 2D and E; Supplementary Table S1).

E-Liquid Effects on Reactive Oxygen Production

Oxidative stress was evaluated using the fluorescent marker 2′,7′-dichlorofluorescin diacetate (Figure 3A). At 1% vol/vol, 34 e-liquids showed values below and above ± 3 SD of the control's ROS production. PG/VG did not show any significant effect when compared with the PBS control (Figure 3B and C). E-liquids that responses above or below the average PBS signal ± 3 SD response were tested in a dose-dependent manner. Figure 3D shows representative curves
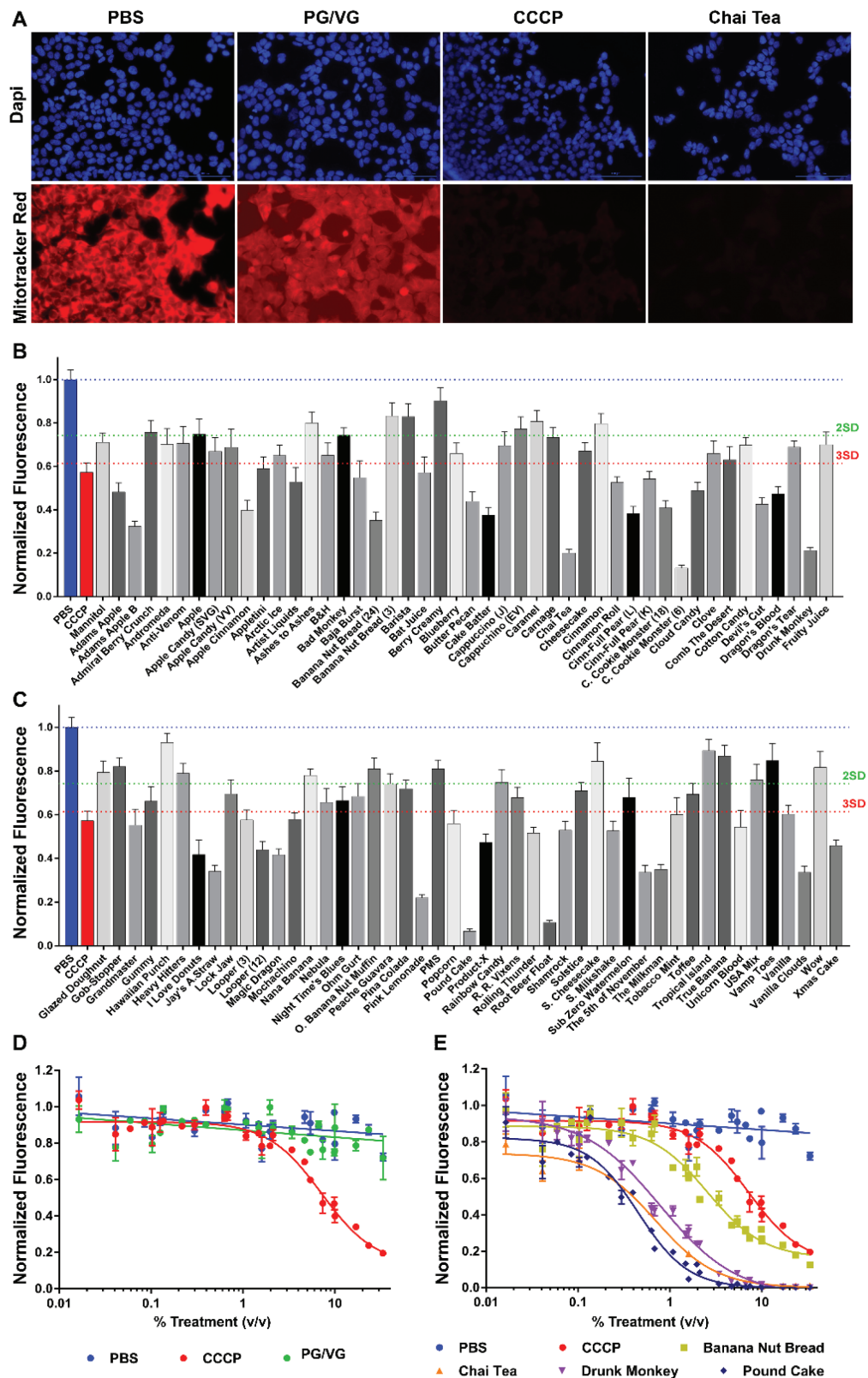
Figure 2. E-liquids impair the mitochondrial membrane potential ($\Delta\Psi_m$). (A) Images showing Mitotracker red and DAPI (blue) staining for PBS, PG/VG, and representative flavors. (B and C) Cells were exposed to e-liquids (1% vol/vol) and then stained. CCCP was used as a positive control (3 µM). Values were normalized to PBS response (blue dotted line). Green and red dotted lines show average response – 2 SD or 3 SD, respectively. $N = 6$. (D, E) E-liquids were tested at several concentrations. Dose-dependent responses are shown. Values were normalized to the PBS response (blue line). All $n = 9$ per group.
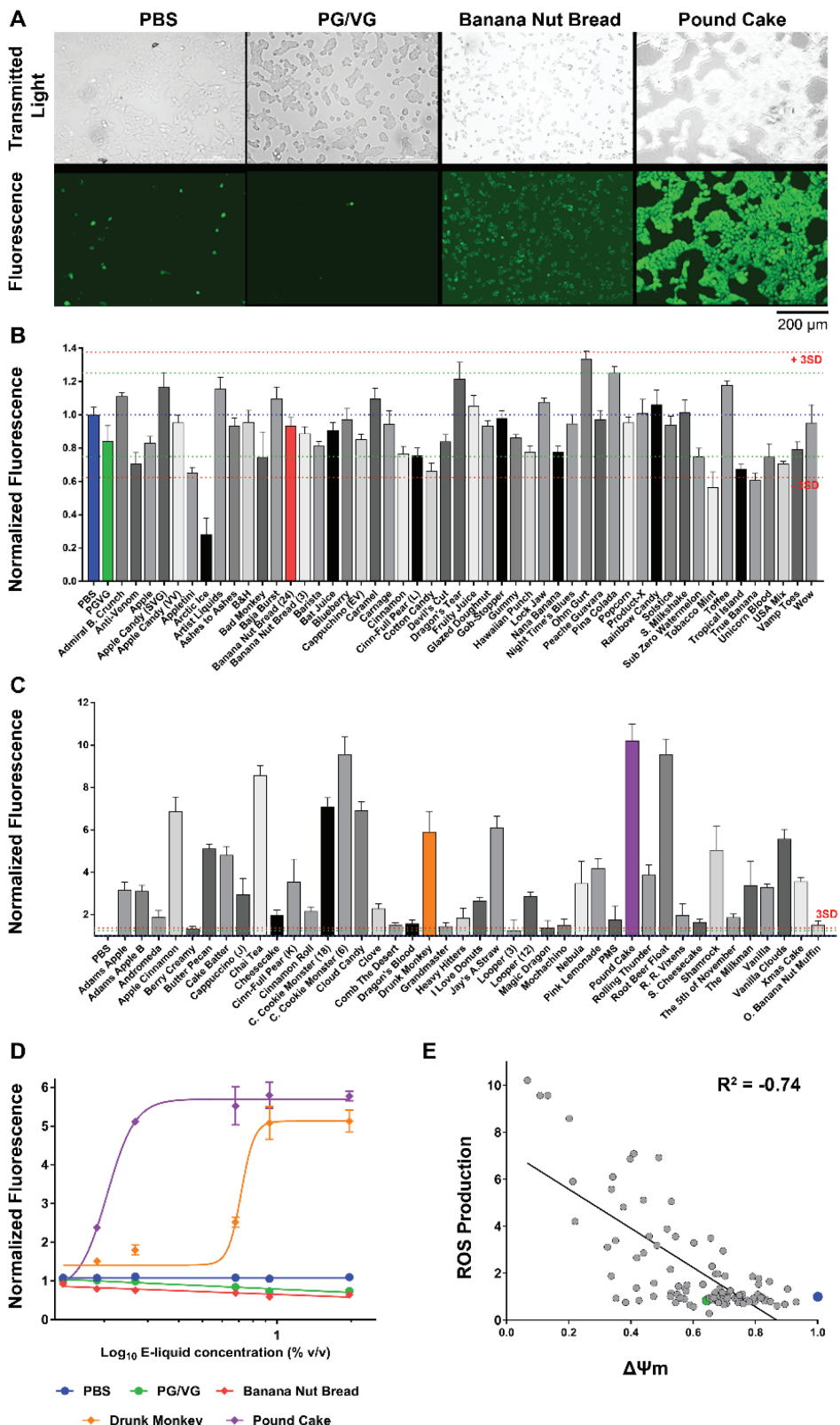
Figure 3. E-liquids effect on ROS production in HEK293T. (A) Transmitted light and fluorescent images (DCFDA stain) for PBS (control), PG/VG (e-liquid base), and representative flavors. (B and C) Primary screen of 98 e-liquids (1% vol/vol). Values normalized to PBS response (blue dotted line). PG/VG is shown in green. Green and red dotted lines show average response ± 2 SD or 3 SD, respectively. $N = 8$. (D) Dose–response curves for PBS (blue), PG/VG (green), and representative e-liquids. (E) ROS production versus $\Delta\Psi_m$ showed a negative correlation ($R = -.74$; $p < .0001$). PG/VG (●) and PBS (●) values are highlighted. All $n = 9$ per group.

for PG/VG, Banana Nut Bread, Drunk Monkey, and Pound Cake. We then analyzed the relationship between ROS production versus $\Delta\Psi_m$, and we found that these are negatively correlated ($R = -.74$; $p < .0001$; Figure 3E).

E-Liquids Depolarize V_m

Several reports have shown a relationship between ROS production and V_m depolarization, so we followed our ROS production findings with V_m assessments.[17,18] An appropriate resting V_m is critical for cell survival. V_m is typically approximately –70 mV, and persistent depolarizations are an early indicator of apoptosis.[19] We therefore investigated the effects of e-liquids on V_m by measuring changes in fluorescence. After incubating HEK293T cells with e-liquids, V_m was measured before ($V_{m\text{-}pre}$) and after ($V_{m\text{-}post}$) adding KCl, which induced membrane depolarization. Before adding KCl ($V_{m\text{-}pre}$), several e-liquids ($n = 39$) caused significant increase in fluorescence (above 3SD compared with the control), indicating spontaneous depolarization compared with the PBS control (Figure 4A and B). After KCl addition ($V_{m\text{-}post}$), PBS-treated cells showed depolarization, as expected, whereas 22 e-liquids showed a decrease in $V_{m\text{-}post}$, suggesting that these e-liquids induced repolarization of V_m or, at a minimum, those e-liquids prevented the cell membrane to depolarize after KCl exposure (Figure 4A and B). E-liquids that showed an effect in the primary assay were used to exposed cell to a range of doses (Figure 4A and B). PG/VG induced a dose-dependent increase in both $V_{m\text{-}pre}$ and $V_{m\text{-}post}$ (Figure 4C and D). To assess the effect after spontaneous depolarization by e-liquids, we plotted $V_{m\text{-}pre}$ and ΔV_m ($V_{m\text{-}post} - V_{m\text{-}pre}$) (Supplementary Figure S1). By comparison to PBS (normal V_m), e-liquids aligned into three distnt groups: (1) depolarization after KCl exposure (normal V_m, like PBS control); (2) spontaneous depolarization and no polarization after KCl exposure (compared with PBS control), and (3) spontaneous depolarization and hyperpolarization due to KCl addition (Supplementary Figure S1, red, blue, and green, respectively).

E-liquid Chemical Composition Is Varied

Gas chromatography–mass spectrometry was used to identify e-liquids chemical constituents, as described.[7] Chromatograms obtained using this approach were compared with the NIST mass spectral database for compound identification. As shown in Supplementary Figure S2, distinct peaks, corresponding to unique chemicals, were readily identifiable. Indeed, a total of 128 different chemicals were identified in the 92 e-liquids (Supplementary Table S3). However, only 30 chemicals were present in more than 10 e-liquids.

Analysis of Chemical Composition in relation with E-Liquids Toxicity

We analyzed the relationship between chemical composition and cellular effects using PCA. The first dimension PC1 included the original variables: $\Delta\Psi_m$, ROS production, live/dead ratio, and LC_{50} values. The correlation or redundancy value was .75, and the contribution of the variables was 23%. This dimension showed that $\Delta\Psi_m$ and ROS were inversely associated with toxicity (live/dead ratio), whereas live/dead ratio were directly associated to LC_{50} values (Supplementary Figure S3). The analysis also separated e-liquids according to their toxicity within these traits, with the most toxic e-liquids being on the left side of the axis (more negative axis values) and the least toxic on the right side (more positive axis values)

(Supplementary Figure S3). The second dimension, or PC2, represented e-liquid toxicity according to the cellular membrane potential change after adding KCl ($V_{m\text{-}post}$) or ΔV_m ($V_{m\text{-}post} - V_{m\text{-}pre}$). These variables had a correlation of .73, and their contribution to variability was 50%. This dimension showed a direct correlation between these two traits; it ordered e-liquids from most toxic at the bottom of the axis to least toxic toward the top. The two first PCA dimensions accounted for 54% and 21%, respectively, of the total variance in e-liquids toxicity in all cellular traits (Supplementary Figure S3).

The non-metric multidimensional scaling ordination of the e-liquids showed an acceptable stress level of 0.20 (>0.3 is a poor representation of the data). The ordination of PC1 indicated a clear separation between two groups of chemicals that show below or above toxicity score when compared with the average number of chemical components present in each e-liquid (Supplementary Figure S3). To compare e-liquids grouped according to traits, we used PERMANOVA to define the space between the groups' centroids and dispersion. The centroid is defined as the mean location of all data points and dispersion accounts for the variability in measured space from the centroid.[20] Analysis of PC1 showed a significant difference between the two groups regarding their location ($p = .0066$) and dispersion ($p = .026$), thus revealing that the least toxic group is more variable than the other. On the other hand, the same analysis of PC2 did not show significant differences ($p = .60$ and .61, respectively). These results could tentatively explain that the number of chemical components in an e-liquid could be directly related to its toxicity.

Finally, a linear model was used to assess toxicity ranking scores according to the presence and/or absence of certain chemical components in a e-liquid formulation. The model used 6 degrees of freedom (independent variables) in the analysis and had an adjusted R^2 value (goodness of fit for this model) of .401 and a p-value lower than .001. With this analysis, we got a final model that best explained the variability in toxicity among e-liquids. We identified five chemicals that significantly increased the toxicity score (PC1) when present in e-liquids (Supplementary Table S2). These chemicals and their % presence in e-liquids were: ethyl-vanillin and vanillin, 33% incidence; benzyl alcohol, 31%; acetoin, 19%; cinnamaldehyde, 10%; and methyl-cyclopentenolone, 8%. Supplementary Table S2 shows a comprehensive list of chemical compounds that showed increased toxicity in e-liquids. For each compound, we have included its flavor category.

Discussion

Increasing evidence shows that exposure to e-cigarette vapor in human models affects pulmonary and vascular functions,[21] which correlate with e-liquids altering a variety of human cells in vitro.[1,6,22] Several studies have shown that e-cigarette vapors trigger apoptosis and necrosis in human endothelial cells, epithelial cells, and alveolar macrophages.[23–25] Human airway epithelia showed decreased ciliary beating, immune defense, and ion channel conductance; increased levels of oxidative stress via amino acids and ADP levels; and apoptosis when exposed to e-cigarette vapors.[26–28]

The transmembrane potential of the mitochondria plays an important role in cell homeostasis as it drives the production of ATP in this organelle. We found that 35 of the e-liquids tested induced a significant decrease in $\Delta\Psi_m$ compared with the PBS control ($p < .05$). Moreover, eight e-liquids showed a greater inhibitory effect than the respiratory chain uncoupling agent, CCCP, suggesting that
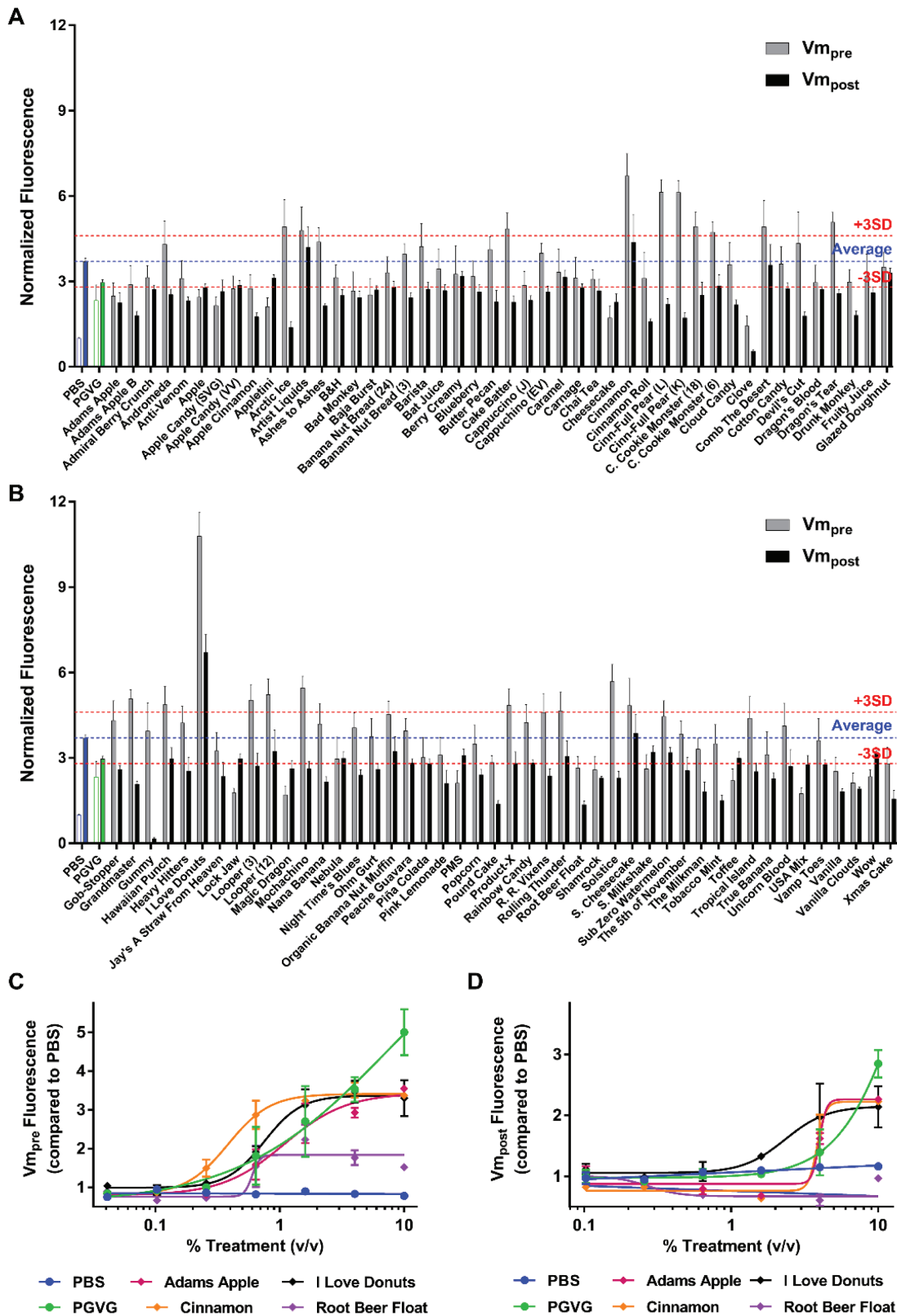
Figure 4. E-liquids effect on V_m. (A–B) Primary screen of 92 e-liquids (1% vol/vol). Values normalized to $V_{m\text{-pre}}$ PBS response. Blue dotted lined represents average PBS $V_{m\text{-post}}$. PG/VG is shown in green. Red dotted lines show average $V_{m\text{-post}}$ response ± 3 SD. $N = 8$. (C) $V_{m\text{-pre}}$ dose–response curves for PBS (●), PG/VG (●), and representative e-liquids. (D) $V_{m\text{-post}}$ dose–response curves for PBS (●), PG/VG (●), and the same representative e-liquids. $N = 4$ per group.

particular flavors, contaminants, or additives may contribute to cellular toxicity by impairing mitochondrial function. Independent of flavors, some authors have reported that e-cigarette vapors disturb the mitochondrial respiration complexes III and IV in human myofibroblasts and primary lung fibroblasts, respectively.[29,30] A recent study showed that e-cigarette aerosols induce mitochondrial depolarization in vascular endothelial cells in a similar way as cigarette smoke.[31] Mitochondrial dysfunction is increasingly recognized as a key component in acute and chronic cellular stress and we propose that screening for e-liquid effects on $\Delta\Psi_m$ become standard due to the importance of this measure.

Mitochondrial respiration chain inhibition leads to the formation of ROS.[32] We have reported that 64% of the e-liquids tested, induced a significant change in cellular ROS levels in a concentration dependent manner. PG/VG (55:45) did not induce ROS production suggesting that it is not responsible for this effect. Conceivably, it is a combination of nicotine, flavoring chemicals, and/or nonflavor components of e-liquids that cause changes in ROS production. Some studies showed that unvaporized e-liquids were oxidative, depending on the presence of PG and glycerin[33] and that some flavored e-cigarette aerosols increased ROS independently of nicotine.[34] Altered ROS in the lung can lead to the increased expression of proteins that regulate $\Delta\Psi_m$. In turn, these changes can cause fibrosis, inflammation, structural damage, promoted airway remodeling, and bronchial hyperresponsiveness, which are involved in the development of asthma, chronic obstructive pulmonary disease, and lung cancer.[35–37] Lipid-soluble components in cigarette smoke induce mitochondrial production of ROS in lung epithelial cells.[32]

Regarding the effects of e-liquids on V_m, 42% of tested e-liquids induced a decrease in V_m followed by a lack of response to KCl, suggesting that they had caused spontaneous depolarization. In most cell types, the plasma membrane Na⁺/K⁺-ATPase establishes the ionic gradients, and then K⁺ channels are responsible for determining the resting V_m, due to K⁺'s Nernst potential across the plasma membrane.[38] It is currently unclear whether the observed effects on V_m were due to alterations in the Na⁺/K⁺-ATPase or K⁺ channels. However, given that we observed a depolarization with extracellular K⁺ addition that was diminished after e-liquid addition; these data suggest that K⁺ channel activity has been altered. E-liquids have previously been shown to affect ion channels. For example, the cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel was affected by both nicotine and flavors.[39] Moreover, transient receptor potential channels, which are nonselective cation channels, can be directly stimulated by certain flavors like menthol[40] and more recently have been shown to be activated by nicotine.[41] An appropriate V_m is important for multiple aspects of cell homeostasis. For example, if the cell is chronically depolarized, this would disrupt influx of the key second messenger Ca²⁺. Moreover, dysregulation of ionic gradients and V_m could alter intracellular ion concentrations, which could have ramifications for everything from cell growth to lysosomal functions.[42,43] However, more testing is needed to validate these results and to elucidate the underlying mechanism.

The e-liquids tested in this study also caused cell death at higher concentrations. However, both their LC_{50} and their chemical constituents greatly varied, suggesting that e-liquid composition has a direct impact on toxicity. In agreement with these results, other studies revealed that chemical composition had an influence on the extent of human endothelial cells apoptosis.[44] Here, we identified flavoring compounds that were associated with $\Delta\Psi_m$, ROS production, and cell death. As an example, ethyl-vanillin has been identified

as one of the most common flavoring compounds used in e-liquid formulation with concentrations ranging from 0.5 to 335 mg/mL.[45] Ethyl-vanillin is known as a potent agonist of several transient receptor potential channels in rat neurons, leading to inflammation.[46] Moreover, methyl-vanillin, which is widely found in e-liquids, increases ROS production, promoting inflammation and cell death in human monocytes and endothelial cells.[47,48] As a whole, there is sufficient evidence that the "vanillin" family of flavoring compounds is involved in cell apoptosis; consequently, these class of chemicals should be banned from e-liquid formulations.

Acetoin, also related with toxicity according to our study, was identified in vapor mixtures associated with "Popcorn workers' lung,"[13,49] an obstructive pulmonary disease, and it has been classified as a "High Priority Substance" in the Federal Emergency Management Agency (FEMA) report on respiratory health and safety in the food manufacturing workplace.[50] In addition to its use in the food industry, this compound is one of the most prevalent flavors in e-cigarettes, being detected in e-liquids at up to 529 μg per e-liquid vial.[14] In agreement with our results, it has been recently found that acetoin increases ROS levels in human monocytic cell lines leading to inflammatory responses and that this effect is independent of nicotine presence.[48] Similarly, we did not find an effect due to nicotine presence in our model.

We found benzyl alcohol was present in 31% of our samples. In support of our results, other studies have reported that this chemical reduces $\Delta\Psi_m$ and induces cell death when applied in high doses to mice and human hepatocytes, respectively.[51] Last, methylcyclopentenolone (coffee flavor) has been found in e-liquids although to our knowledge this is the only study that has evaluated its cytotoxicity in e-liquids.[52] Benzyl alcohol is known to cause respiratory failure, vasodilation, hypotension, and convulsions in adults[53] and gasping syndrome in neonates,[11,54] and methyl-cyclopentenolone was deemed toxic by the European Union.[55] Chemical diversity in e-liquid formulation is a key component of e-liquid safety.

We recognize that our study presents a limitation as the e-liquids were tested neat and not vaped. Several researchers have now found evidence of chemical transformation (ie, pyrolysis) after vaping.[56,57] In agreement with our previous studies,[7,58] we did not find that vaping e-liquids changed their relative toxicity. Indeed, we have found that vaping is correlated with direct e-liquid addition in several pulmonary cell types,[7] suggesting that this phenomenon may be flavor dependent. In our experience, vaping is more variable and less amenable to HTS. We have also reported that e-liquids have a LC_{50}s of ~6% or less; this would suggest that e-liquids may reach biologically relevant levels in the lung. Indeed, it has recently been reported that vaping significantly alters the secreted human airway proteome, suggesting that this may be the case.[59] In addition, we recognize that our approach uses HEK293T cells and not air–liquid interface cultures of pulmonary epithelial primary cells or cell lines. With over 8000 different flavors on the market, we believe that it is not be possible to study all e-liquids under vaped/"physiological conditions." We have previously demonstrated that HEK293T cells are suitable to compare the cytotoxic effects of many e-liquids in a quick and reliable way that is representative of the toxicity induced in pulmonary cell types, including air–liquid interface epithelia.[7] Other groups have reported cytotoxicity of e-liquids, some using air–liquid interface cultures, but their screening capacity was 40 e-liquids or less.[8,39,60] Our study provides a first step to identify toxic effects of e-liquids that would require further investigation.

A founding principle of toxicology is "the dose makes the poison." This study was FDA funded, and knowing the upper limit of toxicity will be useful to the FDA even if it is not achieved during normal vaping. Moreover, although every e-liquid is toxic at high doses, individual e-liquids are differentiated based on the LC_{50} and whether or not the curves are left-shifted relative to PG/VG. Indeed, as per Figure 1, "Devils' Cut" has an LC_{50} of ~0.1%, whereas PG/VG has an LC_{50} of ~10%.

Diversity in flavor composition is one of the main marketing strategies practiced by e-liquid vendors. The appeal of new flavors creates collateral consequences, including nicotine addiction.[61] The US FDA has been taking steps to regulate e-cigarette sales: first by deeming electronic nicotine delivery systems to be tobacco products and thus including them in 2009 Family Smoking Prevention and Tobacco Control Act, and subsequently by issuing an "Advanced Notice of Proposed Rulemaking" that gives importance to the regulation of flavors in tobacco products.[62] Our study provides new cytotoxic information of a varied and representative sample of flavored e-liquids, and importantly, we have incorporated this information into our publicly available database (https://eliquidinfo.org/). This website allows searching the LC_{50} value, chemical composition, PG/VG ratio, and vendor information for 320+ e-liquids, and thus, it can inform users, academic researchers, and government researchers. Remarkably, we have revealed that the presence of specific flavors is associated with higher levels of toxicity regarding oxidative stress, $\Delta\Psi_m$, and cell death. Thus, given their heterogeneous nature, the overall goal of this project was to screen a greater number of neat e-liquids to identify flavors and/or chemical constituents that are more toxic and prompt new research on the potential effects of flavor chemical components on human health in order to limit their use on e-liquid formulation.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

Funding

Declaration of Interests

None declared.

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