clear translocation of NF-κB were not increased, although pro-inflammatory genes were upregulated. However, another inflammation-related transcription factor, activation protein 1 (AP-1), was induced by AqCSE. Gene classification analysis suggested that induction of the inflammatory response by AqCSE was dependent on NRF2 and AP-1 rather than NF-κB.

1928 Developing a Versatile Exposure System for the Analysis of the Effects of Electronic Cigarettes

G. E. Nipp, Y. Escobar, I. Jaspers, and J. Surratt. *University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Use of electronic cigarettes (e-cigs), particularly among youth, has proliferated in recent years. However, there remains little consensus on e-cigs' health effects. With the plethora of devices, e-liquids, and flavorings available on the US market, there is a need for a controlled yet flexible *in vitro* exposure system which can accommodate the versatility of devices. This standardized exposure system will allow for more meaningful comparison between e-cig devices, e-liquid components, and devices operated at varying settings. The goal of this study was to develop and optimize a versatile *in vitro* e-cig exposure system and perform initial experiments to determine biological effects. Our exposure chamber is composed of a 3 L Plexiglas cube with an inlet for e-cig operation, an outlet attached to a vacuum line, and a fan for aerosol distribution. A series of mock exposures using a third-generation e-cig device at a flow rate of 2.5 LPM were performed in order to determine chamber parameters which yield even aerosol deposition while minimizing gas loss and disruption of aerosol nucleation. Average aerosol deposition across the 12 well plate was found to be 1.634 mg/cm^2 for propylene glycol (PG), 1.600 mg/cm^2 for glycerin (GLY), and 1.707 mg/cm^2 for a 55:45 PG/GLY mixture with no significant difference in deposition between the wells of the plate. Initial applications of this exposure chamber included exposure of human bronchial epithelial cells to vaped PG and GLY at ratios of 100% PG, 55:45 PG/GLY, and 100% GLY. PG and GLY are universal components of e-liquids, with 55:45 being a commonly used ratio among e-cig users. *IL-6* and *IL-8* transcript levels were analyzed in the exposed cells at varying time points. Our results indicate that exposure to PG aerosols increased *IL-6* and *IL-8* transcript level at 2 hours post-exposure, suggesting an inflammatory response. Liquid Chromatography interfaced to Electrospray Ionization Mass Spectrometry is used to analyze the aerosol composition. The results of this study describe a novel and versatile *in vitro* e-cig exposure system that will allow for a controlled, replicable exposure to a variety of different e-cig aerosols, thus producing meaningful *in vitro* assessment of e-cig toxicity.

1929 Analyzing the Cellular Stress Response in Airway Epithelial Cells to Vaporized Propylene Glycol and Glycerol

Y. H. Escobar, G. Nipp, J. Surratt, and I. Jaspers. *University of North Carolina at Chapel Hill, Chapel Hill, NC.*

In the United States the use of electronic cigarettes (e-cigs) has surged since its introduction to the market in 2007. Despite previous claims that e-cig use is 'safer' than traditional cigarette use, there is insufficient data to determine if e-cig generated aerosol itself is safe. To further compound the problem of determining the safety of e-cigs is the sheer number of variations in e-cig use: e-cig device type, wattage or temperature settings, flavorings and nicotine concentrations. However, one standard component of e-cigs, is its use of the humectants propylene glycol (PG) and glycerol (GLY). All e-cig devices heat the humectants to the point of vaporization, which can cause the formation of aldehydes and free radicals. The goal of this study is to determine the health effects of vaporized PG and GLY on airway epithelial cells. We exposed airway epithelial cells (16HBE cells) at air liquid interface to the vaporized humectants: 100% PG, 100% GLY, and a PG/GLY mixture at a 55/45 (v/v) ratio, at low (45W) and high (80W) wattage settings on the e-cig device. Each exposure consisted of 20 puffs at a flow rate of 2.5 LPM. Our data demonstrate that 100% GLY at the 80W setting caused a significant upregulation of cellular stress related gene heme oxygenase 1 (HMOX-1) and HO-1 protein, while 100% PG increased the expression of NAD(P)H Quinone Dehydrogenase 1 (NQO1) and the corresponding protein. These responses were not observed in 16HBE cells exposed to the aerosol generated at the 45W setting of the e-cig device. Additionally, we repeated the 80W exposure with primary airway epithelial cells from non-smokers and smokers. The 100% GLY exposure caused an upregulation of HMOX-1 in the cells from non-smokers but not smokers. Furthermore the PG/GLY exposure caused an upregulation of glutamate-cysteine ligase catalytic (GCLC), which is the rate limiting step of glutathione synthesis, in non-smokers and not smokers. These data suggest that aerosols generated from e-cig devices at high temperatures, regardless of flavoring content, are likely to cause cellular stress responses. Additionally non-smokers are likely to experience more cellular stress from e-cig use than smokers.

1930 Effects of E-cigarette Flavoring Chemicals on Human Macrophages and Bronchial Epithelial Cells

A. Morris[1,2], N. Olgun[1], K. Attfield[3], J. Fowles[3], and S. Leonard[1]. *[1]NIOSH, Morgantown, WV; [2]West Virginia University Health Sciences Center, Morgantown, WV; and [3]California Department of Public Health, Richmond, CA.*

E-cigarettes are a relatively new and popular alternative to tobacco cigarettes used by many young adults and teens. Due to their novelty, the respiratory health effects of e-cigarette flavoring components are not well understood. There are thousands of e-cigarette flavors available on the market, many of which have the potential for toxicity. The majority of e-cigarette flavoring chemicals (ECFCs) have not been tested for inhalation safety. In this study, we used pulmonary-associated cell lines to assess the *in vitro* effects of thirty ECFCs to determine their potential cytotoxicity at various concentrations. The ECFC vehicles, propylene glycol (PG) and vegetable glycerin (VG), were tested individually and as mixtures that mirrored common ratios found in e-liquids (50/50 and 30/70 PG/VG, respectively). Cultured human monocytes (THP-1) were differentiated into a macrophage phenotype with vitamin D_3 before treatment. Cultured human bronchial epithelial cells (BEAS-2B) and differentiated macrophages were treated with 10, 100, and 1000 μM of ECFC and analyzed for cytotoxicity and inflammatory markers, including changes in viability, cell membrane damage, reactive oxygen species (ROS) production, and inflammatory cytokine release. The ECFCs that caused the most cell death in both cell types (eugenol, linalool, and nonanol) were primarily classified as hydroxyls. A number of aldehydes (cinnamaldehyde, decanal, and trans-2-hexen-1-al) also caused significant cell death. Cell membrane damage, as measured by lactate dehydrogenase release, was elevated in both cell lines after treatment with eugenol, linalool, and nonanol. Decanal also caused membrane damage to BEAS-2B cells, while vanillin was damaging to THP-1 cells. Vanillin elicited high amounts of ROS from both cell lines, with the BEAS-2B also producing ROS after exposure to diketones (2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanedione). These findings provide insight into the potential tissue damage that e-cigarette users are at risk for and provide a basis for future experiments with ECFC exposures.

1931 Systems Toxicology Assessment of a Representative E-liquid Formulation Using Human Primary Bronchial Epithelial Cells

D. Marescotti, C. Mathis, V. Belcastro, S. Acali, D. Peric, R. Dulize, D. Bornard, E. Guedj, M. Biasoli, F. Frauendorfer, D. Sciuscio, M. Esposito, N. V. Ivanov, M. C. Peitsch, and J. Hoeng. *PMI R&D, Philip Morris Products S.A., Neuchatel, Switzerland.*

Cigarette smoke (CS) is a risk factor for respiratory and systemic diseases, smoking cessation remains the most effective approach for risk reduction. Innovative products with the potential to reduce the risk of smoking related diseases are being developed with the aim to provide a better alternative to smokers who would otherwise continue to smoke. Literature suggests that e-vapor products likely have a significantly lower risk profile than cigarettes. This is based on the notion that chemical composition of e-vapor aerosols is much simpler than that of CS, and ingredients are generally regarded as safe given their use in the food industry. Yet, data available for safety assessment in the context of inhalation toxicology are limited. A list of flavors used in e-liquids was grouped based on common physicochemical properties and available toxicological data, as defined by the European Food Safety Authority. Within each group, at least one representative flavor was selected to create a mixture, with 28 representative flavors dissolved in a matrix containing 41% propylene glycol, 38% vegetable glycerin, and 0.6% nicotine. We evaluated the effects of exposing normal human bronchial epithelial cells in submerged condition to 1) the e-liquid/flavor mix and 2) the 28 individual flavors. Finally, new mixtures were generated by selectively removing those flavors exhibiting the largest cytotoxic effects. Each e-liquid solution was tested over a 24-hour period using a real-time, impedance-based assay. Phenotypic effects were further evaluated using a battery of high-content screening endpoints. For additional mechanistic insights, effects of the flavor mixture and its corresponding matrix were investigated using gene expression analysis combined with a computational approach leveraging mechanistic network models to identify and quantify perturbed molecular pathways. The 28-flavor mixture (flavor content ≥5%) was more cytotoxic than the matrix following a 24-hour exposure. Assessing each flavor individually, D-L-citronellol (0.48%)

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