**Electronic Cigarette Flavorings Alter Lung Function at the Cellular Level**

ATS 2015, DENVER — Certain flavorings used in electronic cigarette liquid may alter important cellular functions in lung tissue, according to new research presented at the 2015 American Thoracic Society International Conference. These changes in cell viability, cell proliferation, and calcium signaling are flavor-dependent. Coupling these results with chemicals identified in each flavor could prove useful in identifying flavors or chemical constituents that produce adverse effects in users.

“The effects of the various chemical components of e-cigarette vapor on lung tissue are largely unknown,” said lead author Temperance Rowell, a graduate student in the Cell Biology and Physiology Department of the University of North Carolina at Chapel Hill. “In our study using human lung epithelial cells, a number of cell viability and toxicity parameters pointed to 5 of 13 flavors tested showing overall adverse effects to cells in a dose-dependent manner.”

In the study, cultured human airway epithelial cells were exposed to various doses of the 13 e-cigarette liquid flavors for 30 minutes or 24 hours. During the 30 minute exposure test, the flavors Hot Cinnamon Candies, Banana Pudding (Southern Style), and Menthol Tobacco elicited a dose-dependent calcium response and were toxic to the cells at higher doses.

During the 24 hour exposure test, these same three favors decreased cell proliferation and cell viability in a dose-dependent manner.

The toxic effects of these flavorings were not seen with either nicotine or the e-liquid vehicle, which consisted of propylene glycol and vegetable glycerin.
Additional experiments testing the aerosolized product of e-liquid flavors on cultured primary human bronchial epithelial cells are ongoing. Flavors being tested were selected from the findings in this study.

“The specific chemical components underlying the toxic effects of these e-cigarette flavors on cell viability, proliferation, and calcium signaling in airway epithelia are undergoing further study in our lab,” said Ms. Rowell. “Given the increasing popularity of flavored e-cigarettes, a better understanding of their ingredients, the potential health risks of these ingredients, and the causes of these risks is urgently needed.”

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* Please note that numbers in this release may differ slightly from those in the abstract. Many of these investigations are ongoing; the release represents the most up-to-date data available at press time.

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Select E-Cigarette Flavors Alter Calcium Signaling, Cell Viability and Proliferation in Lung Epithelia

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Abstract Body

**Rationale:** Flavored e-cigarettes are becoming increasingly popular. However, little is known about their constituent chemicals or their effect on the pulmonary epithelia. We purchased 13 representative flavors of e-cigarette liquid from the Vapor Girl (http://www.thevaporgirl.com/) and tested their effects on airway epithelial calcium signaling, cell viability, and cell proliferation. We know that calcium homeostasis is deranged following tobacco exposure, leading to airway epithelial abnormalities. Since calcium is an important cell signal that regulates secretion, protein trafficking, cell division and death among other functions, we measured changes in cytoplasmic calcium levels following treatments with different flavors.

**Methods:** Calu3 cells were seeded into 96-well microplates. Cells were exposed to various doses of the 13 e-liquid flavors diluted in cell culture media for 30 minutes or 24 hours. Calcium signaling was measured using Fluo-4, a calcium indicator. Cell viability was assessed using LDH release, propidium iodide uptake, and trypan blue exclusion. Cell proliferation was measured using LDH release. Mass spectrometry was used to determine presence of chemical constituents in each flavor reported.
**Results:** Flavors such as Hot Cinnamon Candies, Banana Pudding (Southern Style), and Menthol Tobacco evoked a strong calcium response and cytotoxicity in higher doses during the 30 minute exposure. These same flavors also decreased cell proliferation and the ability of cells to respond to a pharmacological agent that releases internal calcium stores in a dose-dependent manner after the 24 hour exposure. Moreover, these effects were not reprised by nicotine or the e-liquid vehicle for chemical constituents (propylene glycol and vegetable glycerin).

**Conclusions:** Only select flavors of the 13 screened evoked alterations in calcium signaling, which could affect the changes in cell viability and proliferation that were also measured. There could be chemical constituents present in particular flavors that we can identify via mass spectrometry that affect calcium signaling, cell viability and proliferation in airway epithelia. In this way, we aim to determine which chemicals in flavored e-cigarette liquids are associated with toxicity.