Metals in Electronic Cigarette Aerosol
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Abstract:

Electronic cigarettes (ECs) use heat to aerosolize “e-liquid” mixtures, which we have found often contain high concentrations the flavor chemicals menthol (M-ol) and/or menthone (M-one), and cinnamaldehyde (CAD). This supplemental project will: a) identify and quantify reaction products formed by vaping e-liquids containing M-ol, M-one, and CAD; and b) determine which *in vitro* assays best assess toxicity of EC aerosols *and* which reaction products cause toxicity. Work will be conducted at two campuses with expertise in analytical chemistry (Portland State University-PSU) and human toxicology (University of California Riverside-UCR).
Aim #1 (PSU). Degradation products formed by pyrolysis/oxidation of M-ol, M-one, and CAD will be identified and quantified when vaped in propylene glycol (PG)/glycerol(GL) at concentrations ranging from 1 to 300 mg/mL (~0.1 to 30% by weight). Popular tank ECs will be used at high and low voltages, as defined by the manufacturers and in postings on user websites. Aerosols will be quantitatively collected in isopropanol (IPA) and analyzed using gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/MS/MS. Unknown peaks will be identified by comparison to: a) MS “libraries” and b) results with authentic standards. The formation of flavor chemical degradation products will be correlated with starting flavor chemicals and with formation of known PG and GL degradation products (acetaldehyde, acrolein, glycidol, etc.).
Aim #2 (UCR). Authentic standards of reaction products identified in Aim #1 in aerosolized EC fluids containing M-ol, M-one, or CAD will be screened for cytotoxicity in dose response experiments using the MTT assay in conjunction with BEAS-2B lung epithelial cells from normal human adults. Lowest adverse effect levels, no adverse effect levels, and IC_{50} values will be used to identify those reaction products that are most cytotoxic. Cytotoxicity of flavor degradation products will be tested individually, and, in search of synergistic effects, together with other major flavor degradation products and with PG and GL degradation products.
Aim # 3 (UCR). M-ol, M-one, CAD, authentic standards of the most cytotoxic chemicals (Aim #2), and PG/GL degradation products will be tested *in vitro* using a multiplexing air-liquid interface (ALI) system. BEAS-2B cells (monolayer) or 3D human primary bronchial epithelium will be exposed at an ALI using a system that produces aerosols without heating to eliminate concern that heating will alter test chemicals. Assays will examine: 1) survival, 2) stress protein synthesis, 3) inflammation, 4) ROS production, 5) ATP production, and 6) DNA damage. Data will establish the relative cytoxicity of each chemical in six mode-of-action assays and identify those assays that are best for detecting toxicity of flavor chemicals/reaction products using a lung model.
In summary, while focusing on prevalent and high concentration flavor chemicals, this project will identify and quantify flavor chemical degradation products produced during EC heating and determine which in vitro assays are capable of examining the harm potential of these flavorings in ECs.