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The Importance of Conventional Toxicological Metrics of Aerosol Characterization

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E-cigarettes are new but rapidly evolving class of products on the U.S. market. When Food and Drug Administration's Center for Tobacco Products (FDA CTP) was given regulatory authority over all e-cigarettes on August 8, 2016, it began to generate and utilize aerosol science to effectively regulate this evolving class of tobacco products. We believe that, for the FDA CTP to utilize independent e-cigarette aerosol research data (ie, data created by entities other than FDA) in its scientific review process, independent researchers must return to complete, conventional metrics of aerosol characterization.

Early research comparing e-cigarette aerosol exposures with combustible cigarette smoke exposures in nonclinical and clinical studies justifiably focused on the internal dose of nicotine as a principal means of balancing exposures between e-cigarette aerosols and combustible cigarette smoke. Humans self-medicate and therefore titrate to an effective dose of nicotine (Benowitz, 2009). However, as many research studies are now focused on identifying potential health effects components of e-liquid aerosols, tetrahydrocannabinol (THC), and cannabinoid (CBD) inhalation products (ie, not in comparison with tobacco combustion products), greater attention is needed to understand dosimetry, chemical uptake, and biological interactions of the varied substances used in vape devices. Cell culture models add important complexities for dosimetry such that simple measurements of "puffs" are uninformative in terms of actual cellular dose. Many recent publications fall well short of comprehensive characterization of exposures, which limits the rigor, reproducibility, generalizability, and value to other labs, regulatory agencies, and assorted stakeholders of such research.

We propose that the following measures should be considered for *in vivo* and *in vitro* exposure systems to effectively characterize e-cigarette aerosols and combustible cigarette smoke to appropriately understand exposure and dose:

1. **Aerosol mass concentration characterization.** Gravimetric determination of aerosol mass concentration is recommended as a minimum characteristic for each exposure period. Contemporary methods of reporting only "number of puffs" are highly inadequate to compare doses across systems or even across devices within the same system because air flows and chamber volumes differ across systems. Ideally, a real-time (eg, DustTrak or similar product) and cumulative (ie, gravimetric) aerosol mass characterization would be determined for all exposures. The real-time analysis is essential to understand the peak concentrations while gravimetric (filter collections) will provide a cumulative exposure.
2. **Aerosol/particle size characterization.** Characterizing the aerosol particle size at the point of inhalation is valuable to ensure comparable deposition. Respiratory deposition of aerosols is largely dependent on size, with smaller droplets penetrating deeper into the lungs than larger droplets, while particles larger than 5 μm may have limited respiratory deposition. E-cigarette devices that do not allow consumers to change device parameters consistently produce a very small droplet. Our inhalation labs routinely see the output in the 80–140 nm range. However, condensation and agglomeration can occur with longer residence times in an exposure chamber, depending on the distance from the droplet generation to the point of inhalation. Because the aerosol droplet size produced by most e-cigarettes is consistently small, routinely used inhalation systems may not need frequent

size distribution analysis (such as, on a daily basis). However, changes in the e-cigarette device, vehicle, or other changes to the exposure system would necessitate a revalidation of aerosol droplet size at the point of inhalation.

3. **Aerosol harmful and potentially harmful gas and nongas phase constituent characterization.** The physicochemical properties of an e-cigarette aerosol will be influenced by the e-liquid composition, including nicotine, the humectant ratios, the presence of flavoring chemicals, the pH, as well as by the power output of the device (Gholap *et al.*, 2020). The resulting ENDS aerosol may be a mixture of particle and gas phases, with the latter containing several volatile organic compounds and semivolatile organic compounds, such as acetaldehyde, formaldehyde, and acrolein, which are known respiratory irritants (Eshraghian and Al-Delaimy, 2021). Additionally, overheating of the e-cigarette coils may increase metals in the aerosol (Gray *et al.*, 2022). It is thus valuable to understand the operational limits of the device to be tested and to analyze chemical constituents especially *after* the aerosolization during the initial characterization of a given exposure system.
4. **Aerosol uptake (pulmonary function)**. While more challenging, measuring pulmonary function in real-time is arguably the most valuable tool to understand and control dosimetry. Pulmonary function measurements in preclinical models allow better estimates of the pulmonary uptake of vaped substances and thereby support a more accurate assessment of dose-response. Laboratory rodents are notable for their ability to alter respiration to reduce internal exposure to toxicants (Heck *et al.*, 2002); as novel vape ingredients or specific conditions (eg, subohm vaping) may generate irritant chemicals, mice may downregulate respiration and thereby provide a lower-than-expected toxic outcome. Pulmonary function testing can be optional or complementary if the ENDS aerosol internal dose is assessed.
5. **Aerosol internal dose (exposure biomarkers).** Rodents, as a major model for preclinical research, have anatomical and physiological differences from humans that can impact the relative internal uptake of ENDS aerosols. Rigorous assessment of internal dose can bridge this interspecies gap. An ideal scenario would be to accurately measure the inhaled chemicals in the lung tissue and systemically (eg, in plasma or target organs). Many studies only measure urinary cotinine, which has tremendous value in comparing e-cigarette exposure with tobacco smoke. But again, as many studies are exploring the health impacts of the other ingredients (eg, propylene glycol, vegetable glycerin THC, and CBD), cotinine use alone has limits. In addition to cotinine, volatile organic compounds biomarkers of ENDS exposure are also of significant interest. A more accurate assessment of internal dose will have tremendous value for comparing toxicity across products and among different laboratories.

The 2019 E-cigarette and Vaping-Associated Lung Injury scare caused by counterfeit THC vaping products containing vitamin E acetate (Ghinai *et al.*, 2019) was a sentinel event that highlights the need to properly evaluate the toxicity of new products and ingredients, as well as to better understand long-term consequences of vaping. However, in order to properly couch results as well as to compare findings across studies and laboratories, greater attention to fundamental exposure characterization is needed.

DECLARATION OF CONFLICTING INTERESTS

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