ASSESSMENT OF IN VITRO TOXICITY/GENOTOXICITY OF ENDS AND COMBUSTIBLE PRODUCTS

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Abstract

In May 2016, the U.S. Food and Drug Administration Center for Tobacco Products (CTP) issued draft guidance for Premarket Tobacco Product Applications (PTPA) for Electronic Nicotine Delivery Systems (ENDS), which requires inclusion of in vitro toxicology testing (e.g., genotoxicity and cytotoxicity studies). Specific in vitro toxicological testing, including assays/protocols outlined in the International Conference on Harmonization (ICH) S2R and Organization for Economic Cooperation and Development (OECD) guidelines. The draft guidance also recommends testing multiple test article concentrations in each assay, and using comparator products for hazard identification. We present an example of in vitro toxicology testing for ENDS/PMTA.

Key References


Results

Figure 1: Examples of positive response with market combustible vs. example ENDS (ENDS-1) in Ames TA98 (+S9), NRU and MN assays. The ENDS-1 aerosol and TPM were as toxic as the positive control PCB-56 and generated as described above.

Materials and Methods

Generation/Preparation of Test Materials

Seven different flavor variants of a single ENDS product were assessed under GLP conditions at three separate comparators generated by Dr. Bombick's lab. ENDS products were compared to four different test materials from each flavor variant were used for the in vitro assessments, including: aerosol, and negative control. Theophylline was included as a positive control. The ENDS products were incubated in the test systems for 24 to 48 hours. The ENDS aerosol was generated in vitro with existing test equipment. Theophylline was included as a positive control. The ENDS products were incubated in the test systems for 24 hours. The ENDS aerosol was generated in vitro with existing test equipment. The ENDS products were incubated in the test systems for 24 hours. The ENDS aerosol was generated in vitro with existing test equipment. The ENDS products were incubated in the test systems for 24 hours. The ENDS aerosol was generated in vitro with existing test equipment.

Introduction

Electronic nicotine delivery systems (ENDS), also termed e-cigarettes, typically contain e-liquids with the following components: nicotine, flavoring, propylene glycol, vegetable glycerin, and water. Since the liquid is vaporized and tobacco is not burned as with a traditional combustible cigarette, lower carcinogenic toxicants would be anticipated with ENDS due to the lack of precursors (Royal College of Physicians, 2011). ENDS aerosols have significant reductions in chemical constituents and in vitro toxicity compared to traditional cigarette smoke (National Academy of Sciences, Engineering, and Medicine, 2013). Therefore, ENDS represent great potential for reducing the harm traditionally associated with combustible tobacco products.

Many health-related effects of nicotine smoking have been linked to genetic and cell vulnerability changes. Several mechanisms underlie these changes, including DNA damage, cytotoxicity, and changes in chromosomal structure/function. For decades, standardised in vitro toxicological methods have been used to assess the potential genetic, mutagenic, toxicologic, and carcinogenic effects of ENDS aerosols and other traditional tobacco products. In recent years, external requirements or recommendations for assay battery selection (tobacco products have grown, including recommendations by the CORESTA Task Force (2004) on in vitro Toxicology (Ames mutagenicity assay, the Neutral Red Uptake (NRU) cytotoxicity assay and a mammalian genotoxicity assay [chromosome aberration, in vitro micronucleus genotoxicity assay, and the Neutral Red uptake (NRU) cytotoxicity assay]. In the NRU cytotoxicity assay, only one of seven different e liquids representing seven different flavor variants of the same ENDS product demonstrated positive results in one of the three tests.

Figure 2: Market combustible TPM but not ENDS aerosol samples demonstrate mutagenicity in Ames assay; all ENDS samples are negative.

Summary & Conclusions

• Across all of the in vitro assays conducted, a clear positive response was observed with market combustible TPM.
• A clear positive response was observed with market combustible GFU in the MN and MN assays, but not with the ENDS.
• For ENDS test articles, ranging in flavor strength, ENDS products under the experimental conditions tested. In contrast, with the exception of GVP in the Ames assay, the combustible market cigarette comparator was consistently positive, confirming that the assays are sensitive to potential toxicogeno...