A Method for Comparative In Vitro Hazard Analysis of e-Vapors to Inform Relative Hazards and Bridge Data

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Abstract

Standardized in vitro toxicology assays are useful tools by which comparative hazard assessments between electronic nicotine delivery system (ENDS) emissions and cigarette smoke or other tobacco product emissions can be made in the context of Premarket Tobacco Product Application (PMTAs). Studies to examine the cytotoxic, genotoxic, and mutagenic responses of mainstream gas vapor phase (GVP) and aerosol collected matter (ACM) from NJOY ENDS using a battery of established OECD assays (i.e., neutral red uptake, in vitro micronucleus, and bacterial reverse mutation) are negative compared to the positive responses from cigarette smoke. While factors such as product stability and minor device design change(s) within specifications have the theoretical potential to affect in vitro assay outcomes, we have developed a method that uses a comparative in vitro hazard analysis between tested products and the modified or aged products to determine if additional in vitro testing is warranted. A decision tree was developed to select the analytes among harmful and potentially harmful constituents (HPHCs) and leachables that pose potential hazards. A framework is established to assess the relative hazard of modified products in comparison to the tested products and combustible cigarettes. Specifically in vitro exposure estimates were interpolated by comparing the concentrations of the selected analytes measured from the modified products to the LED or HID which is necessary to elicit a positive response for the respective in vitro toxicity assays. Individual and cumulative hazard quotients (iHQx and ΣHQ) were calculated to determine the margin of exposure. If ΣHQ ≤ 1.0, risk is considered negligible to low, and no unacceptable effects are expected to occur in the exposed population. The in vitro exposure was also extrapolated to in vivo human cigarette particulate matter (PM) and ENDS ACM exposures following a worst-case scenario of PM dosimetry retained in the lung of a "heavy" ENDS user. As an illustrative case, one modified ENDS product was assessed following the framework and the overall in vitro ΣHQ was far less than 1. The highest concentrations used in the in vitro assay were from 294 to 1,052 times more PM than the daily exposure of a heavy cigarette smoker, and 125 to 720 times more PM than what a heavy user of ENDS would be exposed to daily. This assessment indicates that further in vitro toxicology testing is not warranted for the product evaluated. This method results allows an objective determination of when further in vitro toxicity testing is necessary to investigate the potential health impact of modified ENDS products in comparison to cigarette smoke.

Introduction

As a key component of product hazard assessment and regulatory submissions, a battery of in vitro toxicity assays was conducted on NJOY ENDS products via Ames assay, in vitro micronucleus assay, and cytotoxicity testing (e.g. NRU assay); all results were negative. The HPHCs in GVP or ACM and leachables in e-liquid were characterized and included in the cumulative risk assessments to inform the potential human health impacts of NJOY ENDS in comparison to reference cigarette data and other comparative products. The NJOY ENDS consistently demonstrated a significantly reduced health risk compared to combustible cigarettes and less than or similar risks to other ENDS products on the market when the comparative analyses were conducted. However, scenarios exist where not all iterations of the product were tested thus bridging or justification is necessary by leveraging existing testing data that could provide valuable information about the product. The objective of this project is to develop an approach that provides the framework to support product evaluation, leveraging existing empirical testing data and conclusions regarding the potential health impacts of NJOY ENDS, to avoid additional in vitro testing of modified or aged ENDS product (MAEPs). Key tools involved in consideration of framework development included: data on concentrations of HPHCs or leachables in MAEPs and original products, and the highest ineffective dose (HID) and lowest effective dose (LED) for key analytes with toxicity potential in the literature.

Methods

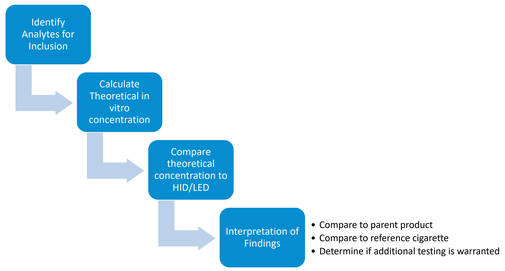
Scenarios that warrant the reevaluation of a modified and/or aged ENDS product (MAEP)

- Changes in aerosol composition over shelf life
- Changes in leachables over shelf life
- Changes in product design or composition

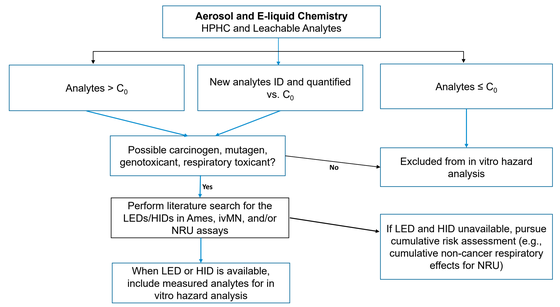
Highest ineffective dose (HID) and lowest effective dose (LED) Identification

- **Literature Search**
  - Searched databases of hazard information and supplemented with search of peer reviewed literature
  - Databases included: ATSDR toxicological profiles, OECD SIDS, ECHA Registered Substances Database, IARC Monographs, EPA IRIS, and WHO IPCS
- **Review of Values**
  - Identified highest ineffective dose (HID) and lowest effective dose (LED) for each substance of interest and assay
- **Selection of HID/LED**
  - LED = Lowest Dose to cause positive response in assay across studies
  - HID = Highest Dose to not cause positive response in assay; highest dose administered in studies that don't report positive responses

Evaluation Framework



Decision Tree for Analyte Selection for In Vitro Hazard Analysis



C_0 = Concentration of analyte at Time 0

Evaluation *In Vitro* Dosimetry Comparison to HID and/or LED

$$Max.\,in\,vitro\,analyte\,dose_x\left(\frac{\mu g\,X}{mL}\right) = measured\,analyte\,concentration\left(\frac{\mu g\,X_{Ti}}{\mu g\,ACM_{Ti}}\right) \times maximum\,in\,vitro\,ACM\,dose\left(\frac{1000\,\mu g\,ACM}{mL}\,or\,\frac{5000\,\mu g\,ACM}{plate}\right)$$

$$HQ_x = \frac{maximum\,in\,vitro\,dose_x}{in\,vitro\,LED\,or\,HID_x}$$

Overall *In vitro* **ΣHQ** (sum of all individual **HQx**) calculated

Ti = Concentration of analyte at Time 0

Results

HQx of Selected Analytes in Original NJOY Product

Analyte	Product	ACM (µg/puff) (a)	[X] µg/puff (b)	µg X/ µg ACM (c = b/a)	ivMN max dose (µg X/mL) (d = c x 1000)	HQ, ivMN (e = d/HID or LED)	Ames max dose (µg X/plate) (f = c x 5000)	HQ, Ames (g=f/LED)
Formalde-hyde	ENDS N% Flavor U	5.56E+03	3.43E-02	6.17E-06	6.17E-03	2.06E-03	3.09E-02	4.94E-03
Nickel		5.56E+03	3.86E-03	6.95E-07	6.95E-04	7.72E-06	3.47E-03	3.47E-06
Dichloro-methane		5.56E+03	1.10E-06	6.11E-05	6.11E-02	3.60E-04	3.06E-01	1.25E-04

HQx of Selected Analytes in a MAEP

Analyte	Product	ACM (µg/puff) (a)	[X] µg/puff (b)	µg X/ µg ACM (c = b/a)	ivMN max dose (µg X/mL) (d = c x 1000)	HQ, ivMN (e = d/HID or LED)	Ames max dose (µg X/plate) (f = c x 5000)	HQ, Ames (g=f/LED)
Formalde-hyde	ENDS N% Flavor U	4.93E+03	1.52E-01	3.08E-05	3.08E-02	**1.03E-02**	1.54E-01	**2.46E-02**
Nickel		4.93E+03	4.12E-03	8.36E-07	8.36E-04	**9.28E-06**	4.18E-03	**4.18E-06**
Dichloro-methane		4.35E+03	8.30E-06	3.61E-04	3.61E-03	**2.12E-05**	1.80E+00	**7.35E-04**

Overall in vitro ΣHQ for an Original NJOY Product and MAEP

Products	Conditions	ivMN Σ(HQ_{iv})	Ames Σ(HQ_{iv})
ENDS N% Flavor U	Original	0.0044	0.0053
	MAEP	**0.0220**	**0.0272**

- The overall in vitro ΣHQ for the product was far less than 1, the threshold at which it is reasonable to expect to be able to observe a potential positive response for in vitro genotoxicity and/or mutagenicity testing
- The highest exposure/concentration used in the in vitro assay was over 200 times more PM than the daily exposure of a heavy cigarette smoker, and over 100 times more PM than what a heavy user of NJOY ENDS would be exposed to daily (Data not shown).
- The same negative responses reported in the in vitro assays for NJOY ENDS would be expected to be observed for the MAEP which indicates no further in vitro toxicity testing is warranted.

Conclusion

The LED/HID-based relative in vitro hazard assessment can serve as a reliable tool to provide sound scientific determination on whether additional in vitro toxicity testing is necessary and estimate the relative hazard and potential health impact of a MAEP in comparison to cigarette smoke and originally tested ENDS product.

References

Bird et al, 1982; Brambilla et al, 2011, 2013; Buxton et al, 2020; Doherty et al 1996; IARC 2016; Latvava et al 2016; Liu et al 2017, Lovschall et al 2002, Migliore et al 1996; WHO IARC Monographs 71, 88, NTP TR 1996, 2016; Zhang et al, 2018.

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