IIVS Workshop Series: Identification, Discussion and Recommendations for the Optimal Generation and Use of In Vitro Genotoxicity Assay Data for Tobacco and Nicotine Products

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INTRODUCTION

The Institute for In Vitro Sciences (IIVS) is sponsoring a series of workshops to identify, discuss and develop recommendations for optimal scientific/technical approaches for utilizing *in vitro* assay data within and across tobacco and nicotine product categories. Workshops provide a unique opportunity for invited expert stakeholders to share experiences and to develop recommendations that may serve as a resource for developing optimal approaches and data to evaluate the toxicity of tobacco and nicotine products. It is envisioned that some of these recommendations would form the basis for the generation of guidance documents and/or serve as authoritative reference publications for optimal methodologies and data interpretation and to support regulatory submissions. Invited experts for the IIVS workshops include scientists from tobacco companies, contract research organizations, US regulatory agencies, and other *in vitro* assay experts with tobacco product and/or genetic toxicology experience. The format for this workshop series is primarily discussion among participants which provides an environment to tackle issues in detail. Participants are expected to actively participate by collecting relevant published and unpublished non-proprietary research information, to offer experiences and expert opinions, and to actively share with other workgroup members. While the focus will be on the widely used regulatory *in vitro* genetic toxicology assays, it is important to note that much of the discussion will be applicable to all *in vitro* assays. As a part of the workshop discussion, data gaps will be identified. Thus, in addition to recommendations based on current information, this workshop series will provide key research questions that need to be addressed by the scientific community. This will provide a useful roadmap for research that can have direct impact on the regulation of tobacco products and on protecting human health related to consumer use of tobacco products. The product of these workshops will be a series of scientific publications and meeting presentations that can be utilized by all stakeholders.

Prior to the first workshop (November 27-28, 2018) important issues for using *in vitro* genotoxicity assays for evaluating tobacco and nicotine products were identified. During the first workshop issues were triaged into three priority categories based on the amount of available information (Tables 1-3).

Table 1. DISCUSSION ITEMS IDENTIFIED AS CATEGORY 1: EXTENSIVE INFORMATION AVAILABLE; RECOMMENDATIONS CAN BE READILY DEVELOPED.

Discussed and consensus reached during the first workshop.

- Include cytotoxicity assays in the workshop discussion
- Endorse the ICH and CORESTA recommended *in vitro* battery of genetic toxicology tests including the Ames test, and one of the following mammalian genetic toxicology tests: the mouse lymphoma gene mutation assay using the thymidine kinase locus (MLA), the *in vitro* micronucleus assay and the *in vitro* chromosome aberration assay

Issues identified as high priority for discussion and development of recommendations at the second and subsequent workshops.

- Relevant test material matrix and test material preparation
- Methods to prepare specific types of samples (i.e. particulate matter, condensates or gas vapor phase from combustibles; particulate matter, condensates or gas vapor phase (aerosol) from electronic nicotine delivery systems (ENDS) including e-cigarettes; extracts from smokeless or smoke).
- Identify appropriate sample types relative to product types and recommend sample preparation methods.
- Appropriate solvents or matrix media for specific types of samples.
- Recommended "puffing/vaping" profile for test material generation (i.e. condensates/particulates, aerosols and smoke).
- Test article characterization that is compliant with GLP.
- Stability assessment and its impact on biology of samples prepared for *in vitro* assays (recommendations on "use by" date/shelf life).
- Determining the appropriate Ames test strains for tobacco product testing

Issues identified for discussion at subsequent workshops.

- Cell lines for use in the *in vitro* micronucleus and *in vitro* chromosome aberration assays.
- Selection of appropriate top concentration when a sample is not sufficiently cytotoxic (and the top concentration is limited by the amount of solvent).

Table 2. DISCUSSION ITEMS IDENTIFIED AS CATEGORY 2: ADDITIONAL SHORT-TERM (<2 YEARS) INFORMATION/RESEARCH REQUIRED

- Promising new *in vitro* assays for genetic damage and recommendations for "validating/qualifying" them for routine use.
- Recommended methods to expose cells to aerosols and smoke (both combustibles and ENDS). This includes generation and handling of the aerosol, exposure and recovery of cells, exposure duration, appropriate cytotoxicity and concentration range, and dosimetry.
- Recommendations for follow-up tests when deconvoluting positive results—potentially generating a decision tree.
- Recommendations for evaluating large numbers of products (particularly ENDS) varying only in single additives such as flavoring compounds.

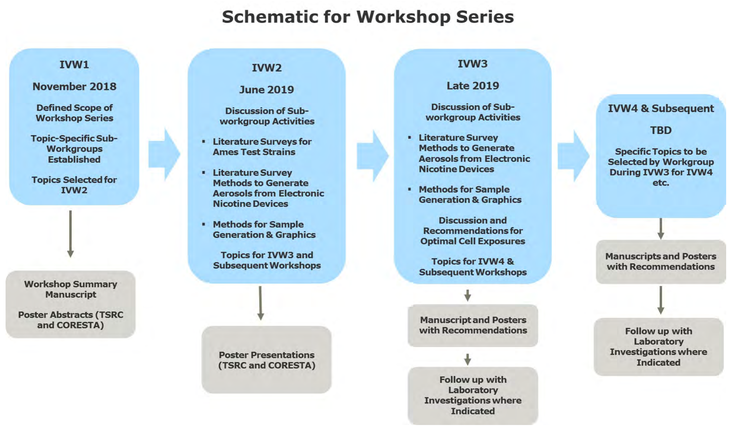
Table 3. DISCUSSION ITEMS IDENTIFIED AS CATEGORY 3: ADDITIONAL LONG-TERM (>2 YEARS) INFORMATION/RESEARCH REQUIRED.

- Recommended experimental design providing appropriate data for quantitative comparisons (i.e. number of replicate cultures, concentration spacing, number of independent experiments etc.)
- Recommended methods to express exposure, particularly with comparing different types of products (i.e. combustible cigarettes vs. smokeless vs. ENDS).
- Recommended methods to make quantitative comparisons for *in vitro* responses (i.e. potency, graphical, BMD or another metric).
- What are the specific issues (and potential solutions) associated with evaluating and comparing relative potency of complex mixtures? What is the minimum amount of genotoxicity/toxicity that can be detected in a mixture?
- Extrapolation of *in vitro* results to *in vivo*.
- Use of bridging biomarkers from *in vitro* to human.

CURRENT AND FUTURE WORKGROUP ACTIVITIES (FIGURE 1)

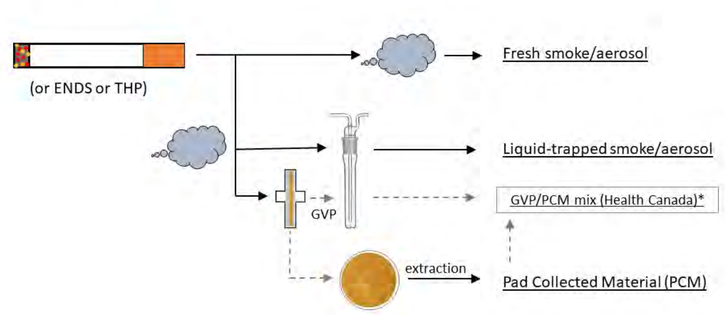
During the second workshop (June 4-5, 2019) the group discussed methods that have been used to generate samples from combustible cigarettes and a review of the literature to outline methods used to generate aerosols from ENDS. The workgroup plans to continue the literature review and to generate a summary publication. The next workshops (Figure 1) in the series will focus on developing recommendations for optimal methods to expose cells to the various types of samples and to generate optimal data from the standard genetic toxicology assays. Future workshops will tackle issues such as: (1) recommended cell types for *in vitro* cytogenetic evaluations, (2) recommendations for expressing exposure when comparing products within and among product categories, (3) recommendations for comparing toxicological responses within and among product categories, and (4) applying new or existing methods for assessing genotoxicity and other toxicological effects of tobacco and nicotine products using cells in culture.

Figure 1. Overview of future workshops in this series.



During a short meeting (March 7, 2019) and the second workshop (June 2019) the workgroup discussed ongoing issue evaluations. During these meetings the workgroup developed a schematic (Figure 2) to visualize the types of samples that can be generated from inhaled tobacco products.

Figure 2: Schematic for the types of samples that can be generated from inhaled tobacco products including combustible cigarettes, Tobacco Heating Products (THPs) or ENDS. These types of samples can be evaluated in most of the standard regulatory genetic toxicology assays. *Note that Health Canada recommends testing a combination of the GVP and the PCM for the Neutral Red Uptake assay.



CONCLUSION

The IIVS Workshop series provides the opportunity for experts to share their experiences and knowledge to provide recommendations that will be useful to the broader scientific and regulatory communities seeking to evaluate the toxicity of tobacco products. The triaged list of topics developed in the first workshop have been prioritized for detailed discussions during subsequent workshops. The workgroup strategy is to collect information prior to each convened meeting in preparation for detailed workshop discussions. The goal of the workgroup discussions will be to share insights and to develop recommendations that will be shared in a series of publications and in presentations at scientific meetings.

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