In Vitro Toxicity Testing (IVT) SG
Annual Report

Cancun, Mexico

11 October 2023

- **Current Objectives**
  1. To compile and review information on in vitro toxicity testing and apply learnings to further biological research.
  2. To organise and conduct periodically proficiency testing of tobacco and tobacco related products.

- **SG Composition**
  - Proposed Coordinator: **Liam Simms (Imperial Brands) awaiting CORESTA review/sign off.**
  - Secretary: Yuki Kanemaru (JT international)

IVT SG – 2/2-5 year plans

- **2 year plan: Core areas**
  - Characterise analytically and biologically trapped ENDS aerosols from sample types assessed in safety toxicology studies.
  - Produce CORESTA recommendations on good technical practices to be factored in when assessing EVP aerosols toxicologically.
  - Produce guidance on recommendations when assessing EVP e-liquid formulations.
  - Support member organisations wishing to participate in in vitro proficiency and ring trial studies using core OECD test methods for toxicological in vitro methods.
- **2-5 year plan: establish longer-term IVT SG core areas**
  - Continue to support member organisations wishing to participate in in vitro proficiency and ring trial studies using core OECD test safety toxicology in vitro methods
  - Support BMK SG and NGTX TF with cross collaboration to develop robust understanding of mechanistic pathways of smoking related diseases and clinical outcomes to better identify future fit-for-purpose biomarkers.
  - Participate with BMK SG and NGTX TF on identifying and assessing Novel Assessment Methodologies for member organisations.
  - Support for future Adverse outcome Pathway (AOP) developments (to be fully scoped)

IVT SG – Additional ideas (to be scoped)

- **Additional ideas captured during the Antibes meeting in April, among the group for 2 - 5 year plan**
  - **Enlarge the scope of the group (e.g. EVP, HTP, and oral products)**
  - **Use of human cells for routine assays**
  - **Increased method harmonization (e.g. cell lines, study conditions)**
  - **Building regulatory acceptance and decision making for Tobacco Testing (processes and robustness)**

IVT SG – Completed projects

Project No.	Activity Report	Leader	Publication Date
311	In vitro Micronucleus Assay, Inter-Laboratory Proficiency Study	E. Weber	April, 2023

IVT SG – On-going proficiency testing

Project No.	Activity Report	Leader	Publication Deadline
354	Neutral Red Uptake Assay, Inter-Laboratory Proficiency Study	R. Wieczorek	June 2024
	Ames ring trial	R. Wieczorek	2024/25

- **Current status**
  - **354 NRU Study: Draft results were shared. Each lab is using their own preferred cell line (7 different labs and 5 different cell lines), but it was agreed that a common cell line (BEAS2B) will be added to the next ring trial.**
  - **Ames ring trial, proposals put forward to the team for potential test cigarettes (Flue-cured or Burley cigarettes) HTP, and possible study designs (35 and/or 55ml puff). Team to decide the way forward.**

IVT SG – Current projects

Description	Leader	Timeline
MLA, Inter-Laboratory Proficiency Study (on hold)	TBD	?
Ames, Inter-Laboratory Proficiency Study	Roman / Elisabeth	2024
ToxTracker, Inter-laboratory Proficiency Study, Collaboration with Biomarkers and NGTX groups	Kirk Newland	2024
ENDs toxicity of EVP and HTP (quantifying markers trapped in media representative of gas vapor/particulate phase). Guidance for EVP testing	M Hollings / Doshi	2024
Recommended methods for smoke free product testing (OND) exact project goals being scoped	S Moses	TBD

- **Current state:**
  - **MLA**: On hold. Currently only 2 labs require the ring trial for the ISO accreditation. The product does not need to be tobacco related.
  - **Ames**: [PMI, Imperial, JT, Altria, Labcorp, (BAT)] Strains (5 or 2) and samples (TPM/HTP) to be determined.
  - **ToxTracker**: [Labcorp, BAT, Imperial, Altria, JTI, Labstat] Smoker concentrated urine samples to be sent to labstat for analysis
  - **ENDs toxicity of EVP and HTP**: [Altria; Imperial; JTI, JT PMI, BAT] The aim is to identify/characterize chemicals in samples for better interpretation. **List of analytes to be refined,** updated survey to companies.
  - **OND method Standardisation, Company current extraction methods have been collected. Initial Meeting was held to discuss current similarities and differences in methods and report to larger team.**

EVP and HTP quantifying markers to establish a quality sample is obtained

- **Initial work identified a list of possible chemicals**
  - **Select HTP/EVP reference product(s) -> e.g. IQOS; Glo; reference EVP?**
    - Select trapping methods -> CFP + impinger; aerosol-bubbled PBS, ethanol DMSO or cell culture medium; Etc (to be selected as will not capture all)
    - Select aerosol generation/trapping conditions -> smoking regime; puff number; solvent volume; Etc.
    - Aim to Select a short list (6-12) of representative constituents ->
  - **Action:**
    - A survey will determine what chemicals can be measured at the respective companies and in which solvents

Constituent category	Representative constituents
Product-specific	Water, nicotine, carbon monoxide, glycerol, PG
Volatiles & semi-volatiles	1,3-Butadiene, isoprene, benzene, toluene, styrene, pyridine, quinoline, acrylonitrile
Carbonyls	Acetaldehyde, acetone, acrolein, butyraldehyde, crotonaldehyde, formaldehyde, methyl ethyl ketone, propionaldehyde
Aromatic amines	1-Aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 4-aminobiphenyl, o-toluidine
Nitrogen oxides	Nitric oxide (NO), oxides of nitrogen (NOx)
Epoxides & vinyl chloride	Ethylene oxide, propylene oxide, vinyl chloride
Tobacco-specific nitrosamines	N-Nitrosoanabasine (NAB), N-nitrosoanatabine (NAT), N-nitrosonornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)
Phenols & acid derivatives	Catechol, o-cresol, m-cresol, p-cresol, hydroquinone, phenol, resorcinol, acetamide, acrylamide
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, pyrene
Nitrobenzene	Nitrobenzene
Elements	Arsenic, cadmium, chromium, lead, nickel, selenium, mercury

- **Initial recommendations for EVP testing** have been drafted and circulated to the working group for review/discussion.

IVT SG Collaboration: BMK-367-NWIP-Evaluation of ToxTracker for applicability in tobacco related clinical research

K Newland, M McEwan (BMK), M Gaca, D Breheny, L Simms (NGTX), K Aleksa, M Hollings(IVT)

- The project will determine if the biomarkers measured are appropriate for use in a clinical study designed to evaluate exposure to tobacco or nicotine related products.
- The ToxTracker assay will be used to assess biomarkers of DNA damage, protein misfolding, oxidative and cellular stress.
- The evaluation will occur in 3 phases.

October 2023

Phase 1: The initial evaluation will be to determine the sensitivity of the assay using concentrated urine from smokers.

- The volume, the level of concentration, the dosing will be determined.
- Acidic hydrolysis step prior to urine concentration will be evaluated to determine if it provides improved sensitivity

Q1 2024

Phase 2: Urine samples from non-smokers and smokers will be compared to determine if biomarkers tested with the ToxTracker assay demonstrate reasonable differentiation. If successful an interim report will be prepared.

2024

Phase 3: If Phase 1 and Phase 2 are successful, an evaluation of a more broad range of samples from subjects with a more broad range of exposures may be evaluated

- Outcome: CORESTA report, technical guidance and publication in a peer-reviewed journal

IVT SG – OND pouch extraction survey

- **Survey (Lead by Sara Moses): How do you extract nicotine pouches for toxicology studies?**
  - Is there common best practice?
  - Key information needed to compare data?

Company	Extraction time	Extraction performed on a mass or pouch number basis?	Cell lines used	Solvent volume (Mls)	Final stock target concentration (e.g. X grammes/mL; X pouches/mL	Extraction temperature	Solvent type (e.g. CAS, culture medium, PBS…)	How is pouch processed (e.g. whole pouch extraction; fleece removed; pouch cut up etc.)?	Extraction conditions (e.g. shaking, no shaking, other)	Wet versus dry pouch method (i.e. do you use different approaches depending on whether pouch is wet or dry)?	Additional information e.g any publications on the methodology

  - Team: Sara Moses (Swedish Match), Elisabeth Weber (JTI), Brian Keyser (RAIS), Robert Leverette (RAIS) Emma Cheung (BAT) Altria, Sarah Jean Pour (ITG)

- **Survey results are being summarized:**
  - What cell lines used; what extraction media (volume, temp, duration); whole pouch or cut open; pH; osmolarity effects.
  - Where can methods be standardised?
  - Deliverables : Guidance document on best practices, e.g. generation of extracts for biological and toxicological testing, appropriate in vitro assays to use and publication on data.

Next IVT SG meeting

- **Coordinator change:**
  - Damian McHugh has changed roles and is no longer able to hold the role as coordinator. The new co-ordinator is **Liam Simms (Imperial Brands)** subject to approval by CORESTA.
- **Meetings:**
  - Last: **October 8th, Cancun**
  - Next: **Feb 2024 to be decided if virtual or face to face.**