NGTX: 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGPs) Annual Report

Cancun / Mexico

10th October 2023

NGTX TF: 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGPs)

- **Current Objectives**

To review, assess, apply and harmonize 21st century toxicology (TT21C) approaches to tobacco and nicotine products, including but not limited to screening approaches, AOP development, organs on a chip and systems biology to support quantitative risk assessment.

  - To review emerging technologies and their application to NGP testing
  - To identify appropriate **methodology** and application of emerging technologies to NGP testing
  - To provide guidance documents to support assay application for NGP testing using TT21C relevant assays

- **TF Roles**

  - Coordinator: Marianna GAÇA (BAT)
  - Secretary: Liam SIMMS (Imperial **Brands plc**)

NGTX TF: Scoping areas of interest

NGTX TF

1.Alignment of current non- regulatory methods including:
- -High content screening
- -Primary/ cell lines
- -3D models

2. Oral nicotine products: guidance documents and best practices for toxicology testing models
3. Non-standard genetox assays
- -Toxtracker collaboration with BMK/IVT SG

5 years +
Translational data/ activities
Adverse Outcome Pathways (AOPs)
In vivo / in vitro extrapolation (IVIVE) ***currently considered under BMK collaboration***

OTHER CORESTA SG
1. Adaptation of models for CROs -IVTSG
2. Regulatory acceptance following NGTX methods assessment-IVTSG
3. Reference products (EVP, HTP, OTDN etc)

Other non-CORESTA working groups ie IIVS workshops
1. Standardisation of in vitro whole aerosol exposures
2. Screening technologies for whole aerosols
3. Dosimetry
4. Regulatory education ie NICETAM etc

NGTX TF: 2- and 5-year Plan
(NWIPs to initiate research projects)

- **2 year plan: establish NGTX TF and core areas**
  - Review on 21st century toxicology approaches methods for NGP testing
    - CORESTA Review, External publication and presentation of review findings at future CORESTA meeting
  - Review of OECD's Guidance Document Good In Vitro Method Practices (GIVIMP) guidance
    - Guidance document and potential external publication on best practices for NGP *in vitro* studies
  - High Content Screening (HCS) working group method standardisation
    - HCS guidance document for NGP testing and publication on data sets generated
  - New Approach Methodologies (NAMS) 2:
    - To develop a session for the CORESTA annual conference, with a series of talks on the use of NAMS by industry in coordination with NGTX
- **2-5 year plan: establish longer-term NGTX TF core areas**
  - Guidance on testing approaches using 3D reconstituted human tissue models
    - Guidance document on how to use 3D model systems, and recommended approaches
  - Oral nicotine pouches toxicology approaches
    - Guidance documents on best practices, eg generation of extract for biological and toxicological testing, appropriate *in vitro* assays to use, publication on datasets generated
- **2-5 year plan: NGTX TF, BMK SG, IVT SG collaborative study**
  - Assessment of urinary biomarkers using a non-standards genotoxicity reporter-assay testing (Toxtracker).
    - Development of approaches to assess the human relevance to urinary biomarkers; exploring the use of reporter assays to detect urine mutagenicity and potential mechanistic endpoints (including inflammation, oxidative stress); publication on datasets generated

NGTX TF: Identified activities

- **Priorities:**
  - “Good In Vitro Method Practices ” (GIVIMP: OECD Guidance Document) guidance: review of document for NGP testing
  - High Content Screening (HCS) working group method standardisation
  - 3D models guidance on how to use them (a new item to be confirmed)
  - Oral nicotine pouches: potential link into BMK group

- **On going projects:**
  - **NWIP#221:** Review emerging technologies and application to NGP testing- review document
  - **NWIP#** BMK-367-Evaluation of ToxTracker assay for applicability in tobacco related clinical research
  - **New Approach Methodologies (NAMs)2**

NGTX TF: On-going Projects

Project No.	Activity Report	Leader	Publication Deadline
#221	Review emerging technologies and application to NGP testing-review document	M. Gaca	TR expected Q1 2024 External publication 2024

Team: Altria, BAT,JT, ITG,PMI, Reemtsma Cigarettenfabriken GmbH, RAIS, Swedish Match

- Draft manuscript for the journal « Frontiers Special Edition » was shared before the 2023 Spring meeting. There was a request for any others that want to join the group to add their names. The team reviewed the paper and to update with recent papers by the end of 2023, with the aim to publish the paper in 2024.

NGTX TF 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

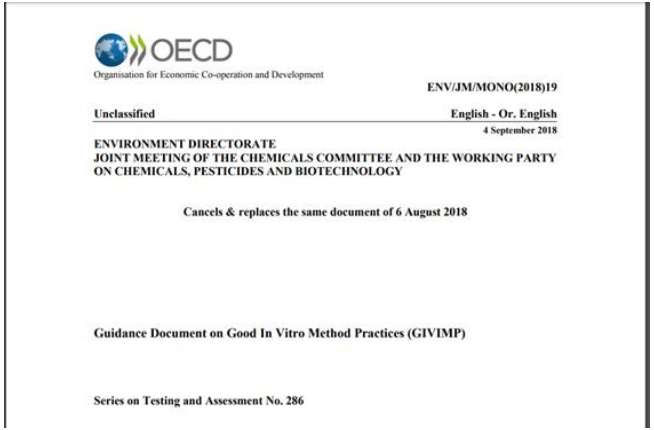
Phase 3: If Phase 1 and Phase 2 are successful, an evaluation of a more broad range of samples from subjects with a more broad range of exposures may be evaluated

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

NGTX TF: Workstreams (New NWIP)
Good *In Vitro* Method Practices

- **Review of OECD's Guidance Document Good *In Vitro* Method Practices (GIVIMP) guidance (BAT, Vitrocell, CRL, JTI, PMI, Labcorp, JT, Altria, JUUL)**
  - Guidance document and potential external publication on best practices for NGP *in vitro* studies
  - Amanda Ulrey from Institute for In Vitro Sciences (IIVS) will collaborate with the working group while developing a recommendation
  - Currently chapters in review and aligning if fit for purpose

Executive summary	Marianna Gaca
Chapter 1. Roles and responsibilities	Tobias Krebbs
Chapter 2. Quality considerations	Leon Stankowski and Robert Leverette
Chapter 3. Facilities	Elisabeth Weber
Chapter 4. Apparatus, materials, and reagents	Michael Hollings
Chapter 5. Test systems	Stefan Frentzel and Hitoshi Fujimoto
Chapter 6. Test and reference/ control items	Monica Lee and Kubilay Demir
Chapter 7. Standard operating procedures	Leon Stankowski and Michael Hollings
Chapter 8. Performance of the method	Damien McHugh and Robert Leverette
Chapter 9. Reporting of results	David Thorne
Chapter 10. Storage and retention of records and materials	Marianna Gaca



OECD
Organisation for Economic Co-operation and Development

ENV/JM/MONO(2018)19

Unclassified

English - Or. English

4 September 2018

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

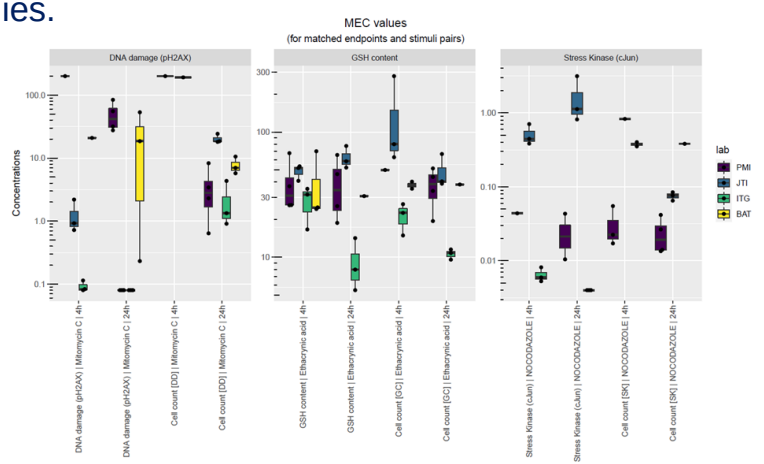
Cancels & replaces the same document of 6 August 2018

Guidance Document on Good In Vitro Method Practices (GIVIMP)

Series on Testing and Assessment No. 286

- Outputs- CORESTA report, publication, and conference presentation
- Timings 2023/2024

- **High Content Screening (HCS) working group method standardisation (PMI, JT, IB, BAT)**
  - To draft HCS guidance document for NGP testing and HCS and publication on data sets generate
  - Approach: to explore HCS data variance across the companies and how HCS can be accepted for NGP testing.
    - Stage 1: Review the data already held by individual companies.
    - Stage 2: Further standardisation of methodology needed (work in progress)
      - All data analysed by PMI using GladiaTOX to derive the minimum effective concentrations (MEC) for each of the endpoints. There are clear differences in results each company used own methodology and cells and further standardization of methodology is required, this is work in progress
      - Intention to draft CORESTA recommendation / guidance for those new to HCS



NGTX TF: Workstreams (New NWIP)
New Approach Methodologies (NAMs)

- **Symposium: NAMs session 2 (Industry Perspectives): M.Lee (Altria) + Speakers**
  - In response to the “NAMs session - 1” from regulators at CORESTA 2021 conference. To develop a session for CORESTA (2023), with a series of talks on the use of NAMS by the industry in coordination with IVT SG.
  - Discussion at SOT 2023 meetings
  - Program established. A total of (1+6) presentations plus, an invited panelist, FDA/CTP (TC)
  - Adding Context in Each Talk: Set the stage at introduction – Followup at Panel discussion
    - What products - RR products, test materials, or ingredients
    - What question(s) or hypothesis to test
    - How NAM tools are used – Case examples
    - What are the critical gaps and opportunities - Panel discussion
    - See the next set of presentations


SOT March.2023 – Nashville, TN US

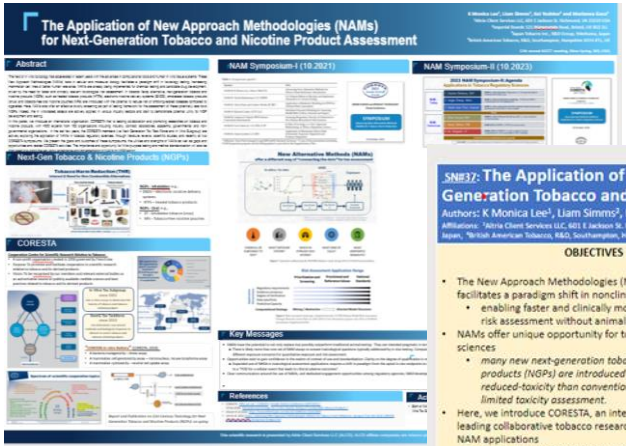
NGTX TF: Future Projects + NWIPs

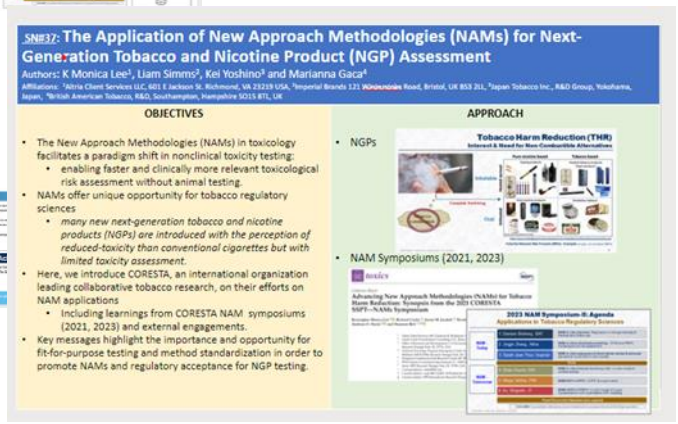
Description	Leader	Timeline
Humanised in vitro approaches	TBD	TBD
Oral nicotine pouches	Sara Moses	TBD

- **Humanised *in vitro* approaches: Guidance on testing approaches using 3D reconstituted human tissue models (cell lines and primary cells to be considered).**
  - Outcomes: Guidance document on how to use 3D model systems, and recommended approaches and publication
  - Team: Elisabeth Weber (JTI), Kirsten Jordan (RAIS), David Thorne (BAT), Damien Breheny (BAT), Shigeaki Ito (JT), Roman Wieczorek (ITG), Sarah Jean Pour (ITG)
- **Oral nicotine pouches: potential link into BMK group**
  - Outcomes: Guidance document on best practices, eg generation of extract for biological and toxicological testing, appropriate in vitro assays to use and publication on data
  - Initial survey complete, comparing current methods for pouch extraction, maximal worst case vs consumer usage; Includes extraction time; extraction volume; temperature; solvent used PBS vs artificial saliva etc
  - Team: Sara Moses (Swedish Match), Elisabeth Weber (JTI), Brian Keyser (RAIS), Robert Leverette (RAIS), Emma Cheung (BAT) Altria, Sarah Jean Pour (ITG)

NGTX TF: Science engagement and comms
ASCCT 12th Annual Meeting (Oct 2023)

- CORESTA Poster and Flash Poster presentation
  Monica Lee, Liam Simms, Kei Yoshino, Marianna Gaca





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The application of new approach methodologies (NAMs) for next-generation tobacco and nicotine products assessment

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Abstract

The field of in vitro toxicology has accelerated in recent years with the advances in computational tools and human in vitro tissue systems. These New Approach Methodologies (NAMs) in cellular and molecular biology facilitate a paradigm shift in toxicity testing, harnessing mammalian cell lines of increased human relevance. These in vitro-based tools are implemented in chemical and candidate drug screening, also driven by the need for faster and clinically relevant toxicological assessment.

NGTX TF: Science engagement and comms

- **Science engagement and Comms**
  - Potential Posters for future Conferences: an interest to present posters at CORESTA and external scientific meeting (e.g. SOT, EUROTOX, American Collage of Toxicology) to explain the areas NGTX are working on.

SOT SALT LAKE CITY MARCH 10–14

- **Meetings:**
  - Spring meeting 2023 :Antibes
  - October 8th Cancun
  - Next Meeting Online date to be agreed