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

Virtual Autumn Meeting
November 2022

Sub-Group Composition

- Coordinator : Marianna Gaca (BAT)
- Secretary : Liam Simms (ITG)

Objectives

To review, assess, apply and harmonize 21st century toxicology 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 application to NGP testing- review document to be prepared (NWIP#221)
- To identify appropriate approaches and application of emerging technologies to NGP testing
- To provide guidance documents to support assay application for NGP testing using TT21C relevant assays

❖ 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)
- ➢ External publication on 21st century toxicology approaches for NGP testing and presentation at future CORESTA meeting

❖ On going projects:

Project No.	Project Name: Activity	Leader	Status
NWIP#221	Review emerging technologies and application to NGP testing- review document	M. Gaca	TR expected end of Q1 2023 External publication Q1 2023

NWIP #221 'Report and Publication on 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGPs)

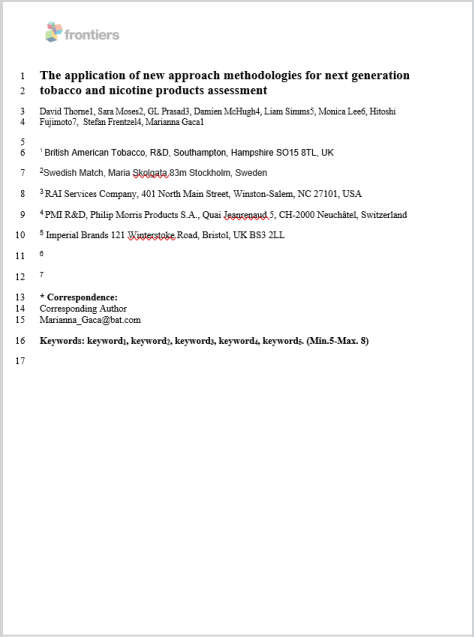
- A review document outlining the emerging 21st century *in vitro* toxicology tools and their potential application for tobacco and nicotine product testing.
- External publication on 21st century toxicology approaches for NGP testing and presentation at future CORESTA meeting

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

As the review will be quite comprehensive, can provide content for NGTX webpage and also abstracts for future toxicology conferences

NWIP #221 'Report and Publication on 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGPs)

- Abstract
- 1. Introduction
- 2. Next Generation Tobacco and Nicotine Products (NGPs
- 3. Test Articles
- 4. Model systems and high content analysis
  - 4.1 High content screening
  - 4.2 Toxtracker™
  - 4.3 MultiFlow®
  - 4.4 In vitro airway models
  - 4.5 Ex-vivo models and lung-on-a-chip
  - 4.6. Organ-on-a-chip and multi-organs-on-a-chip
- 5. Considerations
  - (AOPs?)
  - 5.1 Acute vs. repeated exposure
    - 5.1.1 Repeated exposure of lung epithelial cells to aerosol or smoke fractions:
    - 5.1.2. Repeated exposure of lung epithelial cells to whole aerosols:
  - 5.2 QIVIVE
  - 5.3. Dosimetry
- 6. Conclusion



frontiers

The application of new approach methodologies for next generation tobacco and nicotine products assessment

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Keywords: keyword$_1$, keyword$_2$, keyword$_3$, keyword$_4$, keyword$_5$. (Min.5-Max. 8)

High Content Screening
(ITG, JT, PMI, BAT)

- **Which approaches to take forward (practical)?**
  - **Use and application of high content screening**
    - common test systems (cells),
    - common endpoints (cell health, oxidative stress, DNA damage),
    - applied to tobacco and nicotine products

- **Use and application of high content screening (HCS)- potential outcomes (TBC)**
  - Explore HCS data variance across the companies and how HCS can be accepted for NGP testing.
  - Review the data already held by individual companies for reference cigarettes, controls etc. Initially focused on 3 endpoints and defined positives with each members' own methodologies and cell lines.
  - Develop guidance how HCS can be used for NGP testing to be placed on the CORESTA website.
  - To be further discussed at Autumn 2022 virtual meeting

SPPOST 39

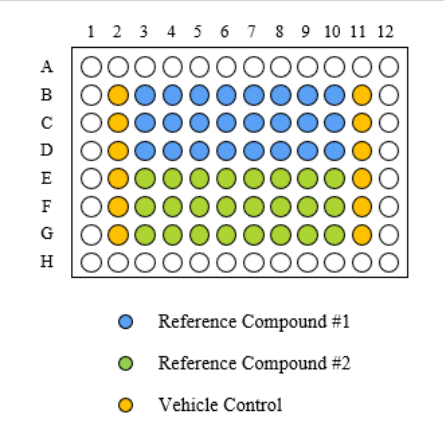
Risk Assessment of a novel tobacco vapour product using ToxTracker® assay and highcontent screening *in vitro*

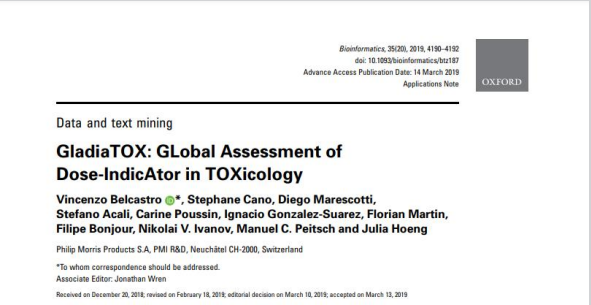
Munakata S, Erami K, Hashizume T

Japan Tobacco Inc, R&D Group, Scientific Product Assessment Centre, Japan

Limited end points and positive controls were agreed:

- Three common end points were chosen
- Group decided to standardise positive controls used.
- All are using and their own normal human Bronchial epithelial (NHBE) cell lines and methodology
- 8 concentrations and 3 replicates per end point positive control, per end point
- A standardised plate layout was agreed
- Data was analysed by PMI using GladiaTOX to derive the minimum effective concentrations (MEC) for each of the endpoints.




Bioinformatics, 35(20), 2019, 4190–4192
doi: 10.1093/bioinformatics/btz187
Advance Access Publication Date: 14 March 2019
Applications Note
OXFORD

Data and text mining

GladiaTOX: GLobal Assessment of Dose-IndicAtor in TOXicology

Vincenzo Belcastro*, Stephane Cano, Diego Marescotti, Stefano Acali, Carine Poussin, Ignacio Gonzalez-Suarez, Florian Martin, Filipe Bonjour, Nikolai V. Ivanov, Manuel C. Peitsch and Julia Hoeng

Philip Morris Products S.A, PMI R&D, Neuchâtel CH-2000, Switzerland

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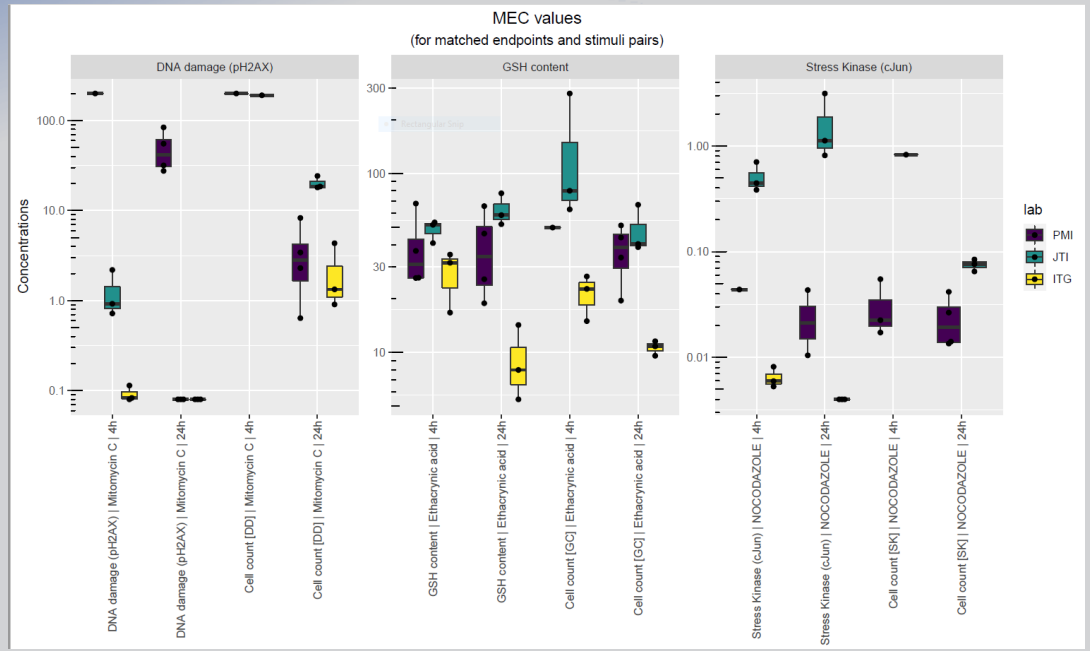
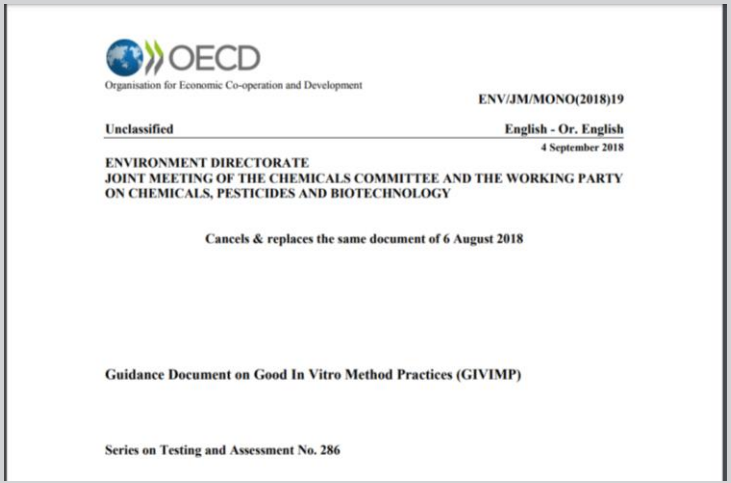
Initial results: MEC values for the three end points and next steps



- Awaiting HCS data from other labs add to initial analyses.
- There are clear differences in results each company used own methodology and cells
- Based on the on the initial results further standardisation of methodology is required, (Stage 2), this is work in progress

Good In Vitro Method Practices (GIVIMP)

- **Guidance documents**
  - **OECD Guidance Document** on Good In Vitro Method Practices (GIVIMP)
  - Team assembled: Vitrocell, CRL, BAT, JTI Oekolab, Labcorp, PMI, JT, Altria, JUUL
  - A guidance document recommending the principles of GIVIMP for non-regulatory assays will be compiled and will be placed on the CORESTA website
  - Autumn 2021 virtual meeting, we reviewed guidance document progress, and guest presentation from Amanda Ulrey, IIVS, “How a GIVIMP certification program can increase confidence of *in vitro* methods”



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

Good In Vitro Method Practices (GIVIMP)

- **Next steps:**
  - **Agree the working group**
  - **Review the current draft**
    -fit for purpose?
    -additions?
    -modifications?
  - **Outputs- CORESTA report, publication, conference?**
  - **Timings- 2023**

Executive summary	Marianna Gaca
Chapter 1. Roles and responsibilities	Tobias Krebbs
Chapter 2. Quality considerations	Leon Stankowski
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
Chapter 8. Performance of the method	Damien McHugh
Chapter 9. Reporting of results	David Thorne
Chapter 10. Storage and retention of records and materials	Marianna Gaca

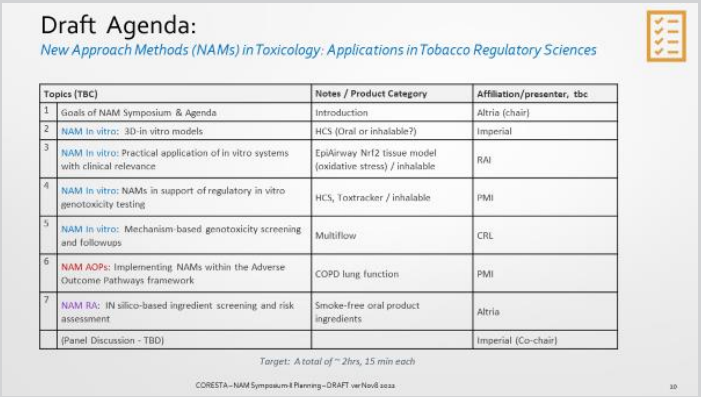
Proposed working group
Monica Lee (Altria), Kubilay Demir (Juul), David Thorne (BAT), Marianna Gaca (BAT) Hitoshi Fujimoto (JT),Damian McHugh (PMI), Edgar Trelles- Sticken (ITG), Tobias Krebbs (Vitrocell), Elisabeth Weber (JTI), Michael Hollings (Labbcorp), Leon Stankowski (CRL), Jenny Yao (Juul), Robert Leverette (RAIS), Diego Marescotti (PMI)

Planning for New Approach Methods (NAMs) Industry Perspectives: Symposium II

Following success of NAMs Symposium-I at CORESTA 2021 and synopsis paper accepted for publication, a second symposium is being considered.

- Series of talks on the use of NAMS by the industry for NGP assessment, in coordination with IVT SG
- Drafting agenda to include speakers from 6+ member companies
- Exploring logistics:
  - In person (TBC)- at the Autumn 2023 CORESTA meeting
  - Virtual (TBC)- at the Spring SG meetings

- Next steps
  - Finalize the agenda / speakers
  - NWIP



Draft Agenda:

New Approach Methods (NAMs) in Toxicology: Applications in Tobacco Regulatory Sciences

	Topics (TBC)	Notes / Product Category	Affiliation/presenter, tbc
1	Goals of NAM Symposium & Agenda	Introduction	Altria (chair)
2	NAM In vitro: 3D-in vitro models	HCS (Oral or inhalable?)	Imperial
3	NAM In vitro: Practical application of in vitro systems with clinical relevance	EpiAirway Nrf2 tissue model (oxidative stress) / inhalable	RAI
4	NAM In vitro: NAMs in support of regulatory in vitro genotoxicity testing	HCS, Toxtracker / inhalable	PMI
5	NAM In vitro: Mechanism-based genotoxicity screening and followups	Multiflow	CRL
6	NAM AOPs: Implementing NAMs within the Adverse Outcome Pathways framework	COPD lung function	PMI
7	NAM RA: IN silico-based ingredient screening and risk assessment	Smoke-free oral product ingredients	Altria
	(Panel Discussion - TBD)		Imperial (Co-chair)

Target: A total of ~ 2hrs, 15 min each

CORESTA – NAM Symposium-II Planning – DRAFT ver Nov8 2022

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. Modern oral products: guidance documents and best practices for toxicology testing models

3. Oral models (2D vr 3D)

4. 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

Interdependencies/ watching brief

Interdependencies/ watching brief

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

- **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