Historical Use of Genetic Toxicology Data for Tobacco Product Stewardship

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Incheon, KOREA
November 15, 2017

Overview

- **Introduction of "CORESTA"**
- ***In Vitro* Toxicity Testing**
- **Task Force Establishment**
- **Proficiency Trials**
- **Whole Smoke**
- **Summary Observations**

CORESTA

Centre de
COopération pour les RECherches Scientifiques
Relatives au TAbac

Cooperation Centre for Scientific Research Relative to Tobacco

The Vision

To be recognised by our members and relevant external bodies as an authoritative source of publically available credible science and best practices related to tobacco and its derived products.

The Purpose of CORESTA

Encourage international cooperation

to actively work

on tobacco-related areas of research

❖ It is an Association:

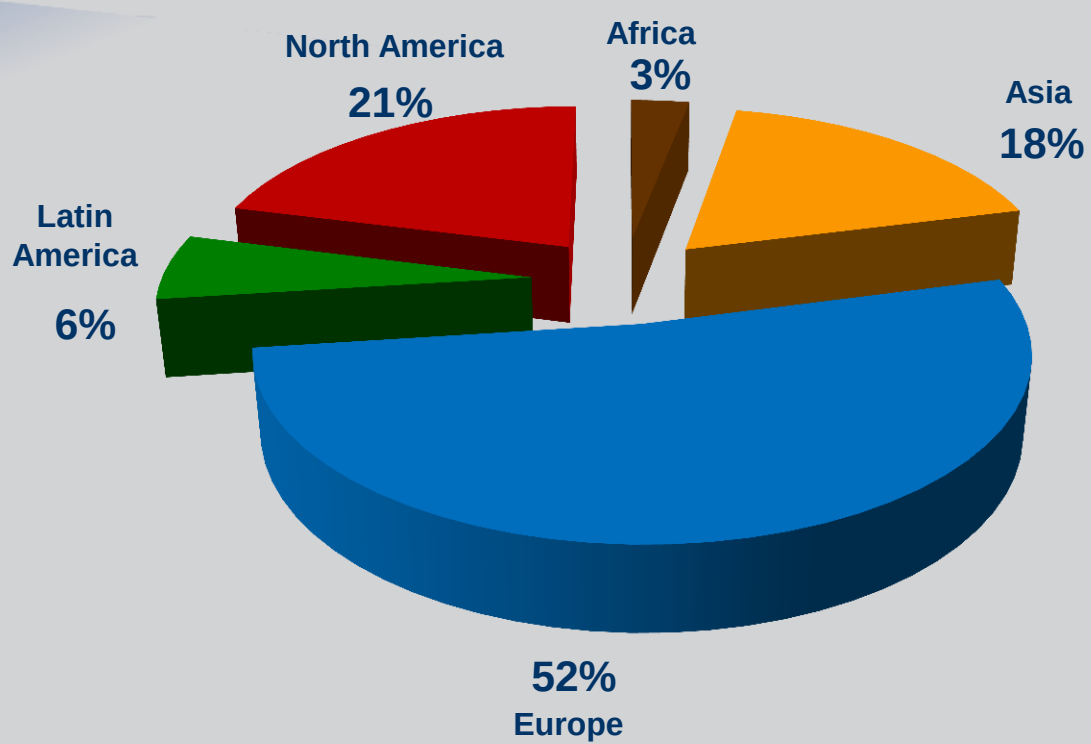
- ➢ Founded in 1956 by 24 organisations from 20 countries
- ➢ Headquartered in Paris and governed under French law
- ➢ Now 150 Member organisations from 38 countries

≈ 600 persons worldwide involved in on-going work

❖ Main bodies

- ➢ Board (12 to 14 organisations)
- ➢ Scientific Commission (20 individuals)
- ➢ General Secretariat (3 persons)
- ➢ 23 Sub-Groups and Task Forces within 4 Study Groups + 3 inter-group committees

Membership
Worldwide distribution



2 + 2 Study Groups

- **Smoke Science, Product Technology**
  - Technical specifications
  - Methods for component and emissions Analysis
  - Consumer behaviour
  - *In Vitro* Toxicology

Smoke - Techno

« SSPT »

- **Agronomy & Leaf Integrity, Phytopathology & Genetics**
  - Agronomy & Breeding
  - Curing
  - Sustainability
  - Pests & plant diseases
  - Agrochemical issues

Agro - Phyto

« AP »

Value of CORESTA

- Global interdisciplinary expertise from different sectors
- Focus on advancing scientific knowledge
- Leadership and coordination of inter-lab studies to recommend analytical methods

www.coresta.org

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In Vitro Toxicity Tests

- **Development of Test Guidelines and Test Batteries**
- ***In vitro* toxicity of cigarette smoke**
  - **Particulate matter**
  - **Whole Smoke**
- **Strengths, Limitations & Context**

Regulatory Standpoint

- **Health Canada:**
- "...annual toxicity testing on cigarette brands… manufacturers and importers are required to perform… three toxicity tests…no later than January 31, 2006"

- **USA FDA Center for Tobacco Products Guidance:**
  - ✓ Modified Risk Tobacco Product Applications: "FDA recommends… nonclinical studies to address the known clinical toxicities of tobacco products"
  - ✓ Premarket Review of New Tobacco Products: "You should generate data to evaluate these product properties using some combination of *in vitro*, *in vivo* and/or *ex vivo* studies"

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CORESTA Toxicity Task Force
Established in 2002

Mandate:

1. **To prepare a report covering the rationale and strategy** for conducting in vitro toxicity testing of tobacco smoke.

2. **To identify key procedures** based upon internationally recognized guidelines, adapted to accommodate the nature and unique properties of tobacco smoke.

Result:

2004 Report "The Rationale and Strategy for Conducting *In Vitro* Toxicology Testing of Tobacco" ***(available on CORESTA website)***

“Rationale & Strategy” Report

- **Recommended a test battery:**
  - **Bacterial mutagenicity assay**
  - **Mammalian cell assay for cytogenetics / mutation**
    - ***In vitro Micronucleus, Chromosome Aberration, or Mouse Lymphoma***
  - **Cytotoxicity assay**
- **Defined test item:**
  - **Cigarette smoke condensates (CSC), i.e., mainstream particulate / Cambridge filter pad / extracted in DMSO**
- **Provided background information, references and recommendations on methodology**

Interlaboratory Study

- **Purpose: To conduct the assays in individual laboratories following the Report recommendations**
  - **4 cigarettes**
    - 100% Flue Cured
    - 50/50 Flue/Burley
    - 100% Burley
    - Kentucky Reference 2R4F
  - **13 laboratories participated**
  - **Each lab smoked cigarettes, prepared extracts and used own internal methods---no common protocol**
    - Many variables
    - Experience
    - Sample preparation
    - Methodology & data analysis

Results & Recommendations

- **Ames: Good concordance**
- **NRU: No overall concordance**
- **MN: Trend but no "complete consensus"**

(Report available on CORESTA website)

Recommendations for Future Studies:

"adequate discussions and attention to experimental design and detail must be given to assure greater concordance"

New Mandate for the Task Force (2011)

“To conduct a proficiency testing programme to evaluate cigarette smoke using a common experimental protocol and the Task Force’s recommended test battery”

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Overview of Proficiency Studies

- **Objective: To improve study conduct & methods**
  - **Assays: Ames, NRU, MN**
  - **Cigarettes: Chosen for each study**
    - Proven to demonstrate differential response in that particular assay
  - **Test Sample Preparation Standardized**
    - Particulate matter extracted in DMSO
  - **Common Study Plans (Protocols) and Worksheets**
  - **Final data was evaluated by a Quality Assurance expert and a Statistician**
  - **All data was evaluated anonymously**

Proficiency Studies
First Round

Assay	Study Design	Conclusions
Ames (2008)	• 2 cigarettes • TA98, TA100 ± S9	• Ames assay was sufficiently sensitive to distinguish the two samples
NRU (2010)	• 3 cigarettes • 4 cell lines	• Cell line had an impact on toxicity ranking: differences were found in the ability of various cell lines to discriminate between samples
MN (2013)	• 3 cigarettes • 2 cell lines ± S9 • Other variables	• The ability to discriminate varied between the different S9 conditions

Proficiency Studies
Observations

- **Proficiency Studies require significant commitment**
  - **Protocol development**
  - **Sample Preparation**
  - **Smoking, Extraction & Shipping**
  - **Lab Study Manager & Personnel**
  - **Trial Coordinator**
  - **Auditors**
  - **Statisticians**
  - **Report Authors/Reviewers**

Proficiency Studies
Observations

- **Individual laboratories have learned from comparisons and discussions**
- **Study quality has improved over time**
- **Further improvements needed**
  - **Study Plans**
    - Balance detail and flexibility
    - Link more clearly to worksheets
  - **Worksheets: more detail/robust formatting**
  - **Documentation**

Proficiency Studies
Observations

- **Statistical analysis is challenging**
  - **Variations in methods & proficiency**
  - **Assay replication**
- **Test sample selection & preparation are an important component**
- **Important to understand/clarify objectives**
  - **Measure general trends**
  - **Determine discriminatory power**

Proficiency Studies
Second Round

- **Test cigarettes: Common test cigarettes produced**
  - 100% Flue
  - 100% Burley
  - Kentucky Reference 2R4F
- **On-going and upcoming studies**
  - MN (2016 - 2017): 9 labs, on-going
  - NRU (2017 -): 11 labs, on-going
  - MLA (2017 -): 4 labs, on-going
  - Ames (2018 -): 8 labs

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In Vitro Whole Smoke: Historical Perspective

- A wide variety of exposure systems
- Smoke exposure conditions not consistent
- Comparisons between set-ups are challenging
- A variety of biological endpoints and methods

2006 NRU Whole Smoke Study

- **Cigarettes were provided**
  - 3R4F; 100% Flue; 100% Burley; 50/50 Flue-Burley
- **Several exposure systems used**
  - Cultex, Borgwaldt, Burghart, TPM/GVP, BAT exposure chamber, Flask/rocker platform
- **Methods varied: cells, exposure, procedures**
  - CHO, HepG2, A549, H292, Balb/C
  - Submerged (fully or partial), air-liquid interface (ALI)
- **Few dosimetry tools available**

(Report available on CORESTA website)

In Vitro Whole Smoke Today

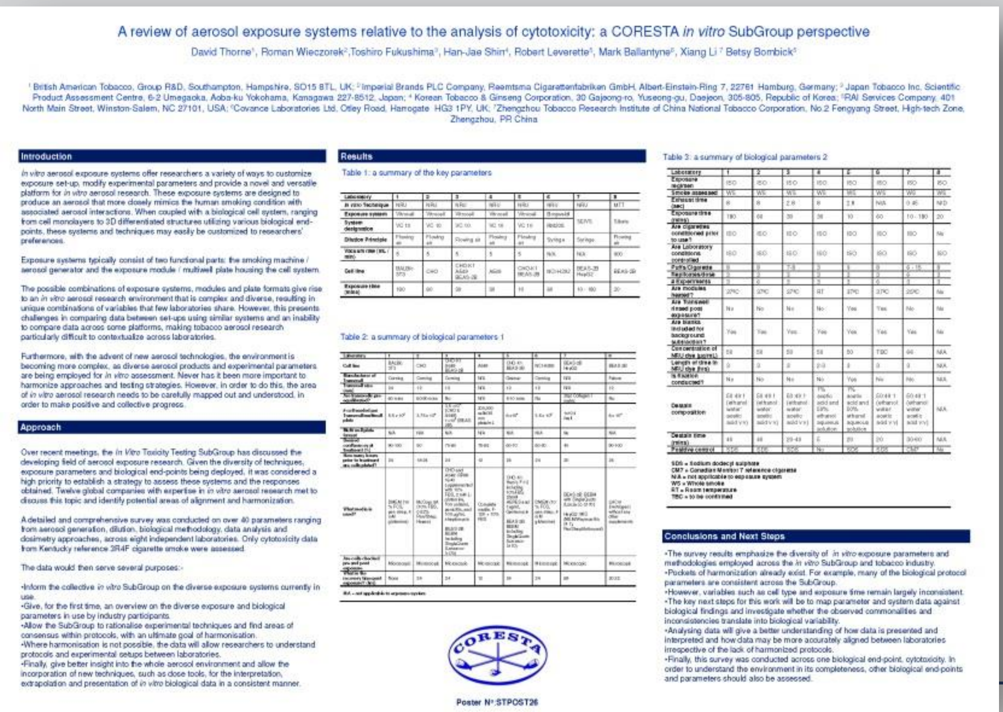
- More laboratories working in this area
- Variety of exposure systems in use
  - VitroCell, CULTEX, Burghart Bt020, Borgwaldt, others
- Greater alignment in technologies
  - More focus on Air-Liquid Interface (ALI)
- Wide variation in experience and understanding
- More dosimetry tools & markers
  - Photometers, CO, Deposited Mass (QCM), Carbonyls, Solanesol, CFD
  - Better understanding of their strengths and limitations is required

In Vitro Whole Smoke Today

- **Significant technical challenges in moving from CSC/TPM to WS**
  - **Characterization of smoke system**
  - **Characterization of exposure**
  - **Dosimetry assessment**
  - **Alignment of biological methodology**

In Vitro Whole Smoke Today

- **Poster: "Review of aerosol exposure systems relative to the analysis of cytotoxicity: a CORESTA *in vitro Toxicity* Sub-Group perspective"**



A review of aerosol exposure systems relative to the analysis of cytotoxicity: a CORESTA *in vitro* SubGroup perspective

David Thorne[1], Roman Wieczorek[2], Toshiro Fukushima[3], Han-Jae Shin[4], Robert Leverette[5], Mark Ballantyne[6], Xiang Li[7], Betsy Bombick[5]

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Introduction

In vitro aerosol exposure systems offer researchers a variety of ways to customize exposure set-up, modify experimental parameters and provide a novel and versatile platform for *in vitro* aerosol research. These exposure systems are designed to produce an aerosol that more closely mimics the human smoking condition with associated aerosol interactions. When coupled with a biological cell system, ranging from cell monolayers to 3D differentiated structures utilizing various biological end-points, these systems and techniques may easily be customized to researchers' preferences.

Exposure systems typically consist of two functional parts: the smoking machine / aerosol generator and the exposure module / multiwell plate housing the cell system.

The possible combinations of exposure systems, modules and plate formats give rise to an *in vitro* aerosol research environment that is complex and diverse, resulting in unique combinations of variables that few laboratories share. However, this presents challenges in comparing data between set-ups using similar systems and an inability to compare data across some platforms, making tobacco aerosol research particularly difficult to contextualize across laboratories.

Furthermore, with the advent of new aerosol technologies, the environment is becoming more complex, as diverse aerosol products and experimental parameters are being employed for *in vitro* assessment. Never has it been more important to harmonize approaches and testing strategies. However, in order to do this, the area of *in vitro* aerosol research needs to be carefully mapped out and understood, in order to make positive and collective progress.

Approach

Over recent meetings, the *In Vitro* Toxicity Testing SubGroup has discussed the developing field of aerosol exposure research. Given the diversity of techniques, exposure parameters and biological end-points being deployed, it was considered a high priority to establish a strategy to assess these systems and the responses obtained. Twelve global companies with expertise in *in vitro* aerosol research met to discuss this topic and identify potential areas of alignment and harmonization.

A detailed and comprehensive survey was conducted on over 40 parameters ranging from aerosol generation, dilution, biological methodology, data analysis and dosimetry approaches, across eight independent laboratories. Only cytotoxicity data from Kentucky reference 3R4F cigarette smoke were assessed.

The data would then serve several purposes:-

- Inform the collective *in vitro* SubGroup on the diverse exposure systems currently in use.
- Give, for the first time, an overview on the diverse exposure and biological parameters in use by industry participants.
- Allow the SubGroup to rationalise experimental techniques and find areas of consensus within protocols, with an ultimate goal of harmonisation.
- Where harmonisation is not possible, the data will allow researchers to understand protocols and experimental setups between laboratories.
- Finally, give better insight into the whole aerosol environment and allow the incorporation of new techniques, such as dose tools, for the interpretation, extrapolation and presentation of *in vitro* biological data in a consistent manner.

Results

Table 1: a summary of the key parameters

Table 2: a summary of biological parameters 1

Table 3: a summary of biological parameters 2

Conclusions and Next Steps

- The survey results emphasize the diversity of *in vitro* exposure parameters and methodologies employed across the *in vitro* SubGroup and tobacco industry.
- Pockets of harmonization already exist. For example, many of the biological protocol parameters are consistent across the SubGroup.
- However, variables such as cell type and exposure time remain largely inconsistent.
- The key next steps for this work will be to map parameter and system data against biological findings and investigate whether the observed commonalities and inconsistencies translate into biological variability.
- Analysing data will give a better understanding of how data is presented and interpreted and how data may be more accurately aligned between laboratories irrespective of the lack of harmonized protocols.
- Finally, this survey was conducted across one biological end-point, cytotoxicity. In order to understand the environment in its completeness, other biological end-points and parameters should also be assessed.

Poster N° STPOST26

(CORESTA Congress 2016)

In Vitro Whole Smoke Today

Key messages

- The survey results emphasize the diversity of in vitro exposure parameters and methodologies employed across the in vitro group and tobacco industry.
- Pockets of harmonization already exist. For example, many of the biological protocol parameters are consistent across the group. However, variables such as cell type and exposure time remain largely inconsistent.
- The key next steps for this work will be to map parameter and system data against biological findings and investigate whether the observed commonalities and inconsistencies translate into biological variability.
- Analyzing data will give a better understanding of how data is presented and interpreted and how data may be more accurately aligned between laboratories irrespective of the lack of harmonized protocols.
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What have we learned?

- **Proficiency Studies**
  - Important to understand specific objectives
  - Paying attention to detail is critical
    - Study Protocols, Worksheets and Documentation
  - There are significant complexities even when using common protocols
- **Understanding test items and smoke exposure systems**
  - Complexities of whole smoke studies
- **Understanding biological systems**
  - Cell lines
  - Variations in methodologies
- **Being open and wise regarding new / emerging *in vitro* models & technologies**

Looking Forward

- **Proficiency Studies: every 3-5 years**
- **Whole Smoke: continue strong emphasis**
  - **System Characterization & Dosimetry**
  - **Data expression**
  - **Future Inter-laboratory studies**
- **Consider other industry products**
  - **Smokeless, e-cigarettes**
- **Consider other biological endpoints**
  - **Satellite Meeting** (under planning):
    "Challenges and opportunities for new approach methodologies (NAMs) for next generation tobacco and nicotine products (NGP) regulatory science"

CORESTA CONGRESS CHINA YUNNAN 2018

At the CORESTA Congress: October 22-26, 2018 in Kunming, China

Between the Past and the Future

- Much has been accomplished, much yet to be done
- The field of *in vitro* toxicology is changing
- It is important to remain both inquisitive and focused

CORESTA Congress: October 22-26, 2018 in Kunming, China

Thank you!

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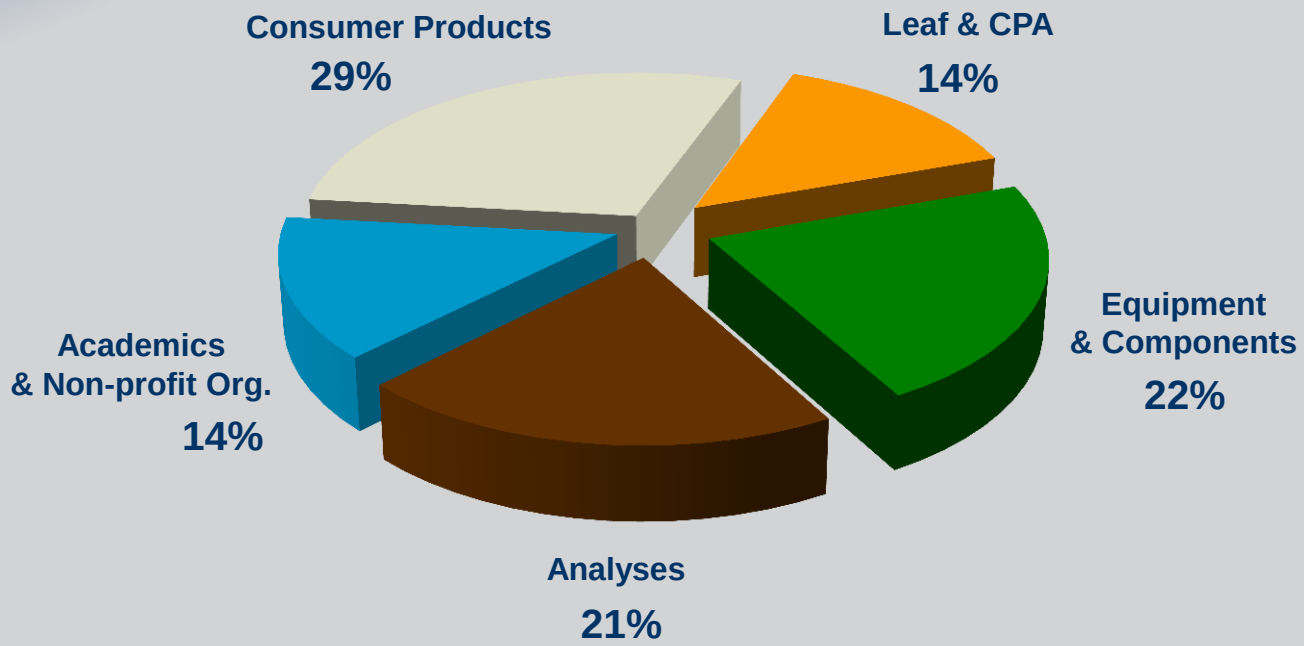
Membership
Core Activity



Stakeholder Engagement

- FDA Workshops
- Standards
  - ISO/TC 126
  - CEN/TC 437
  - AFNOR
- Agrochemical Seminars
- Conferences
  - Global Tobacco & Nicotine Forum
  - Tobacco Campus
  - E-cig Europe
  - US Tobacco Merchants Assoc.
  - Global Forum on Nicotine
  - …