Historical Use of Genetic Toxicology Data for Tobacco Product Stewardship

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2018 Genetic Toxicology Association Meeting
Newark, DE, USA
May 2, 2018

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:

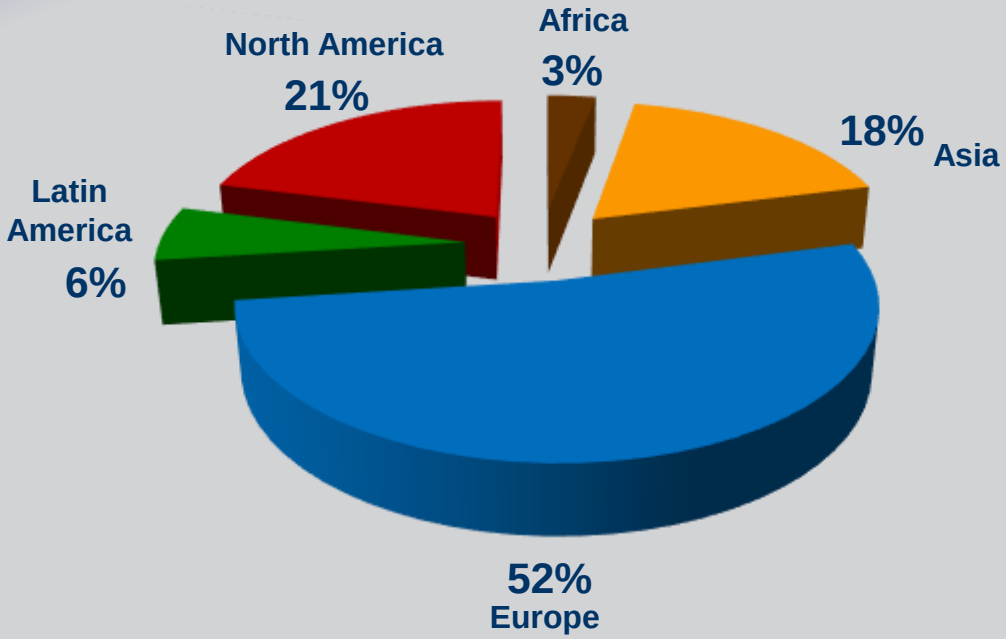
- Founded in 1956 by 24 organisations from 20 countries
- Headquartered in Paris and governed under French law
- Now 150 Member organisations in 38 countries
  involved in over 60 countries through their subsidiaries and affiliates

❖ Main bodies

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

≈ 600 persons worldwide involved in on-going work

Membership
Worldwide distribution



2 + 2 Study Groups

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

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

Agro - Phyto

« AP »

Smoke - Techno

« SSPT »

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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- **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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Establishment of the CORESTA Toxicity Task Force: 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**

- **Purpose: To conduct the assays in individual laboratories following the Report recommendations**
  - **4 cigarettes**

    100% Flue 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 Trials

- **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 Trials

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 Trial Observations

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

Proficiency Trial 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 Trial 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

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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

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

- **Poster: "Review of aerosol exposure systems relative to the analysis of cytotoxicity: a CORESTA in vitro Sub Group perspective": CORESTA Congress 2016**


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

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What have we learned?

- **Proficiency Trials & Interlaboratory 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 Testing: every 3-5 years**
- **Whole Smoke: continue strong emphasis**
  - System Characterization & Dosimetry
  - Data expression
  - Future Interlaboratory studies
- **Consider other industry products**
  - Smokeless, e-cigarettes
- **Consider other biological endpoints**

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

THANK YOU!