



## **Historical Use of Genetic Toxicology Data for Tobacco Product Stewardship**

**Kei Yoshino**

**Coordinator, *In Vitro* Toxicity Testing Sub Group - CORESTA**

**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



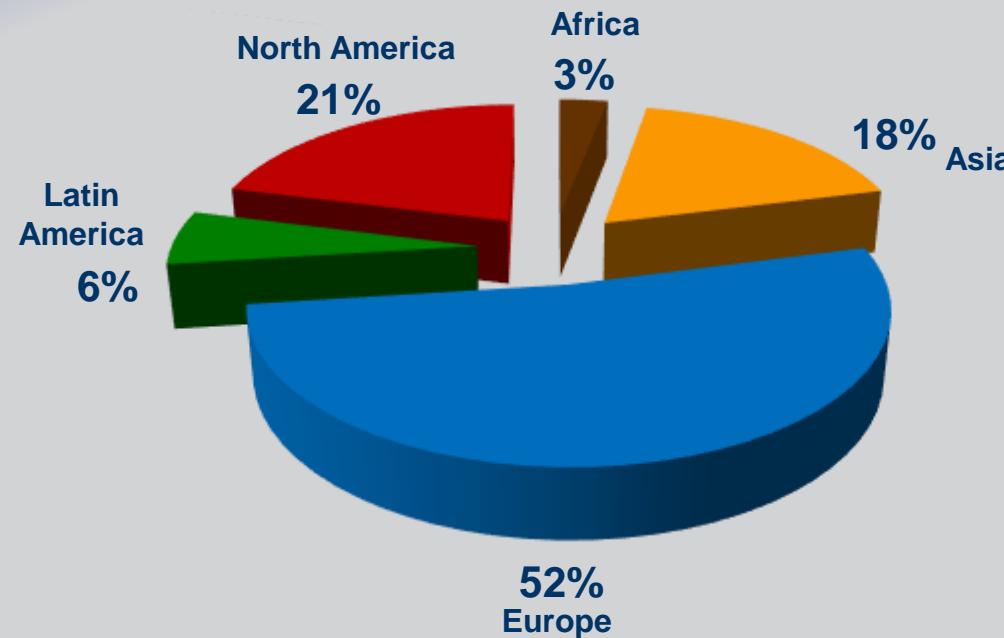
## ❖ It is an Association:

- 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**





## 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](http://www.coresta.org)



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



- ❖ **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”



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



## 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



## Interlaboratory Study

❖ 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”**



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



# 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
<b>Ames (2008)</b>	<ul style="list-style-type: none"><li>• 2 cigarettes</li><li>• TA98, TA100 ± S9</li></ul>	<ul style="list-style-type: none"><li>• Ames assay was sufficiently sensitive to distinguish the two samples</li></ul>
<b>NRU (2010)</b>	<ul style="list-style-type: none"><li>• 3 cigarettes</li><li>• 4 cell lines</li></ul>	<ul style="list-style-type: none"><li>• Cell line had an impact on toxicity ranking: differences were found in the ability of various cell lines to discriminate between samples</li></ul>
<b>MN (2013)</b>	<ul style="list-style-type: none"><li>• 3 cigarettes</li><li>• 2 cell lines ± S9</li><li>• Other variables</li></ul>	<ul style="list-style-type: none"><li>• The ability to discriminate varied between the different S9 conditions</li></ul>



# 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



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



## ***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)*



- ❖ 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



# In Vitro Whole Smoke Today

## ❖ 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 Sub Group perspective**

David Threlfall<sup>1</sup>, Hervé Blascomer<sup>2</sup>, Sohème Pukallusmaa<sup>3</sup>, Hans-Joachim Bönnig<sup>4</sup>, Hélène Lemaire<sup>5</sup>, Mark Ballantyne<sup>6</sup>, Ruiqiang Li<sup>7</sup>, Mandy Bremont<sup>8</sup>

<sup>1</sup> British American Tobacco, Group R&D, Research Triangle, Research Triangle Park, NC 27701, USA; <sup>2</sup> Imperial Tobacco, Wissenschafts- und Entwicklungszentrum (IWE), Adlershofstrasse 9, 12487 Berlin, Germany; <sup>3</sup> Japan Tobacco Inc., Research & Product Development Center, 6-2 Umezono-cho, Kanagawa-ku, Yokohama 221-8522, Japan; <sup>4</sup> British American Tobacco (Ireland) Ltd., Duleek, Co. Meath, Ireland; <sup>5</sup> Philip Morris Companies Europe, 22 Dufour Street, Geneva 1000, Switzerland; <sup>6</sup> Philip Morris Companies Europe, 22 Dufour Street, Geneva 1000, Switzerland; <sup>7</sup> Philip Morris Products Institute, 1250 Peachtree Street, Atlanta, GA 30309, USA; <sup>8</sup> Philip Morris Products Institute, 1250 Peachtree Street, Atlanta, GA 30309, USA

**Abstract:** In vitro aerosol exposure systems offer researchers a variety of ways to measure exposure and/or modify experimental procedures and provide a tool that will enable further research on the effects of smoke. These systems have been developed to address the need to understand the烟道气-dose-response relationship, the mechanisms of action and the toxicological properties of smoke. These systems have been developed to address the need to understand the烟道气-dose-response relationship, the mechanisms of action and the toxicological properties of smoke. These systems have been developed to address the need to understand the烟道气-dose-response relationship, the mechanisms of action and the toxicological properties of smoke.

**Keywords:** The possible constituents of exposure systems, including cell culture methods plus the use of animal models, and their applications in the analysis of cytotoxicity. The use of these systems in exposure systems of variables that has been addressed. However, there is a challenge in comparing data obtained and yet using similar systems and an equally difficult to make an informed assessment of the reliability of different systems. This presentation will highlight some available and forthcoming developments in this field.

**Introduction**

1. Introduction

2. Cell culture

3. Animal models

4. Summary

**Results**

Table 1: A summary of the key parameters

Parameter	Value	Comments
Exposure time	30 min	Most studies
Exposure conditions	100% smoke	Most studies
Concentration	100% smoke	Most studies
Cell type	Human fibroblasts	Most studies
Cell density	10,000 cells/cm²	Most studies
Dose	100% smoke	Most studies
Incubation time	24 h	Most studies
Incubation conditions	37°C, 5% CO <sub>2</sub>	Most studies
Assay	MTT	Most studies
Significance	P < 0.05	Most studies

Table 2: A summary of biological parameters

Parameter	Value	Comments
Exposure time	30 min	Most studies
Exposure conditions	100% smoke	Most studies
Concentration	100% smoke	Most studies
Cell type	Human fibroblasts	Most studies
Cell density	10,000 cells/cm²	Most studies
Dose	100% smoke	Most studies
Incubation time	24 h	Most studies
Incubation conditions	37°C, 5% CO <sub>2</sub>	Most studies
Assay	MTT	Most studies
Significance	P < 0.05	Most studies

Table 3: A summary of biological parameters II

Parameter	Value	Comments
Exposure time	30 min	Most studies
Exposure conditions	100% smoke	Most studies
Concentration	100% smoke	Most studies
Cell type	Human fibroblasts	Most studies
Cell density	10,000 cells/cm²	Most studies
Dose	100% smoke	Most studies
Incubation time	24 h	Most studies
Incubation conditions	37°C, 5% CO <sub>2</sub>	Most studies
Assay	MTT	Most studies
Significance	P < 0.05	Most studies

**Table 1: A summary of the key parameters**

Parameter	Value	Comments
Exposure time	30 min	Most studies
Exposure conditions	100% smoke	Most studies
Concentration	100% smoke	Most studies
Cell type	Human fibroblasts	Most studies
Cell density	10,000 cells/cm²	Most studies
Dose	100% smoke	Most studies
Incubation time	24 h	Most studies
Incubation conditions	37°C, 5% CO <sub>2</sub>	Most studies
Assay	MTT	Most studies
Significance	P < 0.05	Most studies

**Table 2: A summary of biological parameters**

Parameter	Value	Comments
Exposure time	30 min	Most studies
Exposure conditions	100% smoke	Most studies
Concentration	100% smoke	Most studies
Cell type	Human fibroblasts	Most studies
Cell density	10,000 cells/cm²	Most studies
Dose	100% smoke	Most studies
Incubation time	24 h	Most studies
Incubation conditions	37°C, 5% CO <sub>2</sub>	Most studies
Assay	MTT	Most studies
Significance	P < 0.05	Most studies

**Table 3: A summary of biological parameters II**

Parameter	Value	Comments
Exposure time	30 min	Most studies
Exposure conditions	100% smoke	Most studies
Concentration	100% smoke	Most studies
Cell type	Human fibroblasts	Most studies
Cell density	10,000 cells/cm²	Most studies
Dose	100% smoke	Most studies
Incubation time	24 h	Most studies
Incubation conditions	37°C, 5% CO <sub>2</sub>	Most studies
Assay	MTT	Most studies
Significance	P < 0.05	Most studies

**Conclusion and Future Work**

The results clearly emphasize the diversity of in vitro exposure conditions and methodologies employed. Given the in vitro findings and test methods, the influence of human cell lines on烟道气 cytotoxicity is clear. The analysis of the literature suggests that there are significant differences in the way that烟道气 is prepared and tested, with no ultimate point of homogeneity. The development of more reliable and accurate measures of烟道气 cytotoxicity is a necessary objective for烟道气 research. Privately, great effort is being put into烟道气 exposure conditions and other烟道气 parameters, such as dose, time, and exposure conditions. For the moment, it is not possible to present a set of烟道气 parameters or烟道气 biological data as a consensus model.

**CORESTA**  
House # 67000738



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



## 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



- ❖ 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!