# CORESTA

## Sub-Group *In Vitro* Toxicity (IVTSG) Report

**Jeju, South Korea**

**October 8, 2015**

# Agenda

- SG Composition
- History
- Objective 1 : Proficiency testing
- Objective 2 : Direct Exposure Method
- Objective 3: Review available information and suggest suitable work
- Summary

# SG Composition

- **SG Coordinator**
  - Kei Yoshino (JT)
- **SG Secretary**
  - Betsy Bombick (RJRT)
  - ⇩
  - David Thorne (BAT)
- **SG Members:**

| | | |
|---|---|---|
| - PMI | - BAT | - RJRT |
| - ITG | - KT&G | - CNTC |
| - Labstat | - Battelle | - Covance |
| - Enthalpy | - Vitrocell | - JT/JTI/Oekolab |

# History

2002: The first Task Force was established

2004: The Task Force recommended a test battery

2007: Whole smoke exposure system: Preliminary inter-laboratory study was done

2010: Task Force recommended disbanding.

2011: The second phase of the In vitro Task Force was established.

1) 3 Proficiency studies (Ames, NRU, and MN) with CSC
2) Discussion on whole smoke exposure test methods: mainly focusing on Dosimetry

# Objectives

2015: The Task Force was changed to the Sub-Group with the following new objectives:

1) To conduct a proficiency testing programme to evaluate cigarette smoke using common experimental protocols and the SubGroup's recommended test battery.

2) To compile and review information on in vitro whole smoke methodology.

3) To critique and review published papers and other available information on tobacco-related toxicity and suggest suitable work for further biological research and/or proficiency tests.

# Meetings

- May 18, 2015: Neuchâtel (Host: PMI)
- October 3, 2015: Jeju (Host: KT&G)

**Next meeting:**

- March 12, 2016: New Orleans (TBD)
- Host: Battelle & RJRT

# Objective 1

Objective 1:

To conduct a proficiency testing programme to evaluate cigarette smoke using common experimental protocols and the SubGroup's recommended test battery.

- Test cigarettes
- Study Design

# Objective 1 : Test Cigarettes

## ❖ Test Cigarettes used in the past studies

- ➢ Preliminary inter-laboratory study:
  - FC 100%, Bly 100%, FC 50% : Bly 50% (BAT)
- ➢ Proficiency studies
  - Ames: Canadian Monitor 8 & 3R4F
  - NRU: “Base Web” and “Recon” (ITG) + 3R4F
  - MN: FC 100% and BLY 100% (JT) + 3R4F
- ➢ The group prefers to use the same test cigarettes for upcoming studies.

## ❖ Required specifications for future test cigarettes

- ➢ Single grade FC and BLY to be used. Cigarette specifications (paper, filter, etc) other than cut filers should be matched as much as possible.
- ➢ Tar yield should be similar to the market products (not to exceed 10 mg/cig in tar yield)

## ❖ JT Volunteered to produce test cigarettes

# Objective 1 : Test Cigarettes

## ❖ Specification (final)

| Lot # | Type of tobacco | Tobacco weight | Size (Length & Circumference) | Cigarette paper | Filter plug & Tow spec | Vf |
|---|---|---|---|---|---|---|
| Lot 1 | 100% FC (Zimbabwe) | 646 mg | 57+27 mm, ϕ 24.6 mm | 35 CU | Cellulose acetate, 2.8Y35 | 34 |
| Lot 2 | 100% BLY (Brazil) | 614 mg | | | | 37 |
| 3R4F | Blend | 775 mg | 57+27 mm, ϕ 24.9 mm | 24 CU | Cellulose acetate, 2.9Y41 | 29 |

2.09% of glycerin* was added to the tobacco filler to maintain the moisture content of the cut tobacco filler during storage.

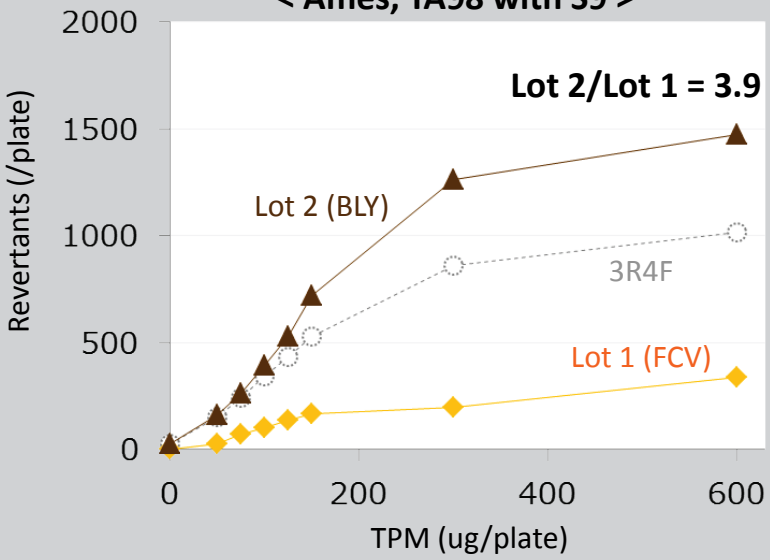
** 2.67% for 3R4F*

# Objective 1 : Test Cigarettes

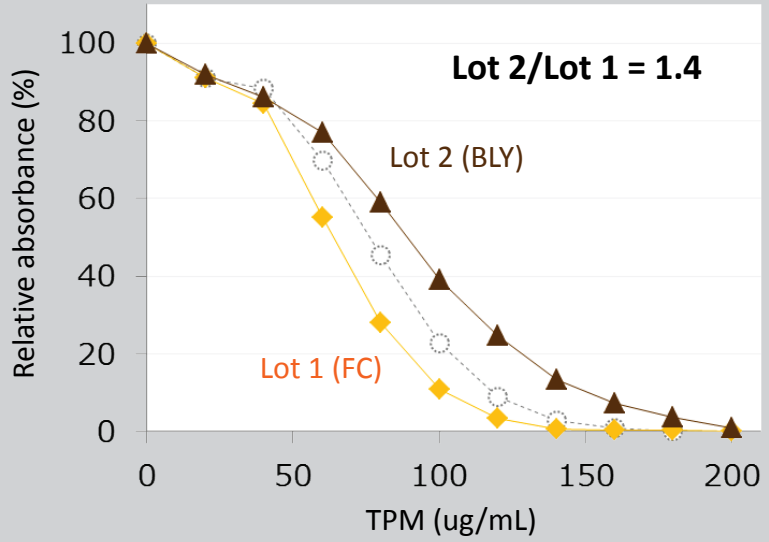
## ❖ Preliminary analysis (small scale production)

| | Component analysis (%-DB) | | | Smoke analysis (mg/cigarette) | | | |
|---|---|---|---|---|---|---|---|
| | VBN | Sugars | Nicotine | TPM | Tar | Nicotine | CO |
| Lot 1 (FC) | 0.41 | 22.4 | 2.25 | 13.1 | 10.9 | 1.07 | 11.0 |
| Lot 2 (BLY) | 1.39 | 0 | 5.00 | 14.1 | 10.8 | 2.19 | 10.5 |
| cf. 3R4F | n.a. | 8.7 | n.a. | 11.0 | 9.4 | 0.73 | 12.0 |

**< Ames, TA98 with S9 >**




**< NRU >**



# Objective 1 : Test Cigarettes

## ❖ Storage and Delivery

- ✓ 60,000 cigarettes for each were produced, expecting to be used for 10 years (expecting one study/year and 10 participants/study)
- ✓ Cigarettes will be stored below -20°C
- ✓ Stability check (SCA) will be performed annually
- ✓ JT will store and deliver test cigarettes.

# Objective 1 : Study Design

## Background

- Provide laboratory performance feedback
- Support ISO accreditation
  - Accept protocol differences in each lab
- “Inter-laboratory study” rather than “Proficiency study”

- Common rationale on study design should be applied to a series of studies : Ames, NRU, Micronucleus (MN) and MLA
- 7 labs are interested in MN assay (Fall 2015)
- NRU (Spring 2016), MLA (Fall 2016) are planned

# Objective 1 : Study Design

## Study Design (MN assay)

- Study objective (TBD): To evaluate the capability of MN testing to discriminate the genotoxic potential of TPM extracts
- Samples: FCV, BLY and 3R4F
- Smoking and extraction will be done in each lab
  - Nicotine in TPM samples will be measured in one lab
- Cell line: CHO, V79 or CHL with or without Cyt.B (Selectable)
- Dose setting: at least 3 doses (non-zero and non-toxic dose) : TBD
  - Maximum dose : laboratory's discretion
  - Doses higher than OECD TG (50-60% of cytotoxicity) will be omitted from overall analysis for reporting

# Objective 1 : Study Design

## Responsibilities (MN assay)

- Study coordinator: E.Weber (JTI/Oekolab)
- Co-coordinator: T.Fukushima (JT)
- Statistician: A.Hauleithner (JTI/Oekolab)
- Nicotine analysis (TPM samples): JT (TBC)

# Objective 2

Objective 2:

To compile and review information on in vitro whole smoke methodology.

# Objective 2

- Following options were discussed at the Fall 2014 meeting
  - Option 1: Summarize current information available
  - Option 2: Conduct exploratory work (NRU) with common cell line (+ each preferred cell line) and summarize power of available dosimetry tools with biological data
- Information (over 40 parameters) was provided from 7 groups (JT, Lorillard, BAT, ITG, RJR/Covance, KT&G, ZTRI) and collated in a spreadsheet by Betsy Bombick (RJRT).
  - Instrumental Set-Up
  - Assay conditions: NRU and Ames (TA98 and TA100)
  - Exposure conditions
- David Thorne (BAT) suggested areas for potential consensus

# Objective 2

## Summary observations

- Many exposure parameters and biological variables already consistent
- Many larger variables, such as cell types and exposure times are not
- Data such as NRU is not comparable as the expression is different from each lab – We need to agree a unified way to express data to make starting comparisons
- Despite the varied exposure conditions, all labs see a consistent positive response in both Ames strains TA98 and TA100
- Alignment of terminology – minimum

- Decide what to do with collated information – this is probably the largest whole smoke collation of information across the 'industry' to date - potentially very powerful.

# Objective 2

## ❖ Next steps / Proposed actions

- ➢ Update collated information with biological data for Ames & NRU
  - 3R4F and CM7 as available
- ➢ Propose a publication (as the first step) to the SC
- ➢ Proficiency Trial based on summary data (if necessary as the second step)

# Objective 3

Objective 3:

To critique and review published papers and other available information on tobacco-related toxicity and suggest suitable work for further biological research and/or proficiency tests.

- Communication with IIVS (Institute of In Vitro Sciences)
- TPD2
- Literature survey scheme

# Objective 3: IIVS

## Institute for In Vitro Sciences (IIVS)

- Non-profit ("charity") laboratory in 1997 which promotes the use and acceptance of in vitro (non-animal) methods for toxicology
- IIVS interacts internationally with governments, regulatory agencies and NGOs to identify non-animal approaches to resolve complex problems
- Non-CORESTA Member

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

## Workshop "Assessment of In Vitro COPD models for Tobacco Regulatory Science": December 8-10, 2014 Bethesda, MD

- Supported by FDA-CTP R13 grant
- Attended by over 60 participants (academia, industry, government, vendors & stakeholders)
- Candidates of Assays: Goblet cell hyperplasia, Mucus production, Ciliary beating frequency

# Objective 3: IIVS

- **Discussion with IIVS (Spring meeting 2015)**
  - Core areas will include:
    - Air liquid interface (ALI)
    - in vitro dosimetry determinations
    - tobacco smoke / e-cigarette aerosols
    - microenvironment exposure/physiology of cells
    - dosimetry approaches / models
  - Validation: Actual validation can take a very long time
  - The final goal is regulatory acceptance of at least some endpoints, with CROs being able to provide these services under GLP conditions.
  - **What does IIVS expect from this Subgroup?**
    - IIVS is very interested in inhalation toxicology and learning from CORESTA members.
    - IIVS is interested in whole smoke/aerosol exposures. IIVS needs to understand what is happening at the exposure/cell surface, and what is impacting dosimetry.
- **The group will keep contact with IIVS.**

# Objective 3: TPD2

## ❖ Knowledge among us (Literature basis)

| | | Altria/PMI | BAT | RJRT | ITG | Lorillard | JT |
|---|---|---|---|---|---|---|---|
| **C** Carcino-genicity | vitro | ・CTA (Bhas 42) | ・CTA(SHE)<br>・AIG | - | - | - | ・CTA (Bhas 42) |
| | vivo | - Skin Painting | - | - Skin Painting | - Skin painting | - Skin painting | - Skin painting |
| **M** Mutagenicity | vitro | ・Ames<br>・MLA<br>・Comet (WS) | ・Ames<br>・MN<br>・MLA<br>・Comet<br>・γH2AX | ・Ames<br>・SCE | ・Ames<br>・MN | ・Ames<br>・MN | ・Ames<br>・MN<br>・MLA |
| | vivo | ・MN (13w inhalation) | - | - | - | - | ・MN (13w inhalation) |
| **R** Reprotox | vitro | - | - | - | - | - | - |
| | vivo | ・One Generation Reproductive Toxicity study*1<br>・Prenatal Development Toxicity Study (modified) *2 | - | - Zebra fish (SOT 2015) | - | - Develop-mental toxicity | - |

# Objective 3: TPD2

## CTA (cell transformation assays)

- Some members expressed interest in CTA
- BAT, PM, JT work, ECVAM protocols
- Guideline situation:
  - Two cell lines: SHE & Bhas42
  - OECD: Guidance document for both cell lines, not for carcinogenicity but as an indicator;  STILL NOT RECOMMENDED
  - Weight of evidence use---could use in WOE context, not as a solo endpoint
- CTA not currently compatible with Air Liquid Interface (ALI)

# Objective 3: TPD2

## Reprotox

- The definitive animal test for evaluating potential developmental toxicity is the prenatal developmental toxicity test [e.g., OECD Test Guideline (TG) 414].
- The latest REACH guidance documentation, broadly describes reproductive toxicity as “effects on fertility and development”.
- Additionally, in Feb (2015) ECHA announced that the “Extended One Generation Reproductive Toxicity study” would be a key information requirement for reproductive toxicity in REACH instead of the two-generation reproductive toxicity study (OECD TG 416). This is a clear indication that for this particular hazard endpoint, there is a trend to reduce animal testing, but as yet, there is no animal free alternative addressing both fertility and development.
- ICH S5(R3) group is also discussing animal replacement with in vitro testing, but it may take long time to reach consensus.

# Objective 3: TPD2

## Reprotox (cont.)

- To date, ECVAM (European Centre for Validation of Alternative Methods) Scientific Advisory Committee (ESAC) has only endorsed the use of three in vitro methods for toxicity testing.
  - Embryonic stem cell test (EST)
  - Micromass test - Note: Animal sacrifice required
  - Whole rat embryo culture (WEC) - Note: Animal sacrifice required

## The Group will keep eyes on recommendation from authorities.

# Objective 3:
# Literature Survey Scheme

## Background

- Guidelines and regulation exist for in vitro assays: OECD & Health Canada
- Authorized sample preparation methods:
  - TPM: ISO and Health Canada
  - GVP: Health Canada
  - Direct exposure (whole smoke): Not available
- No recommended sample preparation methods is available for Emerging Products (EP)

## The group agreed setting up systematic & continuous review scheme on literatures regarding EP (e-cigarettes, heat not burn, e-liquid): monthly basis

- Aerosol Generation
- Collection Method
- Exposure Method
- In vitro Endpoints: Assay & Test System
- Dosimetry

# Objective 3:
# Small Working Groups

- **Information Survey**
  - Kei Yoshino (JT), Roman Wieczorek (ITG), Amit Trivedi (Labstat), Alexandra Martin (Enthalpy), Victoria Ortner (JTI/Oekollab), Oliver Moennikes (PMI)
- **In vitro methods for Emerging Products**
  - Jacqui Miller(JTI), Tobias Krebs(Vitrocell), Kei Yoshino(JT), Gregory Rodrigo(PMI), Tanja Miehl(JTI/Oekolab)
- **TPD2**
  - Jacqui Miller(JTI), Gregory Rodrigo(PMI), April Brys(Battelle), Elisabeth Weber(JTI/Oekolab), Yuki Kanemaru(JT)
- **Whole Smoke**
  - David Thorne(BAT), Toshiro Fukushima(JT), Pingping Shang(ZTRI), Robert Leverette (RJR), others

# Overall Summary

- **Objective 1 : Proficiency testing**
  - Test cigarettes prepared
  - MN inter-laboratory study will be ready to be initiated
- **Objective 2 : Direct Exposure Method**
  - The group will collate needed information and propose a publication
- **Objective 3: Review available information and suggest suitable work**
  - The group will continuously watch science and regulatory updates

# Thank you!