In Vitro Toxicity Testing Sub-Group (IVT SG) 2017 Annual Report

Kitzbühel, Austria

October 12, 2017

IVT SG Membership

- **SG Coordinator: Kei Yoshino (JT)**
- **SG Secretary: David Thorne (BAT)**
- **SC Liaison: Kei Yoshino (JT)**
- **SG Membership**
  - **BAT, Battelle, CNTC, Covance, Enthalpy, ITG, JTI, JTI/Oekolab, KT&G, Labstat, PMI, RAI, Vitrocell, JT, Charles River Laboratories**

Objectives

- **Objective 1: To compile and review information on *in vitro* toxicity testing and apply learnings to further biological research.**
- **Objective 2: To organize and conduct periodically proficiency testing of tobacco and tobacco related products.**

Meetings

Recent Two Meetings

March 4, 2017: Baltimore, US

- 26 delegates (+1 guest) attended the meeting
- Meeting was hosted by Altria Client Services

October 8, 2017: Kitzbühel, Austria

- 31 delegates attended the meeting

Upcoming Meetings

January 2018: Southampton, UK

- Meeting will be hosted by BAT

Projects

Project	Status	Company	Responsibilities
Whole Smoke (#72)	Draft Publication Under Revew	BAT, ITL, JT, KT&G, Covance, CNTC, RAI	Authors = D. Thorne (BAT), R. Wieczorek (ITL), T. Fukushima (JT), H. Shin (KT&G), R.Leverette (RAI), Mark Ballantyne (Covance), Xiang Li (CNTC), Betsy Bombick (RAI)
iVMN (#110)	Draft Report Under Review	ALCS, CNTC, Covance, Enthalpy, Oekolab, Labstat, PMI, ITG, JT	Coordinator = E. Weber (JTI Oekolab) Co-coordinator = T. Fukushima (JT) Statistical analysis = A.hauleithner (JTI Oekolab)
MLA (#164)	2017 start	PMI, Oekolab, Covance, ZTRI	Coordinator = D. Smart (PMI) Co-coordinator = E. Weber (JTI) Statistical Analysis = A.hauleithner (JTI Oekolab)
NRU (#165)	2017 start	ITL, PMI, KT&G, Covance, JTI, Labstat, ZTRI, Altria	Coordinator = K. Yoshino (JT) Co-coordinator = R. Wieczorek (ITG) Statistical Analysis = A.hauleithner (JTI Oekolab)
Ames	2018 start	PMI, KT&G, ITL, Labstat, Covance	Coordinator = R. Wieczorek (ITG) Co-cordinator = E. Weber (JTI Oekolab) Statistical Analysis = A.hauleithner (JTI Oekolab)

Proficiency Studies

Objectives

- Evaluation of the proficiency of the participating laboratories
- Assessment of the discriminatory power of the test towards different tobacco products

Responsibilities

- Coordinator: Elisabeth Weber (JTI/Oekolab)
- Co-Coordinator: Toshiro Fukushima (JT)
- Statistical analysis: Alexander Hauleithner (JTI/Oekolab)

Basic agreement

- Test cigarettes: 3R4F, 100% FCV and BLY
- Study procedure: Follow lab's own protocol
- Lab Performance: Coefficient of Variance (Standard Deviation in percent of Mean)

List of Participants

- Altria Client Services LLC
- China National Tobacco Quality Supervision & Test Center
- Covance UK
- Enthalpy
- Imperial Tobacco
- Labstat
- Phillip Morris International
- JTI Oekolab : Study Coordination, Statistical Analysis, Technical Report
- JT: Study Co-coordination, Providing test pieces, Nicotine analysis

Assay Conditions

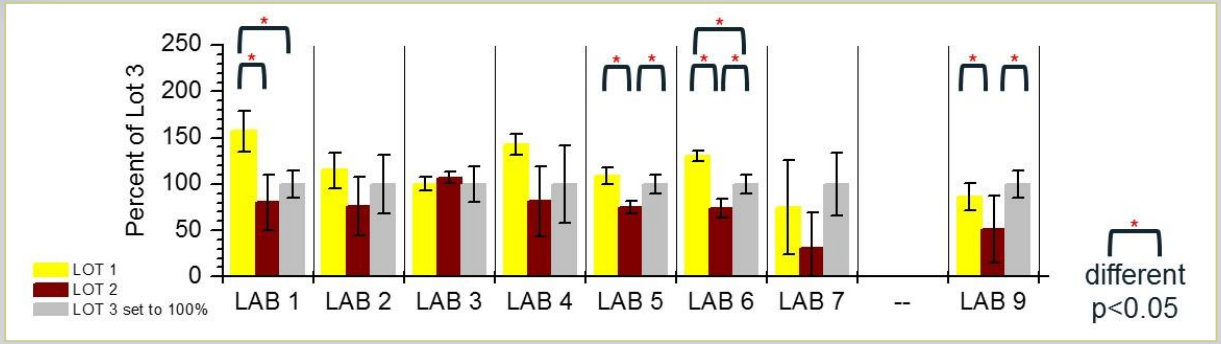
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9
Cell Line	V79	CHO WBL	CHO WBL (IVGT)	V79	CHL/IU	CHO-K1	TK6	V79	CHO-K1
CytB	YES	NO	NO	YES	NO	YES	NO	NO	NO
Scoring	Automated Microscope	Flow Cytometry	Manual	Manual	Automated Microscope	Manual	Manual	Automated Microscope	Manual
Staining	DAPI		Acridine Orange	Acridine Orange	DAPI - Cell Mask Orange	DAPI	Acridine Orange	DAPI	Acridine Orange

Treatments

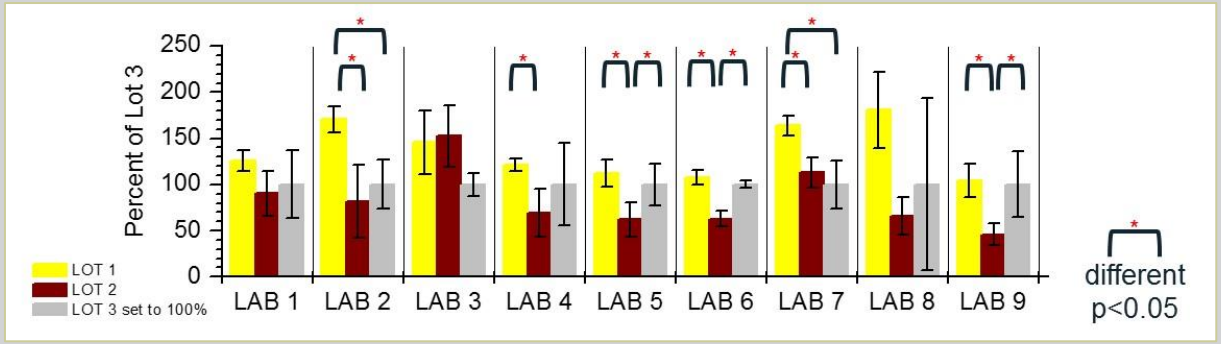
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9
SHORT -S9	X	X	X	X	X	X	X		X
SHORT +S9	X	X	X		X	X	X	X	X
LONG		X	X			X	X		

❖ Ranking

SHORT - S9

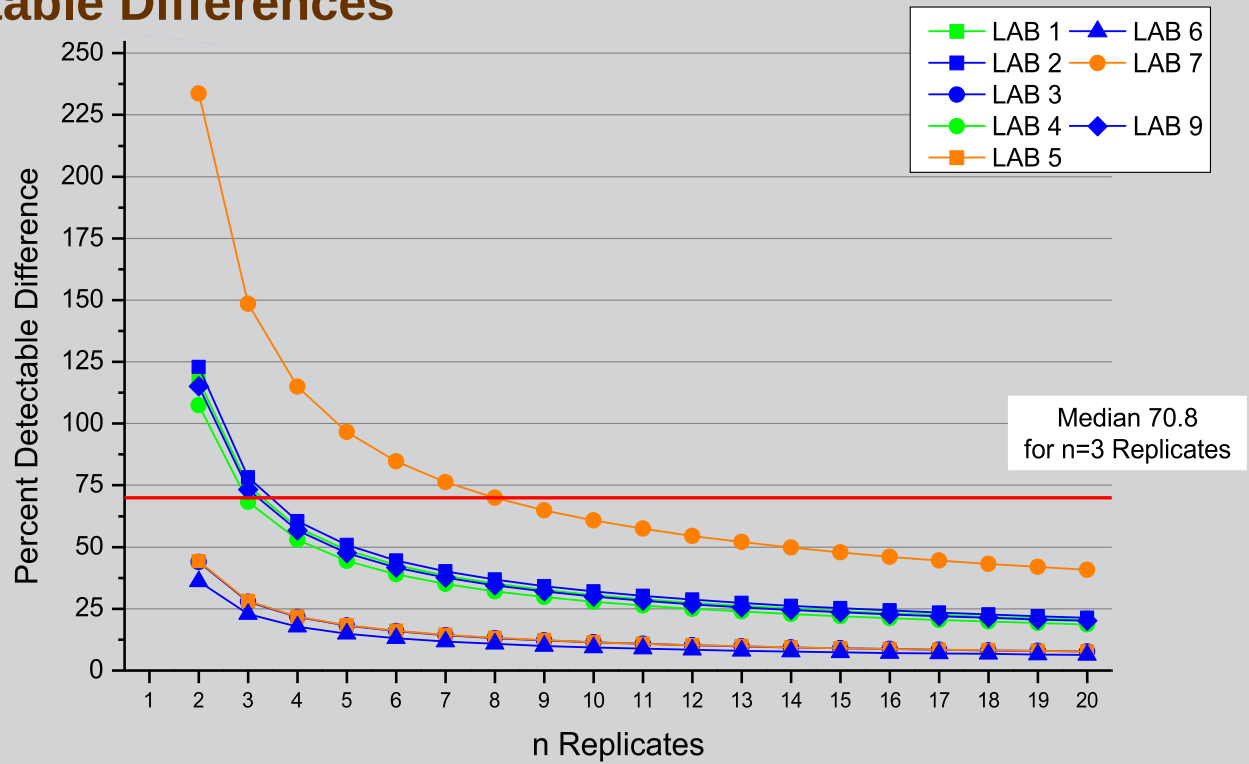


SHORT + S9



Minimum Detectable Differences

Short -S9



❖ MDD

Short -S9

n	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9
	V79	CHO-K1	CHO WBL (IVGT)	V79	CHL/IU	CHO-K1	TK6	V79	CHO-K1
2	117,7	122,9	43,9	107,4	44,3	36,1	233,6		115,1
3	74,8	78,2	27,9	68,3	28,2	22,9	148,5		73,2
4	57,9	60,5	21,6	52,9	21,8	17,8	115,0		56,7
5	48,7	50,8	18,2	44,4	18,3	14,9	96,6		47,6
6	42,7	44,6	15,9	38,9	16,1	13,1	84,7		41,7
7	38,4	40,1	14,3	35,1	14,5	11,8	76,2		37,6
8	35,2	36,8	13,1	32,1	13,3	10,8	69,9		34,4
9	32,7	34,1	12,2	29,8	12,3	10,0	64,9		32,0
10	30,6	32,0	11,4	27,9	11,5	9,4	60,8		30,0
11	28,9	30,2	10,8	26,4	10,9	8,9	57,4		28,3
12	27,4	28,7	10,2	25,0	10,3	8,4	54,5		26,8
13	26,2	27,4	9,8	23,9	9,9	8,0	52,0		25,6
14	25,1	26,2	9,4	22,9	9,4	7,7	49,8		24,5
15	24,1	25,2	9,0	22,0	9,1	7,4	47,9		23,6
16	23,2	24,3	8,7	21,2	8,7	7,1	46,1		22,7
17	22,5	23,5	8,4	20,5	8,5	6,9	44,6		22,0
18	21,7	22,7	8,1	19,8	8,2	6,7	43,2		21,3
19	21,1	22,0	7,9	19,3	7,9	6,5	41,9		20,6
20	20,5	21,4	7,7	18,7	7,7	6,3	40,7		20,1

❖ Results (Draft)

- In SHORT –S9, SHORT +S9 and LONG the mutagenicity ranking of test items was mainly <u>100 FC > KR 3R4F > 100 BLY</u>.
- The Coefficient of Variation (CoV) of the mutagenic rates is used to assess Lab performance. It is mainly below/around 30% for all test items and schedules.
- The median of the Minimal Detectable Difference between the slopes of two test items tested in three replicates is 60-70%.

Workshop “in vitro Toxicology”

Organizing Team

- **Contributors**
  - **BAT: Marianna Gaca**
  - **RAI: Betsy Bombick**
  - **Imperial: Edgar Trelles Sticken**
  - **Altria: Monica Lee**
  - **PMI: Oliver Moennikes**
  - **JTI: Jaqueline Miller**

Outline

- Title: ‘In vitro testing, past, present, and future’.
- Purposes: “Education and Inspiration”
  - To provide an overview of current and developing in vitro science
  - To generate discussion and inspiration for future approaches and actions
- Agenda:
  - Part 1:In Vitro Science Past and Present: current status and challenges (in 2017)
  - Part 2: Emerging in vitro science: development and validation (*Planned* in 2018)

In Vitro Science Past and Present

- **Invited Speakers**

1) Martha M. Moore: Ramboll Environ, US

"Guidances Related to the Use of Genetic Toxicology Assays for International Regulation of Tobacco Products"

2) Julie Clements : Covance Laboratories, UK

"The Application of Genetic Toxicology Assays to Tobacco Products"

Martha Moore

Summary (Dr. Moore)

- There is a “standard” battery of genetic toxicology assays--used for a wide variety of regulated products. (This battery was developed based on extensive discussion involving all the relevant stakeholders.)
- The CORESTA battery is comparable to this “standard” battery
- There are issues that are unique to tobacco products, and we need to develop consensus approaches for these tobacco product unique questions (This needs to be developed involving all the relevant stakeholders)
- Once consensus is reached, guidance dealing with these specific issues can be developed.

Julie Clements

Summary (Dr. Clements)

- **Key Area for Discussion**
  - End Points/Battery
  - Guideline Compliance
  - Same Strategy for all Products?
  - Dosimetry
  - Comparative Testing of Products
  - Top Dose
  - Power and Design
  - Exposure Times
  - Data/Stats Analysis?

Workshop “in vitro Toxicology”

Meeting notes will be available upon request (in December)

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Satellite Meeting “in vitro Toxicology” in China, 2018 (*Under Discussion*)

Satellite Meeting “in vitro Toxicology”

- **Title: Challenges and opportunities for new approach methodologies (NAMs) for next generation tobacco and nicotine products (NGP) regulatory science.**
- **Outcome**
  - **Drawing on the participant’s experiences and knowledge, providing a commentary addressing the challenges and opportunities offered using new approach methodologies for NGP regulatory science.**
  - **Drafting a position paper summarizing the presentations and Q&A from the sessions**

Draft Programme

- **Topic/Title**
  - In vitro testing of tobacco products: history of the CORESTA testing battery, continued relevance today and the current regulatory environment
  - New approach methodologies (NAMs) approaches -use of in vitro for next-generation nicotine product testing
  - Development of new assays for validation and integrated testing strategies
  - Adverse outcome pathways
  - Dosimetry and exposure systems
  - Advances of in vitro systems and applications
  - Regulatory acceptance, dissemination and collaboration
  - Panel discussion and Q&A

Draft Timeline

- Draft contents & NWIP to be reviewed by SC: Mid January 2018
- Estimated budget and financial management scheme to be reviewed by the Board: Mid February 2018
- Request for sponsorship: March 2018
- Draft final program to be reviewed by Reading Committee: May 2018
- Discussion with internal & external speakers until October 2018

Future Actions

Future Actions (Draft)

- *Review* of 2004 IVT Report "The Rationale and Strategy for Conducting In Vitro Toxicology Testing of Tobacco Smoke"
  - Update: testing methods for conventional products
  - Current methods applicable to NGPs?
  - Air Liquid Interface

Acknowledgements

- **Invited Speakers (Tox Workshop)**
  - Martha Moore (Ramboll Environ)
  - Julie Clements (Covance)
- **Altria Client Services (Host of the Spring Meeting in Baltimore)**
  - Monica K. Lee
  - Bonnie G. Coffa
- **Contributors (Discussion for Tox Workshop)**
  - Marianna Gaca (BAT), Betsy Bombick (RAI), Jaqueline Miller (JTI), Oliver Moennikes (PMI), Monica K. Lee (ALCS), Edgar Trelles Sticken (ITG)


UNITED STATES
GOLDEN GLOVES