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

Kunming, China

October 23, 2018

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, Imperial Brands, JTI, JTI/Oekolab, KT&G, Labstat, PMI, RAI, Vitrocell, JT, Charles River Laboratories, ITC**

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

January 22-23, 2018: Southampton, UK

- 34 delegates attended the meeting
- Meeting was hosted by British American Tobacco

October 22, 2017: Kunming, China

- 30 delegates attended the meeting

Upcoming Meetings

March 8, 2019: Baltimore, US

- Meeting will be hosted by JT (TBC)

Projects

Project	Status	Company	Responsibilities
Whole Smoke	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	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	On-going	PMI, Oekolab, Covance, ZTRI	Coordinator : D. Smart (PMI) Co-coordinator = E. Weber (JTI) Statistical Analysis = A.hauleithner (JTI Oekolab)
NRU	On-going	ITL, PMI, KT&G, Covance, JTI, Labstat, ZTRI, Altria, Enthalpy	Coordinator = K. Yoshino (JT) Co-coordinator = R. Wieczorek (ITG) Statistical Analysis = A.hauleithner (JTI Oekolab)
Ames	2018 start	Charles River, CNTQS&TC, Covance, Enthalpy, IB-Reemtsma, JT, KT&G, Labstat, Ökolab, PMI	Coordinator = R. Wieczorek (ITG) Co-cordinator = E. Weber (JTI Oekolab) Statistical Analysis = A.hauleithner (JTI Oekolab)

- Revision of the “Rationale Paper” for combustible products
- Recommendations for NGP (New Generation Products) testing

Inter-laboratory Study

- Mouse Lymphoma Assay (MLA) -

Objectives

- To conduct an inter-laboratory mouse lymphoma assay (MLA) proficiency study using two test items and the Kentucky Reference KR 3R4F in the L5178Y cell line.
- Assessment of the discriminatory power of the test towards different tobacco products.

Responsibilities

- Coordinator: Daniel Smart (PMI)
- Co-Coordinator: Elisabeth Weber (JTI Ökolab)
- Statistical analysis: Alexander Hauleithner (JTI Ökolab)

❖ Test Design

- **Labs use their own protocols**
- **Basic requirements were defined in the study plan (based on OECD TG 490)**
  - Conditioning and smoking of test items according to ISO International Standards
  - Cytotoxicity (as relative total growth; RTG) must be measured concomitantly with mutation frequency (MF).
  - Short +S9 treatment mandatory
  - At least 3 replicates per test item, concentrations as per lab protocol
  - Negative and positive controls (+S9: pro-mutagen: CP, B[a]P; -S9: direct mutagen: e.g. MMS, MMC)
- **Test Pieces: 100 % FC, 100 % Bly, and 3R4F**

❖ Participants' protocol

➢ Cell information

	LAB A	LAB B	LAB C	LAB D
cell line	L5178Y TK+/- 3.7.2C	L5178Y TK+/- 3.7.2C (IVGT)	L5178Y TK+/- 3.7.2C	L5178Y TK+/- 3.7.2C
cells free from contamination / mycoplasma	YES	YES	YES	*NO*

❖ Participants' protocol

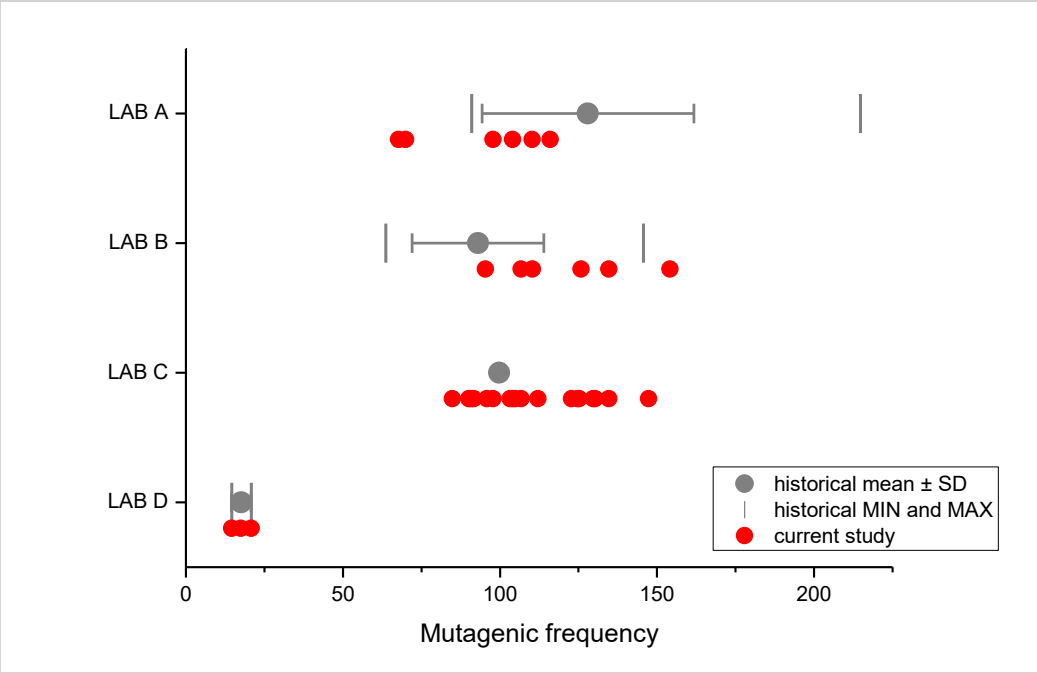
➢ Treatments

Treatment	LAB A	LAB B	LAB C	LAB D
SHORT +S9	X	X	X	X
SHORT -S9	X		X	

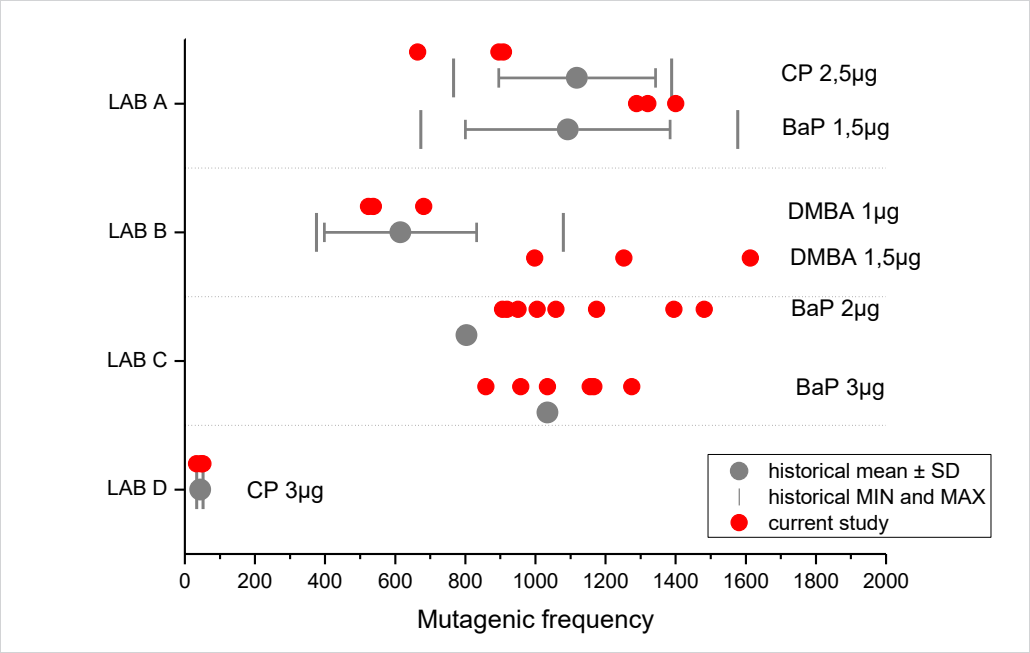
SHORT +S9	LAB A	LAB B	LAB C	LAB D	OECD
Treatment time (h)	3	4	3	3	3-4
Expression time (h)	48	48	48	48	48
Selection time (d)	14	14	12-14	12	10-12

SHORT -S9	LAB A	LAB B	LAB C	LAB D	OECD
Treatment time (h)	3		3		3-4
Expression time (h)	48		48		48
Selection time (d)	14		12-14		10-12

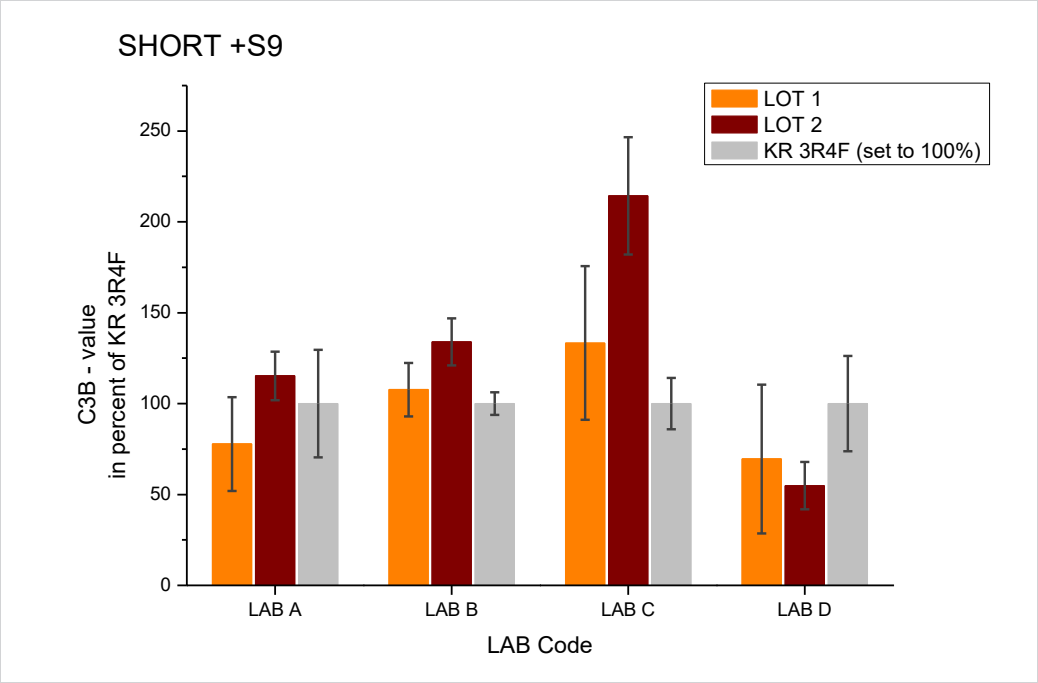
❖ Negative Controls (SHORT +S9)



❖ Positive Controls (SHORT +S9)



❖ Mutagenicity Ranking: SHORT +S9, C3B-values in percent of 3R4F



❖ Mutagenicity Ranking: Summary

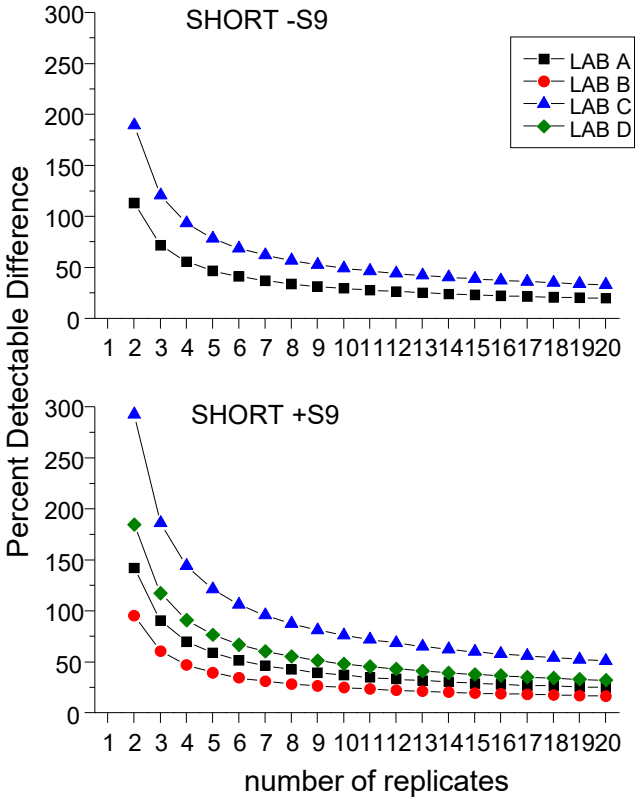
		↑ high		mutagenicity		low ↓
		Rank 1		Rank 2		Rank 3
LAB A	SHORT +S9	**LOT 1**	=	**KR 3R4F**	=	**LOT 2**
	SHORT -S9	**LOT 1**	=	**KR 3R4F**	=	**LOT 2**
LAB B	SHORT +S9	**KR 3R4F**	=	**LOT 1**	=	**LOT 2**
	SHORT -S9	*not tested*				
LAB C	SHORT +S9	**KR 3R4F**	=	**LOT 1**	=	**LOT 2**
	SHORT -S9	**KR 3R4F**	=	**LOT 1**	=	**LOT 2**
LAB D	SHORT +S9	**LOT 2**	=	**LOT 1**	=	**KR 3R4F**
	SHORT -S9	*not tested*				

Percent Detectable Difference between 2 Samples (t-Test with n Replicates)

	LAB A		LAB B		LAB C		LAB D	
n	SHORT +S9	SHORT -S9	SHORT +S9	SHORT -S9	SHORT +S9	SHORT -S9	SHORT +S9	SHORT -S9
2	142,2	113,2	95,3		292,8	189,6	184,7	
3	90,4	72,0	60,6		186,2	120,6	117,5	
4	70,0	55,7	46,9		144,2	93,4	91,0	
5	58,8	46,8	39,4		121,1	78,4	76,4	
6	51,6	41,1	34,5		106,2	68,8	67,0	
7	46,4	37,0	31,1		95,6	61,9	60,3	
8	42,5	33,9	28,5		87,6	56,7	55,3	
9	39,5	31,4	26,5		81,3	52,7	51,3	
10	37,0	29,5	24,8		76,2	49,3	48,1	
11	34,9	27,8	23,4		71,9	46,6	45,4	
12	33,2	26,4	22,2		68,3	44,2	43,1	
13	31,6	25,2	21,2		65,2	42,2	41,1	
14	30,3	24,1	20,3		62,4	40,4	39,4	
15	29,1	23,2	19,5		60,0	38,9	37,8	
16	28,1	22,4	18,8		57,8	37,5	36,5	
17	27,1	21,6	18,2		55,9	36,2	35,2	
18	26,3	20,9	17,6		54,1	35,1	34,1	
19	25,5	20,3	17,1		52,5	34,0	33,1	
20	24,8	19,7	16,6		51,0	33,1	32,2	

not tested (SHORT -S9 for LAB B)

not tested (SHORT +S9 for LAB D)



Summary

- 3 Samples (100 FC, 100 BLY and KR 3R4F) were tested in MLA assay.
- 4 Laboratories participated in the study. Results for SHORT +S9 (4) and SHORT –S9 (2) were reported.
- A nonlinear regression model was used to model the dose response relationship.
- In SHORT +S9 and SHORT –S9 the test piece with the lowest mutagenicity was mainly 100 BLY.
- The test pieces could not be discriminated in neither SHORT +S9 nor SHORT –S9.
- The median of the Minimal Detectable Difference between the slopes of two samples tested in 3 replicates is ~ 90-100%.

Revision of the Rationale Paper

Revision of the Rationale Paper

Background

- The Rationale and Strategy for Conducting In Vitro Toxicity Testing of Tobacco Smoke : Published in May 2004, and need to be revised
- Draft Title: "The Rationale and Strategy for in vitro toxicity testing of combustible products"

Status

- Table of Contents have been agreed
- Major Contributors: Betsy Bombick (RAI), Jacqueline Miller (JTI), Oliver Moennikes (PMI), Tsuneo Hashizume (JT), Doshi Utkarsh (ALCS)
- Timeline: under discussion

Guidance Document for NGP testing

Guidance Documents for NGP testing

- **Background**
  - Need some recommendation to outside of CORESTA for NGP testing conditions
  - "Outline" for each chapter : under discussion
- **Working Group**
  - Redefining CORESTA test Battery recommendation (RAI/Bombick, BAT, ALCS, Covance, ITL, JT/JTI, Enthalpy, Charles River)
  - Aerosol generation & Dosimetry: cig/NGPs, Definitions (ALCS, BAT, Vitrocell, RAI, Enthalpy, Covance, ITL, Labstat, JT/JTI, Battelle)
  - Cell culture/assays (BAT, JT/JTI, ITL, CRL, Covance, RAI)
  - E-liquid (ALCS/Lee, PMI, Covance, ITL, Labstat, CRL, JTgroup, BAT, RAI)

Genetox Workshop in US

Recommendations for the Generation & Use of In Vitro Assay Data for Tobacco Product Regulations

- **Background**
  - Proposed by Dr. Martha Moore (Ramboll Environ)
  - Proposal to undertake a series of discussion workshop (similar to the IWGT approach). Representatives for all the relevant "stakeholders"
  - Identify key issues, discuss and reach consensus on key issues and publish a series of papers presenting the consensus
  - Focus on regulatory issues including those specific to US FDA (and therefore could complement the CORESTA IVTSG efforts)
- **Host organization**
  - IIVS (Institute for In Vitro Sciences): Non-profit organization in US

Recommendations for the Generation & Use of In Vitro Assay Data for Tobacco Product Regulations

- Potential Participants: (Have an "official" workgroup and then open to observers)
  - Tobacco Companies (2-3 key individuals from each organization)
  - CROs
  - FDA: CTP & NCTR
- Goals for the first meeting (November 27-28)
  - Outline "all" the key issues & Prioritize into three priority buckets
- Relationship of this workshop to CORESTA
  - To be an independent exercise. K. Yoshino will serve as a pipeline to CORESTA.
  - Draft discussion topics reviewed/discussed by the IVT members.

Collaboration between BMK SG and IVT SG

Collaboration between BMK SG and IVT SG

Background

- **Collaboration between BMK SG and IVT SG suggested by the SC and the Board.**
- **Representative delegates attend each other's meetings to foster collaboration.**
  - BMK to IVT: Mike McEvan (BAT), G.L.Prasad (RAI), Graham Wood (ACR)
  - IVT to BMK: Kei Yoshino (JT)
- **Find key areas of science in between Clinical (BMK) & in vitro (IVT)**
  - FDA's Predictive Toxicology Roadmap
  - EU-ToxRisk
  - Toxicology Testing in the 21st Century (Tox21)

Collaboration between BMK SG and IVT SG

- **Key concept**
  - IVIVE (in vitro to in vivo extrapolation)
  - Predictive Toxicology
- **Areas in Focus *in general : to be selected by experts***
  - Exposure scenarios / target tissue conc.
  - ADME/PBPK
  - Quantitative AOP
  - Computational toxicology
  - Population / human variability
  - Organ on a chip
  - QSAR
  - Read Across

Collaboration between BMK SG and IVT SG

- « Organizing Team » for « Ad-hoc Group »
  - Members
    - G.L.Prasad (RAI), K. Yoshino (JT), Damian McHugh (PMI), April Brys (Battelle), additional delegates (TBD)
  - Propose areas of common interests
    - Examples
      - Omics : Proteomics / Metabolomics
      - Human Relevant Dose : Computer simulation / organ-on-a-chip
      - AOP (Adverse Outcome Pathways)
  - Find & communicate with experts
  - Plan workshops (TBD)


Sub-Group *In Vitro* Toxicity Testing (IVTSG) – Southampton UK – January 2018