



***In Vitro* Toxicity Testing Sub-Group
(IVTSG)
Annual Report**

Hamburg, Germany

October 8, 2019



SG Composition

- ❖ **SG Coordinator:** Kei Yoshino (JT)
- ❖ **SG Secretary:** David Thorne (BAT)
- ❖ **SG Membership**
 - Altria Client Services, BAT, Battelle, Charles River Laboratories, CNTC, Covance, Enthalpy Analytical, Imperial Brands, JTI, JTI/Oekolab, JUUL Labs, KT&G, Labstat, Millipore Sigma, PMI, RAI, Vitrocell, JT, Charles River Laboratories, ITC, Zhejiang University
- ❖ **SC Liaison:** Kei Yoshino (JT)



- ❖ **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.**



❖ Recent Two Meetings

➤ **March 8, 2019: Baltimore, US**

- 27 delegates attended the meeting
- Meeting was hosted by Japan Tobacco and Altria Client Services

➤ **October 6, 2019: Hamburg, Germany**

- 34 delegates attended the meeting

❖ Upcoming Meetings

➤ **TBD**

- Meeting will be hosted by JT or Vitrocell

❖ Completed project

➤ “Rational and Strategy for In Vitro Testing of Combustible Tobacco Products” (IVT Technical Report)

- Contributors: Lee K.M. (ALCS), Jordan. K.G. (RAI), Wieczorek. R (Imperial Brands), Moennikes O. (PMI), Clements. J (Covance), Hashizume. T (JT), Miller J. (JTI), Weber E. (JTI Oekolab)

REPORTS current

Rationale and Strategy for In Vitro Toxicity Testing of Combustible Tobacco Products

IVT Technical Report

September 2019 *Ref. IVT-225-CTR*

In 2004, the first guideline report was published covering the rationale and strategy for conducting *in vitro* toxicity testing of tobacco smoke and to identify key procedures based upon internationally recognized guidelines, adapted to accommodate the nature and unique properties of tobacco smoke.

In 2018, the CORESTA In Vitro Toxicity Testing Sub-Group decided to review the 2004 guideline in order to: 1) re-evaluate the relevance of the initial rationale and strategy of *in vitro* testing of combustible tobacco products, 2) identify recent and comparable regulatory testing guidelines and examples in publications, and 3) provide a pragmatic summary of key features of each recommended assay.

The review effort revealed the continued usage and reference of the CORESTA *in vitro* test battery, especially where standardized and validated testing is required, upholding that the overall strategy and rationale remains valid and relevant. Sometimes these standardized testing results were supplemented with newer and exploratory *in vitro* assays. However, the *in vitro* tests recommended in 2004 continue to be used in a comparative product testing, such as to evaluate the biological impact of changes in ingredients or product designs against reference tobacco products as part of weight-of-evidence toxicity evaluation.

This report is an update of the 2004 report.



➤ Poster Presentation (POST 49)

- Presented by Lee KM, et al. (ALCS)

The CORESTA *In Vitro* Test Battery for Combustible Tobacco Products: Update from the 2004 Rationale and Strategy Report
LEE KM¹, JORDAN KG², WIECZOREK R³, MOENNIKES D⁴, CLEMENTIS J⁵, CROOKS P⁶, HASHIZUME T⁷, MILLER J⁸, WEBBER E⁹, YOSHINO K¹⁰

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ABSTRACT

In 2004, the CORESTA *In Vitro* Test Battery was established as a rationale and strategy report, commonly known as "the CORESTA *In Vitro* Test Battery guideline". The goals were to: 1) evaluate the relevance and strategy for conducting *in vitro* toxicity testing of tobacco products; 2) identify key procedures (and/or) internationally recognized guidelines, related to experimental design, procedure of tobacco smoke, the Test Form from the Tobacco IVTSC, *In Vitro* Toxicity Subgroup) since 2005 performed a series of scientific studies based on the guideline.

Considering the time elapsed, the IVTSC has revised the guideline to re-evaluate the relevance of the initial rationale and strategy for *in vitro* testing of combustible tobacco products, and to propose a pragmatic summary of key findings of each recommended assay. The author confirmed the continued usage and relevance of the 2004 CORESTA *In Vitro* Test Battery, especially where stakeholders are requesting a regular regulatory submission, updating that the overall strategy and rationale remain valid and relevant. However, some tests identified during the update are no longer used or explored in *in vitro* assays (e.g. *in vitro* DNA damage testing with human whole smoke), however the CORESTA *In Vitro* Test Battery are continuously used in comparative product testing, such as evaluating the biological impact of changes in ingredients or product design as part of a weight-of-evidence toxicity evaluation.

In the updated 2019 guideline, the IVTSC recommends, among other studies for *in vitro* toxicity testing to include the following key components: 1) cytotoxicity assay (Neutral Red Uptake assay with mammalian cells), 2) bacterial reverse mutation (Ames assay), 3) genotoxicity assay (in vitro micronucleus assay), 4) mouse lymphoma assay (in vitro micronucleus assay), the mouse lymphoma assay, or the chromosome aberration (Ames assay), 5) genotoxicity assay (in vitro micronucleus assay), 6) mouse lymphoma assay (in vitro micronucleus assay). The IVTSC considers that the biological significance of the *in vitro* assays must be confirmed in conjunction with available chemical and epidemiological data, in the context of the overall product risk assessment.

BACKGROUND

- The *in vitro* testing framework is built upon standardized and internationally recognized assays that are widely used for toxicity studies (new releases, including CORESTA, cytotoxicity reports).
- Currently, no single *in vitro* assay can provide comprehensive information on toxicity or biological activity. Cytotoxicity assays can be used to support estimation of smoking in vitro to assess toxicological testing and to provide information on adverse effects in smoking and to assess the dose for genetic cytotoxicity. Representative cytotoxicity responses are the toxicity and cellular growth rate.
- Genotoxicity testing is used to evaluate DNA damage or gene mutation and structural or functional chromosome alteration. Bacterial reverse mutation, genotoxicity assay, and comprehensive information on various types of genetic damages, and in order to minimize false positives, a positive test is recommended (e.g. bacterial Ames mutation test and mammalian cell assay detecting chromosomal damage).
- Combustible tobacco test materials (i.e., test particulate matter (TPM)) are already characterized as cytotoxic and genotoxic. Nevertheless, one objective of testing re-evaluable cigarettes is to provide a comparative assessment to determine if a product change of interest has resulted in meaningful modification (no change, increase, or decrease) of biological activity compared with the product without specific change.

REFERENCES

1. Lee KM, et al. (2004) Rationale and Strategy Report for the CORESTA *In Vitro* Test Battery. CORESTA, Paris, France.
2. OECD (2014) Test No. 487: In vitro mammalian cell cytotoxicity assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 487.
3. OECD (2014) Test No. 488: In vitro mammalian cell micronucleus assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 488.
4. OECD (2014) Test No. 489: In vitro mammalian cell chromosome aberration assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 489.
5. OECD (2014) Test No. 490: In vitro mammalian cell gene mutation assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 490.
6. OECD (2014) Test No. 491: In vitro mammalian cell gene mutation assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 491.
7. OECD (2014) Test No. 492: In vitro mammalian cell gene mutation assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 492.
8. OECD (2014) Test No. 493: In vitro mammalian cell gene mutation assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 493.
9. OECD (2014) Test No. 494: In vitro mammalian cell gene mutation assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 494.
10. OECD (2014) Test No. 495: In vitro mammalian cell gene mutation assay. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 495.

RECOMMENDED ASSAYS AND EXAMPLES

For studies that may be classified to regulatory questions and where *in vitro* toxicity testing is deemed appropriate, the CORESTA IVTSC recommends a test battery of:

- 1. **Neutral Red Uptake (NRU) assay**, cytotoxicity with mammalian cell lines
- 2. **Bacterial reverse mutation assay** (Ames assay), cytotoxicity with mammalian cell lines
- 3. **Chromosome aberration assay** with mammalian cell lines (e.g. in vitro chromosome aberration (ICA) assay, the mouse lymphoma assay (MLA), or the chromosome aberration (CA) assay).

SELECTED POINTS	NEUTRAL RED UPTAKE ASSAY (NRU) - EXAMPLES*	SELECTED POINTS	AMES ASSAY - EXAMPLES*
Cell line	HEp-2, A549, CEM3, H460, H460T, H1299	Cell line	TA98, TA978, TA979, TA998, TA999, TA1000
Cell density at seeding	1.0E+06 (cell/ml), 1.0E+05 (cells/well)	Cell density	1E7 cells/well
Medium volume	100 μ l/well	Medium addition	10 μ l/well
Exposure time	24 h, 48 h, 72 h	Incubation period	48 h (24 h for TA98, TA998, TA999, TA1000)
Time of harvest	24 h, 48 h, 72 h	Softest control	TA98, TA998, TA999, TA1000
Gene of interest	None	Maximum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Maximum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000
Gene of interest	None	Minimum values control	TA98, TA998, TA999, TA1000

ACKNOWLEDGMENT

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❖ Update of the on-going projects

➤ Whole Smoke (Publication)

- Lead: David Thorne (BAT)
- Michael Hollings (Covance), Tobias Krebs (Vitrocell) and Robert Leverette (RAI) volunteered to review whole smoke publication for NGP gap analysis. This will be followed by an author review and prior to submission the journal - submit for review to authors by end of the year.

➤ In Vitro Micronucleus Inter-laboratory Study

- Lead: E. Weber (JTI Oekolab)
- Draft report under review by the SC

❖ Update of the on-going projects (continued)

➤ **MLA Inter-laboratory Study**

- Lead: D. Smart (PMI)
- Additional Data to be input (Nicotine analysis)

➤ **NRU Inter-laboratory Study**

- Lead: K. Yoshino (JT)
- Draft report circulated before end of October

➤ **Ames Inter-laboratory Study**

- Lead: R. Wieczorek (Imperial Brand) and E. Weber (JTI Oekolab)
- Draft report circulated before end of October



❖ Upcoming projects

- **Rational and Strategy for in vitro Toxicity Testing of e-Vapor Products**
 - Lead: U. Doshi (ALCS)
 - Outline under discussion
- **Inter-laboratory Study**
 - Based on the members needs



Inter-laboratory Study

- Ames Assay -

❖ Objectives

- To conduct an inter-laboratory proficiency testing programme on two test items and the Kentucky References 3R4F and 1R6F using a common experimental design for the AMES Test.
- Assessment of the discriminatory power of the test towards different tobacco products.

❖ Responsibilities

- **Coordinator:** Roman Wieczorek (Imperial Brands)
- **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 471)**
 - Conditioning and smoking of test items according to ISO International Standards
 - At least 3 replicates per test item, concentrations as per lab protocol
 - Salmonella typhimurium TA98 / TA100 mandatory, \pm S9
 - Negative and positive controls
- **Test Pieces: 100 % FC, 100 % Bly, 3R4F, and 1R6F**

❖ Participating Labs (10 labs)





- **Altria-CRL, CNTQS&TC, Covance, Enthalpy, IB-Reemtsma, JT, KT&G, Labstat, JTI Oekolab, PMI**

❖ Ranking in mutagenic rate (TA98 +S9)

 LOT 1 (100% FC)	 LOT 3 (KR 3R4F)
 LOT 2 (100% BY)	 LOT 4 (KR 1R6F)

LAB ID	[lower mR]	Ranking in mutagenic rate					[higher mR]
LAB A	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB B	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB C	LOT 1	<	LOT 3	=	LOT 4	<	LOT 2
LAB D	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB E	LOT 1	<	LOT 3	=	LOT 4	<	LOT 2
LAB F	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB G	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB H	LOT 3	=	LOT 4	=	LOT 1	=	LOT 2
LAB I	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB K	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2

❖ Ranking in mutagenic rate (TA100 +S9)

	LOT 1 (100% FC)		LOT 3 (KR 3R4F)
	LOT 2 (100% BY)		LOT 4 (KR 1R6F)

LAB ID	[lower mR]		Ranking in mutagenic rate					[higher mR]
LAB A	LOT 1	<	LOT 4	=	LOT 3	=	LOT 2	
LAB B	LOT 1	=	LOT 3	<	LOT 2	=	LOT 4	
LAB C	LOT 1	=	LOT 2	=	LOT 4	=	LOT 3	
LAB D	LOT 1	<	LOT 4	=	LOT 3	=	LOT 2	
LAB E	LOT 1	=	LOT 3	=	LOT 2	=	LOT 4	
LAB F	LOT 1	=	LOT 3	=	LOT 4	=	LOT 2	
LAB G	LOT 1	=	LOT 4	=	LOT 2	=	LOT 3	
LAB H	LOT 1	=	LOT 3	=	LOT 2	=	LOT 4	
LAB I	LOT 1	<	LOT 3	=	LOT 4	=	LOT 2	
LAB K	LOT 1	=	LOT 3	=	LOT 2	=	LOT 4	

❖ Ranking in mutagenic rate (TA97a +S9)

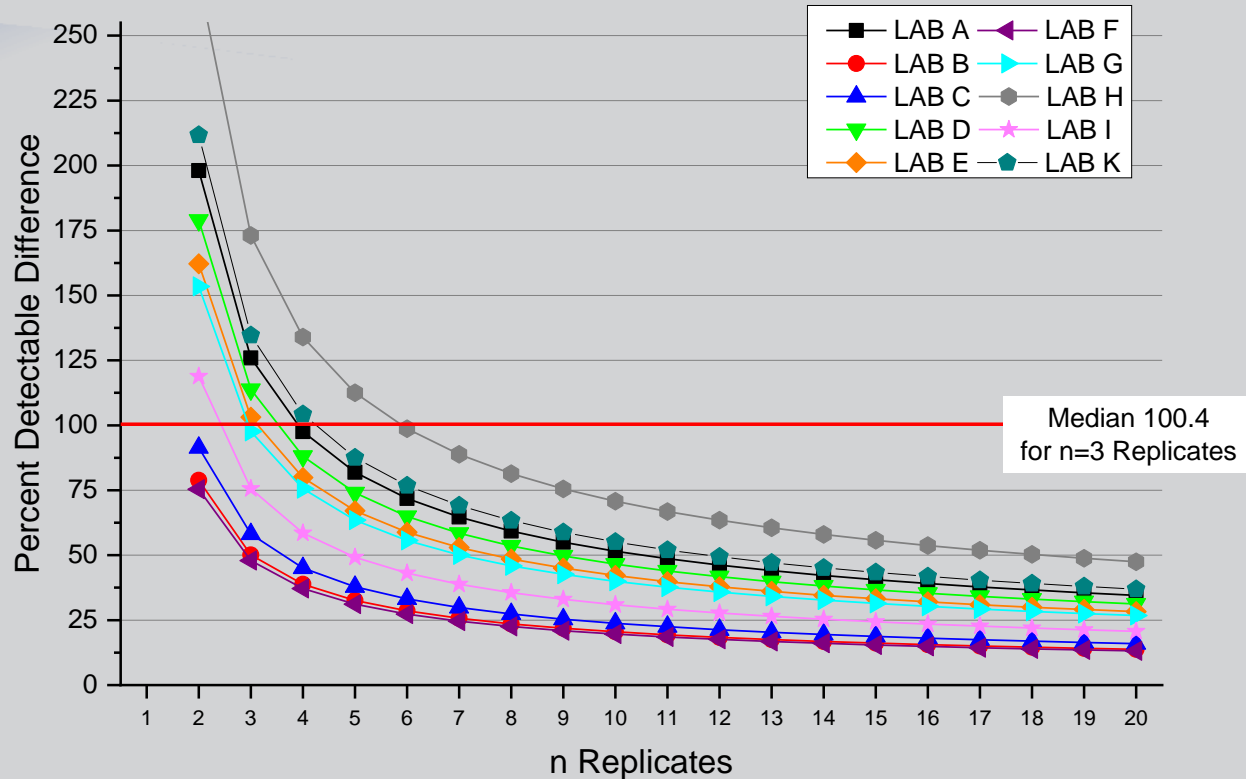
 LOT 1 (100% FC)	 LOT 3 (KR 3R4F)
 LOT 2 (100% BY)	 LOT 4 (KR 1R6F)

LAB ID	[lower mR] Ranking in mutagenic rate [higher mR]						
LAB A	<i>not tested</i>						
LAB B	LOT 1	<	LOT 4	=	LOT 3	<	LOT 2
LAB C	LOT 1	<	LOT 2	=	LOT 4	=	LOT 3
LAB D	LOT 1	<	LOT 3	=	LOT 4	<	LOT 2
LAB E	<i>not tested</i>						
LAB F	LOT 1	<	LOT 3	=	LOT 4	=	LOT 2
LAB G	<i>not tested</i>						
LAB H	<i>not tested</i>						
LAB I	<i>not tested</i>						
LAB K	<i>not tested</i>						



Minimum Detectable Difference (MDD)

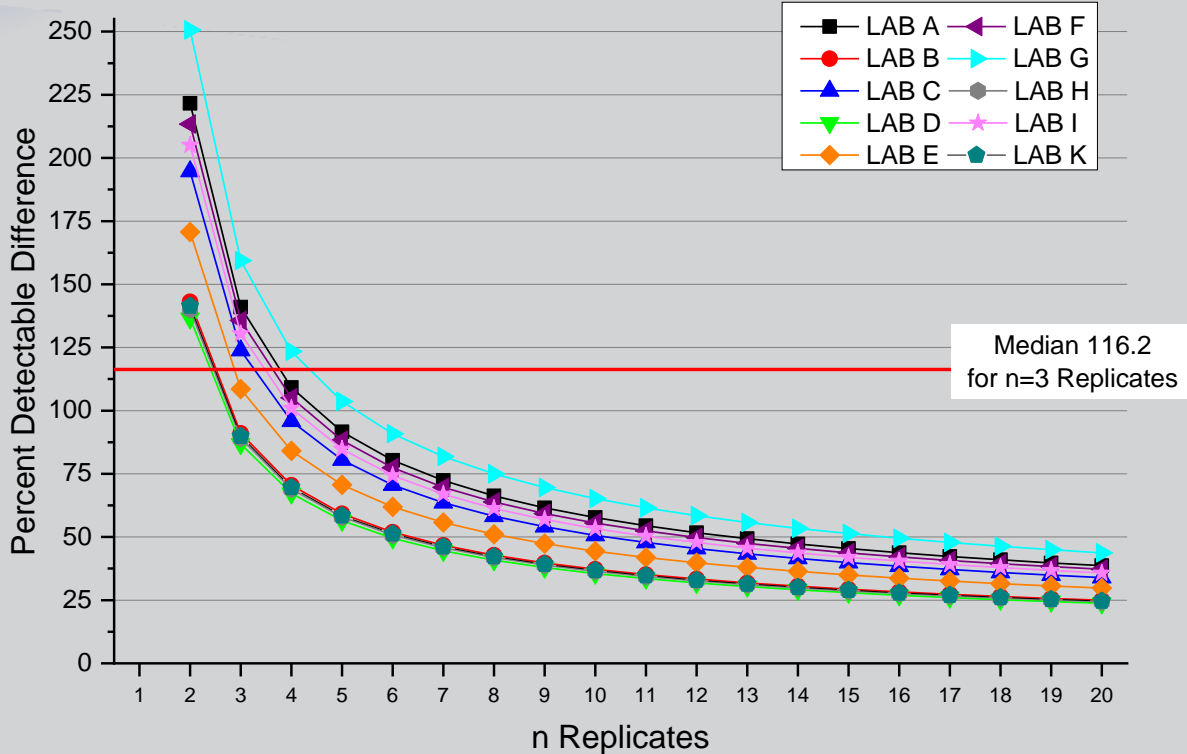
❖ TA98 +S9





Minimum Detectable Difference (MDD)

❖ TA100 +S9



❖ Summary

- 4 Samples (100% FC, 100% BY, KR 3R4F and KR 1R6F) were tested in AMES assay.
- 10 Laboratories reported data.
- Test were performed for *S. typhimurium* strains TA98, TA100 and TA97a in presence and absence of S9.
- TA98 and TA97 showed in comparison to TA100 significant higher increase in revertants.
- In TA98+S9, laboratories could significantly discriminate FC and BY samples, the Reference Cigarettes could not be discriminated.
- If the samples differ ~100-120% in mutagenic rate in the presence of S9, the participating labs can detect that difference (median) in a t-test with 3 replicates.



Independent Genetox Workshop in US

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



Independent Genetox Workshop in US

❖ 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

❖ **Participants:**

- **Tobacco Companies (2-3 key individuals from each organization)**
- **CROs**
- **FDA: CTP & NCTR**

❖ **Goals for the first meeting (November 27-28, 2018)**

- **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.**



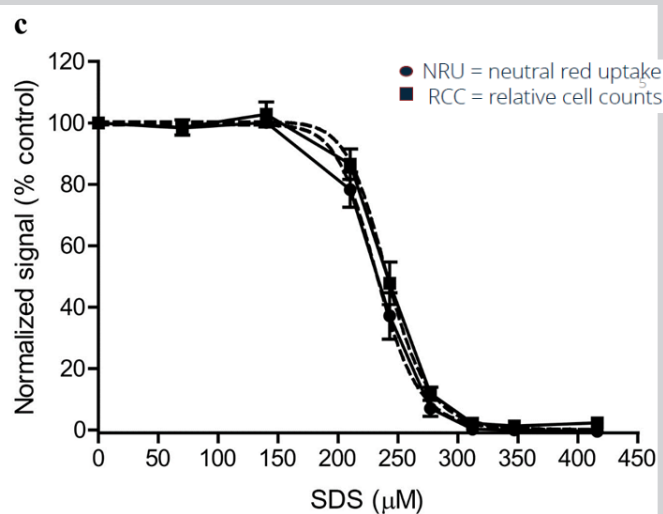
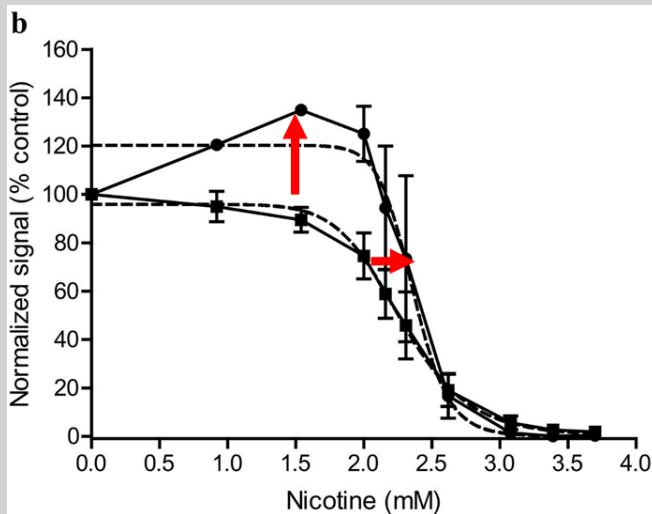
Independent Genetox Workshop in US

- ❖ **Second meeting (June 4 – 5, 2019)**
 - Updates on the grant and comments from FDA
 - Ames strains
 - Sample preparation
 - Issues/considerations for the preparation of samples from combustibles
 - E-cig aerosol trapping
- ❖ **Seek feedback form Dr. Moore regarding Poster Presentation**
- ❖ **Third meeting is expected to be held next spring**



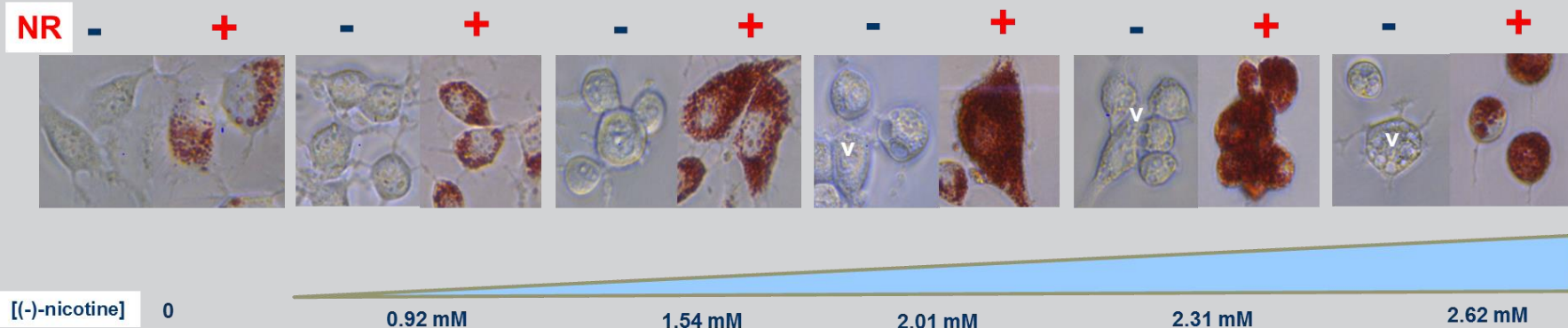
NRU Protocol investigation with BEAS-2B & Balbc/3T3 cells

- ❖ **Apparent increase in neutral red uptake following exposure to eliquids containing (-)-nicotine (PMI presentation, CORESTA Kunming 2018)**
 - **Balb/c 3T3 cells treated for 24 h to eliquids + nicotine**
 - **Increase in NR uptake apparent vs non-nicotinated eliquid controls**

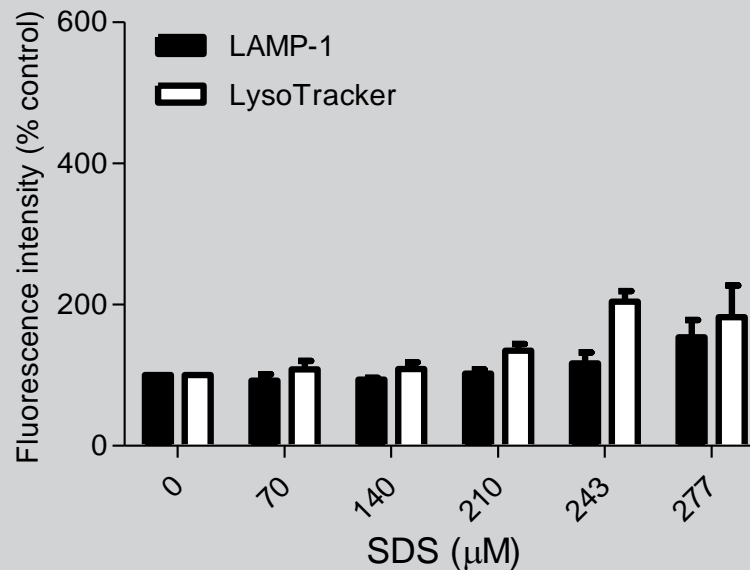
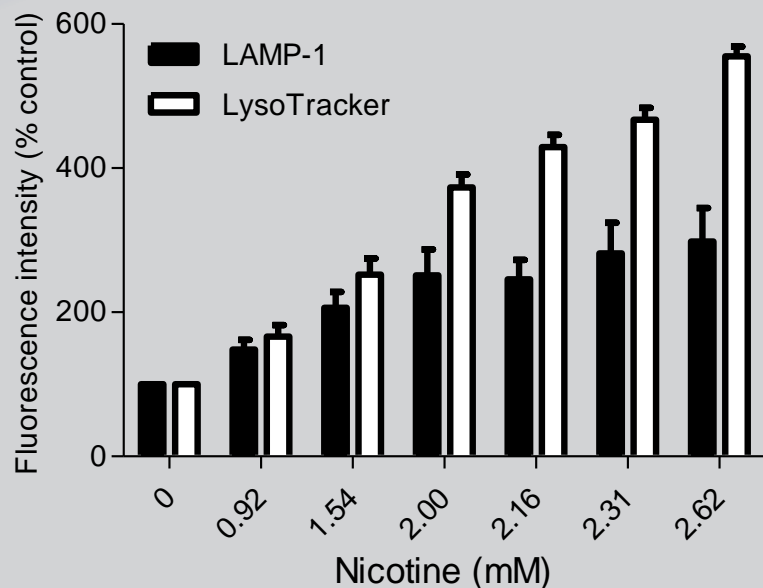


❖ Macroscopic changes apparent in nicotine treated cells

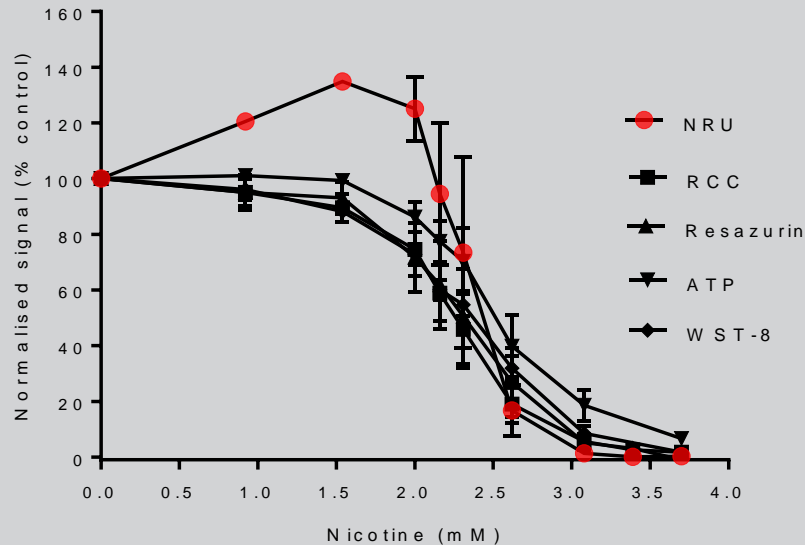
- Enhanced NR uptake coincident with macroscopic changes to cell ultrastructure



❖ Lysosome analysis via FACS confirms perturbation by (-)-nicotine



❖ High-throughput compatible approaches to determine cytotoxicity in Balb/c 3T3 cells



- Nicotine cytotoxicity was successfully evaluated with all assays using metabolic measures of viability
- No nicotine-induced artefacts were detected
- Agreement between the direct (RCC) and metabolic assay processes estimation of the cytotoxicity EC50

- ❖ **OECD Draft guidance No. 129 : Guidance Document On Using Cytotoxicity Tests To Estimate Starting Doses For Acute Oral Systemic Toxicity Tests : ENV/JM/MONO(2010)20**
 - **Caution for the chemical affecting lysosome was already noticed.**
 - **1.3.3 Range of Substances Amenable to the In Vitro NRU Test Methods**
... The toxicity of substances that specifically affect lysosomes may be overestimated because they may affect NRU binding...

	Lab 1				Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 8		Lab 9	
Cell line	CHO-WBL	CHO-WBL	Balb/c3T3	Balb/c3T3	CHO-K1	Balb/c3T3	BEAS-2B	HepG2	CHO-K1	CHO-K1	CHO-WBL (IVGT)	Balb/c3T3	CHO-K1							
Cell density for seeding	1.25 x 10 ⁴ cells/mL	1.25 x 10 ⁴ cells/mL	1.0 x 10 ⁵ cells/mL	1.0 x 10 ⁵ cells/mL	5.0 x 10 ⁴ cells/mL	3.0 x 10 ³ cells/mL	4.0 x 10 ⁴ cells/mL	6.7 x 10 ⁴ cells/mL	1.0 x 10 ⁴ cells/mL	1.0 x 10 ⁵ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	5.0 x 10 ⁴ cells/mL	
Medium Volume	200 uL/well	200 uL/well	100 uL/well	100 uL/well	200 uL/well	100 uL/well	150 uL/well	150 uL/well	200 uL	100 uL	200 uL	100 uL	200 uL							
Pre-culture	23 - 24 hrs.	22 - 24 hrs.	24 hrs.	24 hrs.	20 - 25.5 hrs.	24 - 27 hrs.	20 - 21 hrs.	20 - 21 hrs.	24 hrs.	27 hrs.	22 - 26 hrs.	24 - 25 hrs.	24 hrs.							
Exposure time	24 hrs.	48 hrs.	48 - 49 hrs.	48 - 49 hrs.	24 - 25 hrs.	23 - 24 hrs.	69 - 70 hrs.	69 - 70 hrs.	24 hrs.	24 hrs.	24 hrs.	23 - 24 hrs.	24 hrs.							
Conc. Of serum	10%	10%	10%	0%	10%	10%	0%	0%	10%	10%	10%	10%	10%							
Conc. Of DMSO	0.5%	0.5%	1.0%	1.0%	0.25 - 2%	0.5%	0.42%	0.45%	2.0%	1.5%	2.0%	0.33%	2.0%							
Conc. Of NR dye	50 ug/mL	50 ug/mL	25 ug/mL	25 ug/mL	10%	25 ug/mL	66 mg/mL?	66 mg/mL?	33 ug/mL	50 ug/mL	50 ug/mL	50 ug/mL	15 ug/mL							
Duration for NR incorporation	3 hrs.	3 hrs.	3.5 hrs.	3.5 hrs.	3 hrs.	3 hrs.	3 hrs.	3 hrs.	3 hrs.	3 hrs.	3 hrs.	3 hrs.	3 hrs.							
Duration for NR extraction	7 - 10 min.	5 - 10 min.	20 min.	20 min.	10 min.	5 - 10 min.	15 min.	15 min.	30 min.	10 min.	6 - 8 min.	7 - 11 min.	10 - 12 min.							
Positive response		SLS			SLS	SLS		SDS	SLS	SDS	SDS	SDS	SDS							
Criteria for positive response		< 70 %			non.	< 70 %		non.	non.	non.	non.	non.	non.							
Sensitivity	Dose at where relative abs. is less than 10% (with 3R4F TPM)	150 ug/mL	150 ug/mL	> 150 ug/mL	150 ug/mL	150 ug/mL	80 - 90 ug/mL	30 ug/mL	30 ug/mL	120 ug/mL	120 ug/mL	120 ug/mL	130 ug/mL	120 ug/mL						

- Cell line: CHO-WBL, Balb/c3T3, CHO-K1, BEAS-2B, HepG2, CHO-WBL(IVGT)
- Cell density for seeding
- Exposure time
- DMSO Conc.
- Dose at where relative abs. is less than 10% (with 3R4F TPM)

- ❖ **Confirmation studies voluntarily conducted by PMI, JT, and IB-Reemtsma**
 - The same findings confirmed with Balb/c 3T3 but not with BEAS-2B cells. Vacuole seen in cytoplasm of both cells at the concentration showing 10-20% cytotoxicity.
 - **Study conditions have impacts on “bi-phasic response” in NRU assay**
- ❖ **NRU should be considered when testing e-liquids to ensure appropriate data. Where possible other cytotoxic methods should be employed or considered.**
- ❖ **The group will address this further based on members interests.**



Collaboration between BMK SG and IVT SG

- ❖ **Areas of science and technologies in between “human” and “in vitro”**
 - **‘Omics**
 - **Organ-on-a-chip**
 - **Human Relevant Dose**
 - **Subpopulations (donor variation)**
 - **IVIVE (in vitro – in vivo extrapolation)**

- ❖ **“Guidance Documents” (under development in NGTX TF) covers all the above.**
⇒ **NGTX locates between IVT and BMK.**

- ❖ **The coordinators of BMK, NGTX, and IVT will discuss together and propose future action plan.**



Acknowledgement



Hosts of the Spring Meeting

2011 : ITG (Hamburg)

2012 : Covance (Harrogate)

2013 : BAT (Brockenhurst)

2014 : JTI (Vienna)

2015 : PMI (Neuchâtel)

2016 : Battelle + RAI (New Orleans)

2017 : ALCS (Baltimore)

2018 : BAT (Southampton)

2019 : JT + ALCS (Baltimore)



“Speak Up!”

Betsy R. Bombick in RAI



Pierre-Marie Guitton



