Cancun • Mexico

# CORESTA SSPT 2023

## SMOKE SCIENCE and PRODUCT TECHNOLOGY

## JOINT STUDY GROUPS CONFERENCE

# PROGRAMME & ABSTRACTS

**Cancún, Mexico**

**8–12 October 2023**

Cooperation Centre for Scientific Research Relative to Tobacco

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# WELCOME MESSAGE

## ON BEHALF OF CORESTA

**Xavier CAHOURS**
**Vice-President of the CORESTA Scientific Commission**

Dear Colleagues

It is a great pleasure to welcome you to the 2023 Joint Meeting of the Smoke Science and Product Technology Study Groups of CORESTA, and to express our gratitude to our hosts, Mother Murphy's Laboratories, for inviting us to the beautiful and vibrant city of Cancún in Mexico. My heartfelt thanks also go to the Organising Committee for their tremendous efforts in making this event possible.

The 2023 Joint Meeting marks a significant occasion as it brings us together for an in-person gathering after two years of navigating the challenges posed by the COVID pandemic. We are excited to have over 48 presentations and 54 posters that will showcase the work programme, complemented by six presentations given in the Symposium on "Advancing New Alternative Methods", and six presentations on "Consumer Reported Outcome Measures".

As we reconvene, all Sub-Groups and Task Forces will report their accomplishments and progress. I extend my appreciation to the Coordinators and Secretaries for their dedication and time in ensuring that all working units align with our organizational vision and meet the expectations of our members.

CORESTA remains steadfast in providing publicly available, credible science, and best practices related to tobacco and its associated products. It continues to serve as a platform for engaging with regulators and a place where participants can exchange ideas, discuss their research, and foster new relationships.

On behalf of the Scientific Commission, I would like to wish all delegates, especially those attending or presenting for the first time, a productive and enjoyable stay in Cancún. Let us make the most of this long-awaited opportunity to come together, learn from one another, and strengthen our shared commitment to advancing the field of tobacco science.

Xavier CAHOURS
Imperial Tobacco - SEITA
Paris, France

# WELCOME MESSAGE

## ON BEHALF OF THE ORGANISERS

**Eduardo BEREA NUÑEZ**
**VP International Sales and Business Development, Mother Murphy's Laboratories, Inc.**

Dear Colleagues and Attendees

Welcome to Cancún!

It is a pleasure and an honor for Mother Murphy's Laboratories and I to host the CORESTA 2023 Smoke Science and Product Technology Conference.

As you all know, after many years of uninterrupted annual conferences and congresses, we had to make an obligatory suspension of activities after 2019 and we are very excited to have the opportunity to meet again, face to face, after three years.

Cancún is an example of change, evolution and adaptation. From being a small fishermen area in the state of Quintana Roo in the early 1960s it changed and became a full-scale tourist destination by the 80s and in the last 10 years has developed and grown into an international conference and exhibition center. Nowadays Cancún is a vibrant city with a population close to one million. This versatile attitude, evokes the one we have at CORESTA, always changing, evolving and adapting to the challenges of our industry. We hope this location will promote and inspire a productive meeting, to share innovative thinking and facilitate the building of more relationships between all our members and participants.

We thank the Scientific Commission and all presenters and contributors for putting together a very interesting program.

All together we are invited to do our best to continue building and reinforcing CORESTA's vision: "To be recognised by our members and relevant external bodies as an authoritative source of publicly available, credible science and best practices related to tobacco and its derived products".

On behalf of the Organizing Committee of the 2023 Smoke Science and Product Technology Conference of CORESTA, I wish you a pleasant meeting and an enjoyable stay in Cancún.

Eduardo BEREA NUÑEZ
Chairman of the SSPT2023 Organising Committee
Greensboro, NC, U.S.A.

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CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France

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| | Johan REDEBY (Swedish Match, Sweden) |

# CORESTA SSPT2023 CONFERENCE

# COMMITTEES

## ORGANISING COMMITTEE

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Bernhard EITZINGER

Bin HU

Cyril JEANNET

Jana JEFFERY

Jutta PANI

Johan REDEBY

Mohamadi SARKAR

Kei YOSHINO

# CORESTA SSPT2023 CONFERENCE

# SUB-GROUP / TASK FORCE MEETINGS

**Meetings Venue**

The Pyramid at Grand Oasis Cancún
Blvd. Kukulcan 5-Km 16, Zona Hotelera
77500 Cancún, Q.R., Mexico

| Date | Time | Meeting | Room |
|---|---|---|---|
| **Saturday, 7 October** | 8:30-12:30 | HTP – Heated Tobacco Products Sub-Group | Yucatán |
| | 8:30-12:30 | BMK – Biomarkers Sub-Group | Mérida |
| | 14:00-18:00 | EVAP – E-Vapour Sub-Group | Yucatán |
| | 14:00-18:00 | PUB – Product Use Behaviour Sub-Group | Mérida |
| **Sunday, 8 October** | 8:30-12:30 | TTPA – Tobacco and Tobacco Products Analysis Sub-Group | Yucatán |
| | 8:30-12:30 | NGTX – 21st Century Toxicology for Next Generation Tobacco and Nicotine Products Task Force | Mérida |
| | 9:00-11:30 | CROM – Consumer Reported Outcome Measures Consortium Task Force | Querétaro |
| | 14:00-18:00 | SA – Smoke Analysis Sub-Group | Yucatán |
| | 14:00-18:00 | CSM – Cigar Smoking Methods Sub-Group | Mérida |
| | 14:00-18:00 | IVT – *In Vitro* Toxicity Testing Sub-Group | Querétaro |
| **Wednesday, 11 October** | 14:00-18:00 | NPSQ – Nicotine Pouches Safety and Quality Guidance Task Force | Guadalajara |

# CORESTA SSPT2023 CONFERENCE

# GENERAL INFORMATION

**Conference Venue**

The Pyramid at Grand Oasis Cancún
Blvd. Kukulcan 5-Km 16, Zona Hotelera, 77500 Cancún, Q.R., Mexico
https://oasishoteles.com/en/hotels/the-pyramid-at-grand-oasis

**Registration**

Saturday, 7 October, 12:00-14:00
Sunday, 8 October, 8:00-10:50 and 12:00-18:00

The Pyramid at Grand Oasis Cancún (Guadalajara Room).

**Presentations**

PowerPoint files should be brought to the Conference Office (Tulum Room) at The Pyramid at Grand Oasis Cancún the day before the scheduled presentation.

**Posters**

Posters are to be brought to the Conference Office (Tulum Room) at The Pyramid at Grand Oasis Cancún on Monday, 9 October. Posters will be mounted on Monday and displayed until after the poster session on Tuesday, 10 October. Posters can be taken back at the end of the poster session, or collected from the Conference Office on Wednesday and Thursday, 11 and 12 October.

The Poster Session is on Tuesday, 10 October, 14:00-17:00 (Mérida/Querétaro Room).

# SOCIAL EVENTS

**Welcome Reception**

Sunday, 8 October, 19:00-22:00
The Pyramid at Grand Oasis Cancún, “The Zocalo” Terrace

**Networking Afternoon**

Tuesday, 10 October, 17:00 ~ 20:00
The Pyramid at Grand Oasis Cancún, “The Zocalo” Terrace

**Closing Dinner**

Thursday, 12 October, 20:00 ~ 23:00
The Pyramid at Grand Oasis Cancún, Oasis Arena
Dress code: Business casual

**CORESTA SSPT2023 CONFERENCE**

# SMOKE SCIENCE and PRODUCT TECHNOLOGY

# PROGRAMME

*Presenter's name is underlined when the main author (listed first) is not presenting the paper*

# MONDAY 9 OCTOBER

## SESSION 1: Opening

Chair: Xavier CAHOURS

Yucatán Room

| | | |
|---|---|---|
| 8:30-8:50 | | **Welcome and Opening Remarks**<br>CAHOURS Xavier<br>*Imperial Tobacco - SEITA, 216 rue Raymond Losserand, 75014 Paris, France* |
| 8:50-9:30 | | **Invited Speaker:**<br>**Bolstering confidence in the reduced harm of combustion-free nicotine products: XXI Century approaches**<br>POLOSA R.<br>*Center of Excellence for the Acceleration of Harm Reduction (CoEHAR) & Department of Clinical and Experimental Medicine, University of Catania, Via S. Sofia, 97, 95123, Catania, Italy* |
| 9:30-9:50 | **IG 01** | **CORESTA Tobacco Harm Reduction Workshop Overview**<br>STEVENS R.(1); FLORA J.(2)<br>*(1) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.*<br>*(2) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 9:50-10:10 | **IG 02** | **Potential ultra-low nicotine limit in tobacco – can we meet it?**<br>FISHER A.M.(1); FISHER C.R.(1); YANG S.(2); PATRA B.(1); SLONE S.(3); JI H.(1); KINNEY J.(1)<br>*(1) Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.*<br>*(2) United States Department of Agriculture, 1616 Albrecht Blvd N, Fargo, ND 58102, U.S.A.*<br>*(3) Dr. Bing Zhang Department of Statistics, University of Kentucky, 725 Rose Street, Lexington, KY 40536, U.S.A.* |
| 10:10-10:30 | | **COFFEE** |

# MONDAY 9 OCTOBER

## SESSION 2: EVAP - Aerosol Assessment

Chair: Cyril JEANNET

Yucatán Room

| | | |
|---|---|---|
| 10:30-10:50 | **Report** | **Sub-Group E-Vapour (EVAP)**<br>GILLMAN I.G.<br>*Juul Labs, Inc., 1000 F Street NW, Washington, DC, 20004, U.S.A.* |
| 10:50-11:10 | **ST 01** | **Workflow assessment of potential leachables in aerosol from ENDS systems**<br>CARTER K.M.; SMITH C.R.; CHEVVA H.; AYALA-FIERRO F.; COOK D.K.; LYNDON M.; GILLMAN I.G.<br>*Juul Labs, Inc., 1000 F Street NW, Washington, DC, 20004, U.S.A.* |
| 11:10-11:30 | **ST 02** | **Analytical investigation of data deficient simulated leachables in ENDS products: case study**<br>SMITH C.R.; LYNDON M.; JEONG L.; LEHMAN D.; JAMESON J.B.; CHEEVA H.; AYALA-FIERRO F.; CARTER K.M.; COOK D.K.; GILLMAN I.G.<br>*Juul Labs, Inc., 1000 F Street NW, Washington, DC, 20004, U.S.A.* |
| 11:30-11:50 | **ST 03** | **Assessment of $SiO_2$ particle in aerosol generated from e-cigarette by single particle ICP-MS method**<br>CHEN P.; LIN W.; LI D.<br>*Shenzhen First Union Technology Co., Ltd, Laboratory of Life and Health Sciences, Shenzhen, 518103, China* |
| 11:50-12:10 | **ST 04** | **A universal smoking machine adaptor for emissions testing with smoking/vaping machines: development, validation, and benchmarking**<br>BRINKMAN M.C.(1,2); WATSON C.(3); HUANG M.(3); TRAN H.(3); EL HELLANI A.(1,2); WILSON C.W.(1,2); FLESHMAN C.C.(1,2); PETITTI R.(4); PANCAKE M.H.(4); BENNETT C.(5); ALLREAD G.(6); KELLER-HAMILTON B.(2); JONES J.(7); McGUIGAN M.(3); BRAVO R.(3); WAGENER T.L.(2)<br>*(1) College of Public Health, The Ohio State University, Columbus, Ohio, U.S.A.*<br>*(2) Center for Tobacco Research, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, U.S.A.*<br>*(3) Tobacco Products Laboratory, Centers for Disease Control and Prevention, Atlanta, Georgia, U.S.A.*<br>*(4) Center for Design and Manufacturing Excellence, The Ohio State University, Columbus, Ohio, U.S.A.*<br>*(5) Medicinal Chemistry Shared Resource, Drug Development Institute, The Ohio State University, Columbus, Ohio, U.S.A.*<br>*(6) Ergonomics, Spine Research Institute, The Ohio State University, Columbus, Ohio, U.S.A.*<br>*(7) Produced Better, Alpharetta, Georgia, U.S.A.* |
| 12:10-12:30 | **ST 05** | **A quantitative risk assessment approach to compare toxic emissions of electronic cigarettes with combustible cigarettes**<br>LIU C.(1); BERNAL A.J.(2); HUANG Yilang(3); LIU Chuan(4); GRAFF D.W.(1); JIAN Xingtao(3)<br>*(1) RiskWise Solution LLC, Princeton, NJ, U.S.A.*<br>*(2) ToxCreative LLC, 22171 Bianco, Laguna Hills, CA 92653, U.S.A.*<br>*(3) RELX Technology, RELX International, Boton Technology Park, 1044 Chaguang Road, Tower B, 7/F, Shenzhen, China*<br>*(4) Pinevale Ltd, 1st Floor Chilworth Point, 1 Chilworth Road, Southampton, SO16 7JQ, U.K.* |
| 12:30-14:00 | | **LUNCH** |

## MONDAY 9 OCTOBER

### SESSION 3: EVAP - Liquid Assessment

Chair: Jutta PANI

Yucatán Room

| | | |
|---|---|---|
| 14:00-14:20 | **ST 06** | **Simultaneous determination of four sweeteners in e-liquids by ultra performance liquid chromatography**<br>WANG Di; YANG Wenbin; DING Cheng; LIN Guifeng; MO Dongni; LI Weibo<br>*Shenzhen Yupeng Technology Co., Ltd., Building A2, Niujiaolong Industrial Zone, 320 Wuhe Avenue, Longhua District, Shenzhen, Guangdong, China* |
| 14:20-14:40 | **ST 07** | **Study on the transfer rate of five sweeteners in disposable e-cigarettes with mesh coil**<br>LI Weibo; WANG Di; YANG Wenbin; DING Cheng; LIU Shaohua; YU Hanmou<br>*R&D Department, Shenzhen Yupeng Technology Co., Ltd, Shenzhen 518110, China* |
| 14:40-15:00 | **ST 08** | **Study on the storage stability of three flavours in e-liquid**<br>XIAO Junfei; LIU Shaohua; LI Weibo; DING Cheng; YANG Wenbin; ZHANG Zhonghu; ZHONG Qiaoxia; YU Hanmou<br>*Shenzhen Yupeng Technology Co., Ltd, Shenzhen, Guangdong 518000, China* |
| 15:00-15:20 | **ST 09** | **Study on the thermal stability and interaction of three kinds of cooling agents in simulated e-liquid system**<br>DING Cheng; YANG Wenbin; LIN Guifeng; ZHONG Qiaoxia; ZHANG Zhonghu; YU Hanmou; LIU Shaohua<br>*Shenzhen Yupeng Technology Co., Ltd, Building A2, No. 320, Wuhe Road, Zhangkengjing Community, Guanhu Street, Longhua District, Shenzhen, Guangdong 518000, China* |
| 15:20-15:40 | **ST 10** | **Innovative design and application: a home-built undiluted system for particle size characterization based on laser diffraction principle**<br>YUAN J.; LI D.; HUANG Z.; LI Y.; LIN F.; LIU S.; PENG H.; CHEN X.; LIANG X.; HU H.; LIU W.; LONG H.; LU X.<br>*Shenzhen First Union Technology Co., Ltd, Laboratory of Life and Health Sciences, Shenzhen, 518103, China* |
| 15:40-16:00 | **ST 11** | **Comparison of the particle size distribution and vapor phase of two electronic nicotine delivery systems using two Impactors**<br>OLDHAM M.J.(1); JEONG L.(1); OZVALD A.(1,2); GILLMAN I.G.(1)<br>*(1) Juul Labs, Inc., 1000 F Street NW, 8th Floor, Washington, DC, 20004, U.S.A.*<br>*(2) Currently at Sepion Technologies Inc., 1198 65th St., Suite 170, Emeryville, CA 94608, U.S.A.* |
| 16:00-16:20 | | **TEA** |

# MONDAY 9 OCTOBER

## SESSION 4a: Tobacco Analysis

Chair: Johan REDEBY

Guadalajara Room

| | | |
|---|---|---|
| 16:20-16:40 | **Report** | **Sub-Group Tobacco and Tobacco Products Analytes (TTPA)**<br>WAGNER K.A.<br>*Altria Client Services LLC., 601 East Jackson St., Richmond, VA 23219, U.S.A.* |
| 16:40-17:00 | **ST 12** | **Differential analysis of aroma components of different tobacco types using GC×GC-TOF-MS combined with chemometrics**<br>XIN Zhongquan(1); SU Dandan(1); HU Zhongyi(1); ZHAO Lu(2); GAO Yulong(2); WANG Bingwu(2); LIAO Xiaoxiang(1); TANG Jiangu(1)<br>*(1) Smoore Technology Ltd., No. 15-18, Dongcai Industrial Zone, Gushi Community, Xixiang Street, Baoan District, Shenzhen, China*<br>*(2) Yunnan Academy of Tobacco Agricultural Sciences, No. 33 Yuantong Street, Wuhua District, Kunming, Yunnan Province, China* |
| 17:00-17:20 | **ST 13** | **Quantitative analysis of mycotoxins in sub-ppb levels in tobacco products by use of two-dimensional liquid chromatography coupled to high resolution mass spectrometry**<br>NGUYEN M.; PATRING J.; REDEBY J.; LINDHOLM J.<br>*Swedish Match, Regulatory & Scientific Affairs, Maria Skolgata 83, 104 62 Stockholm, Sweden* |
| 17:20-17:40 | **ST 14** | **Development of certified reference cigars representing three product categories**<br>McNEES C.R.(1); JI H.(1); SLONE S.(2); CRAFT M.(1); SHELTON B.(3); SHEARER A.(3); HALL J.T.(1); FISHER A.M.(1); FISHER C.R.(1); YUAN L.(1); CHAMBERS O.(4)<br>*(1) Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY, U.S.A.*<br>*(2) Dr. Bing Zhang Department of Statistics, University of Kentucky, Lexington, KY, U.S.A.*<br>*(3) Markey Cancer Center, University of Kentucky, Lexington, KY, U.S.A.*<br>*(4) College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY, U.S.A.* |
| 17:40-18:00 | **ST 44** | **Determination of tar filter efficiency using spectrophotometric method UV-VIS**<br>FERREIRA T.; SCHAEFER F.; NASCIMENTO J.; BRUM F.; GONÇALVES C.; SILVA J.<br>*BAT - BAT Brazil LABS, Av. Frederico Augusto Ritter, 8000, Cachoeirinha, Brazil* |

## MONDAY 9 OCTOBER

### SESSION 4b: Clinical Studies 1

Chair: Mohamadi SARKAR

Yucatán Room

| | | |
|---|---|---|
| 16:20-16:40 | **Report** | **Sub-Group Biomarkers (BMK)**<br>SARKAR M.<br>*Altria Client Services LLC., 601 East Jackson St., Richmond, VA 23219, U.S.A.* |
| 16:40-17:00 | **ST 15** | **Nicotine pharmacokinetics assessments of two types of e-cigarettes compared to conventional cigarettes: two randomised, crossover studies**<br>YUKI D.*; GILES L.; HARBO S.; HEMSLEY A.<br>*JT International SA., Scientific & Regulatory Affairs, 8 rue Kazem Radjavi, Geneva 1202, Switzerland*<br>** Now at Japan Tobacco Inc., Scientific & Regulatory Affairs, 4-1-1 Toranomon, Minato-ku, Tokyo 105-6927, Japan* |
| 17:00-17:20 | **ST 16** | **An approach to review pharmacokinetic studies of tobacco-free oral nicotine delivery products to enable product comparison**<br>VERRON T.(1); CAHOURS X.(1); NAHDE T.(2)<br>*(1) Imperial Tobacco - SEITA, 216 rue Raymond Losserand, 75014 Paris, France*<br>*(2) Reemtsma Cigarettenfabriken GmbH, an Imperial Brands PLC Company, Max-Born-Straße 4, 22761 Hamburg, Germany* |
| 17:20-17:40 | **ST 18** | **An adverse outcome pathway for oxidative stress in plaque formation**<br>MAKENA P.(1); HASWELL L.(2); McEWAN M.(2); KEYSER B.M.(1); SMART D.(2); LEVERETTE R.(1); JORDEN K.(1); BREHENY D.(2); BAXTER S.A.(2)<br>*(1) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.*<br>*(2) BAT Investments Limited, R&D Centre, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.* |

*[**ST 17** – Converted to Poster (STPOST55)]*

## TUESDAY 10 OCTOBER

### SESSION 5: New Approach Methods (NAMs)

Chair: K. Monica LEE

Co-Chair: Liam SIMMS

Yucatán Room

| | | |
|---|---|---|
| 8:30-8:50 | **Report** | **21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGTX)**<br>GACA M.(1); SIMMS L.(2)<br>*(1) British Investments Limited, Regents Park Road, Millbrook, Southampton SO15 8TL, U.K.*<br>*(2) Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, U.K.* |

### NEW APPROACH METHODS (NAMs) SYMPOSIUM-II: Applications in Tobacco Regulatory Sciences

*(please see page 135 for full programme and abstracts)*

Starting at 8:50 — Yucatán Room

| No. | Time | Titles | Lead, Affiliation |
|---|---|---|---|
| **NAM 00** | 10 min. | **Introduction: New Approach Methods (NAMs): Applications in Tobacco Regulatory Sciences** | K. Monica LEE (Chair)<br>*Altria Client Services* |
| **Session I: NAM Today** | | | |
| **NAM 01** | 15 min. + 2 min. Q&A | **The use of NAMs in *in vitro* genotoxicity assessment of nicotine delivery products: ToxTracker assay as a case study** | Robert LEVERETTE<br>*RAI Services Company* |
| **NAM 02** | 15 min. + 2 min. Q&A | **Connecting exposure, dosimetry and toxicity responses in the preclinical evaluation of ingredients: case examples of flavoring chemicals in oral tobacco products** | Jingjie ZHANG<br>*Altria Client Services* |
| **NAM 03** | 15 min. + 2 min. Q&A | **Exposure to fresh whole smoke and aerosols: standard and novel (3D) *in vitro* models** | Liam SIMMS<br>*Imperial Brands* |
| **BREAK – 10 minutes** | | | |
| **Session II: NAM Tomorrow (NAMs without *in vivo* - clinical adverse outcomes)** | | | |
| **NAM 04** | 15 min. + 2 min. Q&A | **EpiAirway Nrf2 – oxidative stress model: Practical application of *in vitro* systems with clinical relevance** | Brian KEYSER<br>*RAI Services Company* |
| **NAM 05** | 15 min. + 2 min. Q&A | **COPD AOP-I: quantitative modeling of *in vitro* data using an adverse outcome pathway for the assessment of decreased lung function risk in humans** | Marja TALIKKA<br>*Philip Morris International* |
| **NAM 06** | 15 min. + 2 min. Q&A | **COPD AOP-II: *In vitro* assessment of mucus hypersecretion with quantitative AOP modeling** | Shigeaki ITO<br>*Japan Tobacco Inc.* |
| **Session III: Panel Discussion** | | | |
| | 20 min. | **NAMs - Applications in Tobacco Regulatory Sciences – Gaps and Opportunities** | Liam SIMMS (Co-chair)<br>*Imperial Brands*<br>All speakers & panelists |
| **Total: 140 minutes** | | | |

# TUESDAY 10 OCTOBER

## SESSION 6: Posters

Mérida & Querétaro Rooms

14:00 – 17:00

**IGPOST 01** **CORESTA strategy, cooperation and achievements**

LINDHOLM J.(1); STEVENS R.(2); DIGARD H.(3); COLARD S.(4)

*(1) Swedish Match AB, Box 17037, SE-104 62 Stockholm, Sweden*
*(2) RAI Services Company, 401 North Main Street, Winston Salem, NC 27101, U.S.A.*
*(3) BAT Investments Limited, R&D Centre, Southampton, U.K.*
*(4) CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France*

**STPOST 01** **Statistical derivation of a fold-change cut-off for non-targeted differential screening studies based on analytical method variability**

KLEINHANS S.; LANG G.; GOUJON-GINGLINGER C.

*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000, Neuchâtel, Switzerland*

**STPOST 03** **Methods for analysis of sweeteners and flavor components in nicotine pouches and saliva samples**

CHALCRAFT K.; RODRIGUEZ-LAFUENTE A.

*Labstat International, Inc., 262 Manitou Drive, Ontario, N2C 1L3, Canada*

**STPOST 04** **Quantitative analysis for metabolic profiling of aroma compounds by near infrared spectroscopy (NIR)**

YANG Panpan(1,2); ZHOU Wenzhong(1,2); YANG Xiaoyun(1,2); LI Ming(1); LU Xiaoting(1,2); LIU Jing(1,2)

*(1) Yunnan Reascend Tobacco Technology (Group) Co., Ltd, Kunming 650000, China*
*(2) Yunnan Comtestor Co., Ltd, Kunming 650106, China*

**STPOST 05** **Identification of a novel mode of action of vanillin derivative compound veratraldehyde**

WATANABE T.; MUNAKATA S.; TAKAHASHI T.; HASHIZUME T.

*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

**STPOST 06** **Validation of extraction method for tobacco-free nicotine pouches and Swedish snus pouch products - towards standardization of *in vitro* testing**

TALIKKA M.; GORALCZYK A.; DOS SANTOS D.; ALRIQUET M.; ZANETTI F.; ORTEGA TORRES L.; PAK C.; BERTHOUZOZ M.

*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*

**STPOST 07** ***In vitro* cytotoxicity and mechanistic insight from MucilAir™ and SmallAir™ tissues exposed to cigarette smoke and next-generation products**

HOLLINGS M.(1); ROTHWELL E.(1); MARTIN S.(1); DAUNT A.(1); FREIBERG G.(2); BEDFORD R.(1)

*(1) Labcorp Early Development Laboratories Ltd., Harrogate, U.K.*
*(2) Labcorp Early Development Laboratories Ltd., Eye, U.K.*

**STPOST 08** **Aerosol generation and exposure atmosphere characterization of VITROCELL Air Liquid Interface (ALI) exposure system using ENDS products**

GUPTA A.; PSURNY E.; NEWSWANGER J.; GRIFFIN C.; AMELUNG A.; BEHRINGER S.; MOYER B.

*Battelle Memorial Institute, 505 King Avenue, Columbus, OH 43201, U.S.A.*

**STPOST 09** **Examination of automated growth inhibition classification in Ames test by machine learning**

KUM R.; KAIYA K.; ITO H.

*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

**STPOST 10** ***In vitro*** **micronucleus in V79 cells using Microflow - treatment at the air liquid interface with aerosol from three different nicotine containing products**

ROTHWELL E.; SMITH G.; MARTIN S.; HOLLINGS M.

*Labcorp Early Development Laboratories Ltd, Otley Road, Harrogate HG3 1PY, U.K.*

**STPOST 11** **Validation of the Vitrocell® HTP 2.0+ 12 well mammalian module for assessing cigarettes, ENDS, and eHTPs: evaluation of dilution airflow, dose resolution, and dose repeatability**

SEYMOUR A.; ROTHWELL E.; MARTIN S.; HOLLINGS M.

*Labcorp Early Development Laboratories Ltd, Otley Road, Harrogate HG3 1PY, U.K.*

**STPOST 12** **Rapid *in vitro* toxicological screening using ToxTracker to determine the effect of repeated freeze/thaw of combustible cigarette extract**

CARMINES E.(2); MISRA M.(2); OH S.(1); HENDRIK G.(3); COFFA B.G.(1,2); ALEKSA K.(1)

*(1) Labstat International, 262 Manitou Dr., Kitchener, ON N2C 1L3, Canada*
*(2) Chemular Inc., 103 Main Street, Hudson MI 49247, U.S.A.*
*(3) Toxys Inc, Robert Boyleweg 4., 2333 CG Leiden, The Netherlands*

**STPOST 13** **Application of rapid *in vitro* toxicological screening using ToxTracker to determine the effect of flavors in snus products.**

CARMINES E.(2); MISRA M.(2); OH S.(1); HENDRIK G.(3); COFFA B.G.(1,2); ALEKSA K.(1)

*(1) Labstat International, 262 Manitou Dr., Kitchener, ON N2C 1L3, Canada*
*(2) Chemular Inc., 103 Main Street, Hudson MI 49247, U.S.A.*
*(3) Toxys Inc, Robert Boyleweg 4., 2333 CG Leiden, The Netherlands*

**STPOST 14** **Comparison of urinary biomarkers of tobacco smoke exposure among different smokers**

GAO Yihan; LI Xiaonan; CUI Peicai; WANG Xuan; SHEN Yi

*Shanghai New Tobacco Product Research Institute, No. 3733, Xiupu Road, Pudong New Area, Shanghai, China*

**STPOST 15** **Effect of cigarette smoke on interfacial chemical properties of pulmonary surfactant**

LI Chao(1,2), ZHAO Qun(2), CHEN Longyu(2), FAN Duoqing(1), GUAN Ying(1), CHEN Fangrui(1), ZHU Zhouhai(1), WANG Lu(1), YANG Lei(1), PENG Qiyuan(1), MA Li(1), PAN Yi(2), LI Meng(1)

*(1) China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, Yunnan, China*
*(2) Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650500, Yunnan, China*

**STPOST 16** **Biomarkers assessment in healthy adult smokers who switched from conventional cigarettes to two types of non-combustible tobacco products: a randomized, controlled study**

YUKI D.*; GILES L.; HARBO S.; HEMSLEY A.

*JT International SA., Scientific & Regulatory Affairs, 8 rue Kazem Radjavi, Geneva 1202, Switzerland*
** Now at Japan Tobacco Inc., Scientific & Regulatory Affairs, 4-1-1 Toranomon, Minato-ku, Tokyo 105-6927, Japan*

**STPOST 17** **Biomarkers assessment in healthy adult smokers who switched from conventional cigarettes to closed tank e-cigarette: a randomized, controlled study**

YUKI D.*; GILES L.; HARBO S.; HEMSLEY A.

*JT International SA., Scientific & Regulatory Affairs, 8 rue Kazem Radjavi, Geneva 1202, Switzerland*

** Now at Japan Tobacco Inc., Scientific & Regulatory Affairs, 4-1-1 Toranomon, Minato-ku, Tokyo 105-6927, Japan*

**STPOST 18** **Comparison of biomarkers of exposure in users of various tobacco/nicotine products**

SCHERER M.; BURKHARDT T.; SCHERER G.; PLUYM N.

*ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany*

**STPOST 19** **Analysis of cannabidiol and its three major metabolites in human plasma by online extraction LC-MS/MS**

PLUYM N.; KANIS R.; RIEDEL K.; BURKHARDT T.; SCHERER G.; SCHERER M.

*ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany*

**STPOST 20** **White blood cell count is a biomarker of a potential harm in short-term smoking abstinence and product switching to electronic nicotine delivery systems**

MAKENA P.(1); CHEN P.(1); PRASAD G.L.(3); BAXTER S.A.(2)

*(1) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.*
*(2) BAT Investments Limited, Research and Development, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*
*(3) Prasad Scientific Consulting LLC, 490 Friendship Place Ct., Lewisville, NC 27023, U.S.A.*

**STPOST 21** **Aromatic amines in the mainstream smoke of commercial cigars**

JI Huihua(1); JIN Zhenyu(1); FENTON L.(1); SLONE S.(2)

*(1) Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY 40546, U.S.A.*
*(2) Dr. Bing Zhang Department of Statistics, University of Kentucky, Lexington, KY 40536, U.S.A.*

**STPOST 22** **The effects of characterizing flavors on mainstream cigarette smoke chemistry and *in vitro* toxicity**

YOO Sohee; LEE Sanghoon; JANG Mi; YOO Jihye; JU Soyoung; CHOI Ikjang; MIN Hyejeong; PARK Chulhoon

*KT&G, R&D Headquarters, 30, Gajeong-ro, Yuseong-gu, Daejeon 34128, South Korea*

**STPOST 23** **A pilot assessment on novel hybrid product: emission analysis of nicotine, volatile aromatic constituents and selected HPHCs**

LIU Shengyi; QING Wei; DOU Jinxi; LIU Weijuan

*(1) Ruvian Technology Ltd., Kunming 650106, China*
*(2) Yunnan Reascend Tobacco Technology (Group) Co., Ltd, Kunming 650000, China*

**STPOST 24** **Humectant ability and bilateral humectant method construction for cigarette components**

QIU Changgui(1,2); LIU Ze(3); ZUO Wen(4); LI Siyuan(4); HUA Yikun(4); LIU Jing(1,2); FU Liang(3); QI Lin(4); MA Ning(3); ZHU Yemei(1,2); HE Banghua(3); YANG Panpan(1,2)

*(1) Yunnan Reascend Tobacco Technology (Group) Co., Ltd., Kunming 650106, China*
*(2) Yunnan Comtestor Co., Ltd., Kunming 650106, China*
*(3) China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, China*
*(4) Hongyun Honghe Tobacco (Group) Co., Ltd., Kunming 650231, China*

**STPOST 25** **Nicotine pharmacokinetics and subjective effects of novel heated tobacco product compared to cigarettes: a randomised, crossover study**

YUKI D.*; GILES L.; HARBO S.; HEMSLEY A.

*JT International SA., Scientific & Regulatory Affairs, 8 rue Kazem Radjavi, Geneva 1202, Switzerland*

** Now at Japan Tobacco Inc., Scientific & Regulatory Affairs, 4-1-1 Toranomon, Minato-ku, Tokyo 105-6927, Japan*

**STPOST 26** **Characterization of nicotine pharmacokinetics from use of a novel heated tobacco capsule prototype in adults who smoke**

EDMISTON J.; LIU J.; WANG J.; LAU R.; PITHAWALLA Y.B.

*Altria Client Services LLC, Center for Research and Technology, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

**STPOST 27** **Monitoring puff-by-puff aerosol generation and delivery when vaping viscous liquids by using in-line pressure drop measurement**

TAYLOR H.; TINDALL I.F.

*Cerulean, Rockingham Drive, Milton Keynes, U.K.*

**STPOST 28** **Determination of nicotine, propylene glycol, menthol, glycerol and water by GC-FID & TCD using nitrogen as the carrier gas**

SAKASHITA R.; NANJO K.; OMORI F.

*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

**STPOST 29** **Formation of small organic acids during ends aerosol collection**

CHEETHAM A.G.; JABLONSKI J.J.

*Enthalpy Specialty Labs, 1470 East Parham Rd, Richmond, VA 23228, U.S.A.*

**STPOST 30** **Comparison of material surface staining following exposure to heated tobacco and e-vapour aerosols versus cigarette smoke**

GOMEZ LUESO M.; RODRIGUES CRESPO K.; GLABASNIA A.; GOUJON-GINGLINGER C.; KLEINHANS S.; MITOVA M.I.; LANG G.

*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*

**STPOST 31** **Simultaneous multisensor physical characterisation of heated tobacco product aerosol and the selection of a reference system**

TINDALL I.F.; REDDY S.; LAIMON H.

*Cerulean, Rockingham Drive, Milton Keynes, U.K.*

**STPOST 32** **Simultaneous determination of six furans in aerosol of heated tobacco products by gas chromatography/mass spectrometry**

ZHANG Li(1); WANG Weiwei(1); WANG Bing(2); DONG Rui(1); YIN Fang(1); TONG Fuqiang(1); JI Houwei(1); LIU Yuming(1)

*(1) Technology Center, China Tobacco Guizhou Industrial Co., Ltd., Guiyang 550009, China*
*(2) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China*

**STPOST 33** **Comparison of HPHCs in aerosol generated from a novel heated tobacco capsule prototype to HPHCs in conventional cigarette smoke**

MELVIN M.; SKAPARS J.A.; PFEIFFER K.; LI Weiling; PITHAWALLA Y.B.; DANIELSON T.

*Altria Client Services LLC, Center for Research and Technology, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

**STPOST 34** **Research on the distribution of nicotine retained by filters of heated tobacco products based on visualization**

WANG Shuaipeng(1,2); HUANG Yuchuan(1,3); LIU Kai(2,4); ZHANG Jie(5); HAN Donglin(1,4); LIANG Kun(1,3); CHAI Yidi(1,2); ZHOU Rong(1,3); GUO Linqing(1,2); SHI Jianyang(2,4)

*(1) China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610066, China*
*(2) Sichuan Sanlian New Material Co., Ltd., Chengdu 610041, China*
*(3) Harmful Components and Tar Reduction in Cigarette Key Laboratory of Sichuan Province, Chengdu 610066, China*
*(4) New Tobacco Products Engineering and Technology Research Center of Sichuan Province, Chengdu 610066, China*
*(5) Core Facility of West China Hospital, Chengdu 610041, China*

**STPOST 35** **Determination of aerosol mass and five primary constituents in aerosols generated from heated tobacco products**

BROWN A.P.(1); INGRAM S.(2); SOLER K.(2); McCUTCHEON N.(1); MELVIN M.(1); PITHAWALLA Y.B.(1); LI W.(1)

*(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
*(2) Eurofins Lancaster Professional Scientific Staffing, U.S.A.*

**STPOST 36** **A comparison of HPHCs in on!® PLUS nicotine pouches to HPHCs in cigarettes, smokeless tobacco including snus and an oral NRT product**

MILLER IV J.H.; GRISEVICH H.; JIN X.C.; SMITH J.H.; SENA E.J.; WAGNER K.A.; BALLENTINE R.M.; SALMON C.K.; WILLIAMS M.S.; YANG L.; MORGAN R.W.; HAASE V.; DANIELSON T.L.

*Altria Client Services LLC, Center for Research and Technology, Richmond, VA 23219, U.S.A.*

**STPOST 37** **Determination of unprotonated nicotine in aqueous extract of snus and nicotine pouches by 1H NMR spectroscopy**

OHASHI S.; KOYAMA R.; YOSHIDA M.

*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

**STPOST 38** **Establishment of analytical method for mycotoxins in smokeless tobacco products**

PARK Hyoung-Joon; LEE Min Kyoung; KIM Min Soo; CHO Eun Sang; HEO Seok; CHOI Jang Duck; CHO Sooyeul

*Centre for Advanced Analysis, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Cheongju-si, South Korea*

**STPOST 39** **Non-target analysis survey of tobacco-free nicotine pouches**

MARTIN A.; COLLINS J.

*Enthalpy Specialty Labs, 1470 East Parham Rd, Richmond, VA 23228, U.S.A.*

**STPOST 40** **Comparison of small cigar smoke yields with and without fitment of supplied plastic tip**

BRINKMAN M.C.(1); EL HELLANI A.(1); WILSON C.W.(1); FLESHMAN C.C.(1); PETITTI R.(3); PANCAKE M.H.(3); BENNETT C.(4); ALLREAD G.(5); KELLER-HAMILTON B.(2); JONES J.(6); WAGENER T.L.(2); TINDALL I.F.(7)

*(1) College of Public Health, The Ohio State University, Columbus, Ohio, U.S.A.*
*(2) Center for Tobacco Research, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, U.S.A.*
*(3) Center for Design and Manufacturing Excellence, The Ohio State University, Columbus, Ohio, U.S.A.*
*(4) Medicinal Chemistry Shared Resource, Drug Development Institute, The Ohio State University, Columbus, Ohio, U.S.A.*
*(5) Ergonomics, Spine Research Institute, The Ohio State University, Columbus, Ohio, U.S.A.*
*(6) Produced Better, Alpharetta, Georgia, U.S.A.*
*(7) Cerulean, Rockingham Drive, Milton Keynes, U.K.*

**STPOST 42** **Survey investigation and methodology results of environmental exposure to heated tobacco products exhaled aerosol in Japan**

TATENO S.; SAKAGUCHI C.; KIMURA Y.

*Japan Tobacco Inc., Kamiyacho Trust Tower, 4-1-1, Toranomon, Minato-ku, Tokyo 105-6927, Japan*

**STPOST 43** **Association between heated tobacco products use and long-term health effects in Japan: Internet-based cross-sectional study**

KIMURA Y.

*Japan Tobacco inc., Kamiyacho Trust Tower, 4-1-1, Toranomon, Minato-ku, Tokyo 105-6927, Japan*

**STPOST 44** **Promotional materials for a novel heated tobacco capsule system increase behavioral intentions to try and use in adult smokers but not in nonusers, and do not impede quit intentions**

McKINNEY D.L.; BECKER E.A.; KARELITZ J.

*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

**STPOST 45** **Promotional materials for a novel heated tobacco capsule system do not alter risk perceptions**

McKINNEY D.L.; BECKER E.A.; KARELITZ J.

*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

**STPOST 46** **Measuring tobacco product experience: CROM adapted from the mCEQ for the assessment of new tobacco products**

MAINY N.(1); BAJEC M.(2); ALVES FAVARO M.(3); SALZBERGER T.(4); ROSE J.(5)

*(1) JT International SA, 8 rue Kazem Radjavi, 1201, Geneva, Switzerland*
*(2) Bajec Senseworks Consulting, Hamilton, L8K 2L7, Ontario, Canada*
*(3) Mapi Research Trust, 27 rue de la Villette, 69003 Lyon, France*
*(4) Institute for Statistics and Mathematics, Institute for Marketing Management, University of Economics and Business (WU Wien), Welthandelsplatz 1, 1020, Vienna, Austria*
*(5) Rose Research Center, Raleigh, NC 27617, U.S.A.*

**STPOST 47** **Systematic review of the design and conduct of actual use studies of new tobacco products**

MAINY N.(1); BAJEC M.(2); RICHARDSON C.(3); RUSSELL C.(4)

*(1) JT International SA, 8 rue Kazem Radjavi, 1201, Geneva, Switzerland*
*(2) Bajec Senseworks Consulting, Hamilton, L8K 2L7, Ontario, Canada*
*(3) PEGUS Research, Inc., 331 South Rio Grande, Suite 100, Salt Lake City, UT 84101, U.S.A.*
*(4) Russell Burnett Research & Consultancy Ltd, Glasgow, U.K.*

**STPOST 48** **Study design of a post market surveillance pilot study on reduced risk tobacco and nicotine products**

PRASAD K.(1); SHETTY M.(1); HART R.(1); CARAWAY J.(2); FIEBELKORN S.(1); ROGERS L.(1); BATTISTA D.(2); SHANE R.(3); MIERA M.(3); SCHWARTZ E.(3); BARLOW K.(3); MONTELONGO M.(3)

*(1) BAT Investments Limited, R&D Centre, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*
*(2) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.*
*(3) ICON plc, U.S.A. & U.K.*

**STPOST 49** **Design of a compact connected puffing topography instrument for real-world measurements of e-cigarette use behaviour**

PRASAD K.; EDWARD L.; HARRIS R.

*BAT Investments Limited, R&D Centre, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*

**STPOST 50** **Data gap analysis and proposal for environmental chemical risk assessment of cigarette butts**

DOMÍNGUEZ ESTÉVEZ M.; SAYERS K.; PRICE L.; GILES L.

*JT International SA, 8 rue Kazem-Radjavi, 1202 Geneva, Switzerland*

**STPOST 51** **Application of the human health risk assessment process for the evaluation of electronic cigarettes**

BERNAL A.J.(1); LIU C.(2); JOHNSON C.(3); YOUNG R.(3)

*(1) ToxCreative LLC, Laguna Hills, CA, U.S.A.*
*(2) RiskWise Solution LLC, Princeton, NJ, U.S.A.*
*(3) Bibra Toxicology Advice & Consulting, U.K.*

**STPOST 52** **Chemical, pharmacological, and toxicological assessment of 6-methylnicotine**

CHEETHAM A.G.(1); PLUNKETT S.E.(1,2); MARKING S.(2); CAMPBELL P.(2); COFFA B.G.(2); GILLILAND III, S.(2); McFADDEN L.(1); SCIAN M.(1)

*(1) Enthalpy Specialty Labs, Richmond, VA, U.S.A.*
*(2) Consilium Sciences, Richmond, VA, U.S.A.*

**STPOST 53** **Effect of ion source type and extractor lens diameter on sensitivity and peak shape of semivolatile organic compounds (pyridine, quinoline, and styrene) in gas chromatography/mass spectrometry analysis using hydrogen carrier gas**

NANJO K.; OMORI F.

*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

**STPOST 54** **Rapid and easy removal or purification of pyridine compounds by phenylboronic acid solid-phase extraction (PBA-SPE) cartridge for compound identification for non-targeted gas chromatography/mass spectrometric analysis of tobacco products**

SHIGETO A.

*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

**STPOST 55** **Assessing nicotine pharmacokinetics of new generation tobacco products and conventional cigarettes: a systematic review and meta-analysis**

CAO Yue(1); HU Chongyi(1); ZHANG Lin(2); LI Jiaxuan(1); CHEN Xi(1); ZHANG Jianqiang(1); LIU Xiaona(1)

*(1) Department of Health Sciences, Smoore Tech Research Institute, No. 16, Dongcai Industrial Zone, Baoan District, Shenzhen, China*
*(2) Institute of Artificial Intelligence, Hefei Comprehensive National Science Center, B1205-B1208, Future Center, Institute of Advanced Technology, University of Science and Technology of China, No. 5089, Wangjiang West Road, Hefei, China*

*[**STPOST 02, STPOST 41** – Withdrawn]*

## WEDNESDAY 11 OCTOBER

### SESSION 7: Biological Assessment

Chair: Kei YOSHINO

Yucatán Room

| | | |
|---|---|---|
| 8:30-8:50 | **Report** | **Sub-Group *In Vitro* Toxicity Testing (IVT)**<br>SIMMS L.<br>*Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, U.K.* |
| 8:50-9:10 | **ST 19** | **High-sensitive exposure system in Ames test using bacteria suspension for fresh smoke and aerosol – showcasing by spiking experiment**<br>WIECZOREK R.; POUR S.J.<br>*Reemtsma Cigarettenfabriken GmbH (an Imperial Brands PLC Company), Albert-Einstein-Ring 7, D-22761 Hamburg, Germany* |
| 9:10-9:30 | **ST 20** | ***In vitro* cytotoxicity assessment of arecoline benzoate aerosol by using an air-liquid interface exposure system**<br>HU Hao; CHU M.; DENG J.; ZHAO J.; LIU Z.; CHEN P.; YUAN J.; LI D.; LU Jin<br>*Shenzhen First Union Technology Co., Ltd, Laboratory of Life and Health Sciences, Shenzhen, 518103, China* |
| 9:30-9:50 | **ST 21** | **Evaluation of mixtures of flavor ingredients in 90-day nose-only inhalation exposures**<br>OLDHAM M.J.(1); DESAI R.W.(1,2); RANDAZZO J.(3); WEIL R.(1,4); LALONDE G.(1)<br>*(1) Juul Labs, Inc., 1000 F Street NW, 8th Floor, Washington, DC, 20004, U.S.A.*<br>*(2) Currently at Syngenta, 140 Research Lane, Research Park, Guelph, Ontario N1G 4Z3, Canada*<br>*(3) Charles River Laboratories, LLC, 1407 George Road, Ashland, OH 44805 U.S.A. (Currently at Attentive Science, LLC, 17745 Metcalf Ave, Bldg #4, Stillwell, KS 66085, U.S.A.)*<br>*(4) Currently at Weil Regulatory & Toxicology Consulting, LLC, 3006 White Pine Dr., Monrovia, MD 21770, U.S.A.* |
| 9:50-10:10 | **ST 22** | **Cytotoxicity evaluation of vanillin in electronic cigarette liquids on human aortic artery endothelial cells**<br>CARUSO M.(1,2); EMMA R.(2,3); DISTEFANO A.(1); PARTSINEVELOS K.(1); RUST S.(4); GIORDANO A.(5); SUN A.(5); VOLAREVIC V.(6); ARSENIJEVIC A.(6); KASTRATOVIC N.(6); LESMANA R.(7,8); BARLIANA M.I.(8,9); POLOSA R.(2,3); LI VOLTI G.(1,2)<br>*(1) Department of Biomedical and Biotechnological Sciences, University of Catania, Via S. Sofia, 97, 95123 Catania, Italy*<br>*(2) Center of Excellence for the Acceleration of Harm Reduction (CoEHAR), University of Catania, Via S. Sofia, 97, 95123, Catania, Italy*<br>*(3) Department of Clinical and Experimental Medicine, University of Catania, Via S. Sofia, 97, 95123, Catania, Italy*<br>*(4) ECLAT Srl, spin off of the University of Catania, Via. S Sofia 89, 95123 Catania, Italy*<br>*(5) Sbarro Institute for Cancer Research and Molecular Medicine, Department of Biology, College of Science and Technology, Temple University, PA, U.S.A.*<br>*(6) Center for Molecular Medicine and Stem Cell Research, Department of Microbiology and Immunology, Faculty of Medical Sciences, University of Kragujevac, Serbia, 69 Svetozara Markovica Street, 34000 Kragujevac, Serbia*<br>*(7) Center of Excellence for Pharmaceutical Care Innovation, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM. 21, Jatinangor 45363, Indonesia*<br>*(8) Department Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM. 21, Jatinangor 45363, Indonesia*<br>*(9) Department of Biological Pharmacy, Biotechnology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM. 21, Jatinangor 45363, Indonesia* |

| | | |
|---|---|---|
| 10:10-10:30 | **ST 23** | **Replication study to evaluate the *in vitro* toxicity profile of the myblu™ electronic cigarette compared to tobacco smoke: the REPLICA project**<br>EMMA R.(1,2); FUOCHI V.(2,3); DISTEFANO A.(3); PARTSINEVELOS K.(3); RUST S.(4); ZADJALI F.(5); AL TOBI M.(5); ZADJALI R.(5); ALHARTHI Z.(5); PULVIRENTI R.(3); FURNERI P.M.(2,3); POLOSA R.(1,2,4); SUN A.(6); CARUSO M.(2,3); LI VOLTI G.(2,3); and the Replica Project Group<br>*(1) Department of Clinical and Experimental Medicine, University of Catania, Via S. Sofia, 97, 95123 Catania, Italy*<br>*(2) Center of Excellence for the Acceleration of Harm Reduction (CoEHAR), University of Catania, Via S. Sofia, 97, 95123, Catania, Italy*<br>*(3) Department of Biomedical and Biotechnological Sciences, University of Catania, Via S. Sofia, 97, 95123, Catania, Italy*<br>*(4) ECLAT Srl, spin off of the University of Catania, Via. S Sofia 89, 95123 Catania, Italy*<br>*(5) College of Medicine and Health Sciences, Department of Clinical Biochemistry, Sultan Qaboos University, P.O. Box 35, P.C 123, Khodh, Oman*<br>*(6) Sbarro Institute for Cancer Research and Molecular Medicine, Department of Biology, College of Science and Technology, Temple University, PA, U.S.A.* |
| 10:30-10:50 | | **COFFEE** |

## WEDNESDAY 11 OCTOBER

### SESSION 8: Clinical Studies 2

Chair: Mohamadi SARKAR

Yucatán Room

| | | |
|---|---|---|
| 10:50-11:10 | **ST 24** | **Comparing adult smokers who switched to JUUL vs continuing smokers: biomarkers of exposure, biomarkers of potential harm and respiratory symptoms**<br>SHIFFMAN S.(1); OLIVERI D.R.(2); GOLDENSON N.I.(2); LIANG Qiwei(2); BLACK R.A.(2); MISHRA S.(2)<br>*(1) Pinney Associates, Inc., Pittsburgh, PA, U.S.A.*<br>*(2) Juul Labs, Inc., Washington, DC, U.S.A.* |
| 11:10-11:30 | **ST 25** | **Switching exclusively from smoking to using glo results in significant, substantial reductions in exposure to cigarette smoke toxicants**<br>GALE N.; AZZOPARDI D.; McEWAN M.; MEICHANETZIDIS F.; VEL S.<br>*BAT Investments Limited, R&D Centre, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.* |
| 11:30-11:50 | **ST 26** | **Non-targeted analysis of exhaled breath to distinguish different nicotine product user groups**<br>BURKHARDT T.; PLUYM N.; SCHERER G.; SIBUL F.; SCHERER M.<br>*ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany* |
| 11:50-12:10 | **ST 27** | **Analysis of the smoke components in smokers' oral fluids by large volume injection-in-column evaporation concentration**<br>WU Bingyu; LIU Baizhan; FEI Ting; BI Yanjiu; LIANG Demin; CAI Zhenbo; WU Da; YANG Kai<br>*Shanghai Tobacco Group Co., Ltd., No. 3733, Xiupu Road, Shanghai 201315, China* |
| 12:10-12:30 | **ST 28** | **Revealing the urinary exposome of smokers, vapers, HTP users and pouch consumers by high-resolution LC-MS/MS – an important step in the identification of use-specific biomarkers of exposure**<br>PLUYM N.; KACHHADIA A.; BURKHARDT T.; SCHERER G.; SCHERER M.<br>*ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany* |
| 12:30-14:00 | | **LUNCH** |

## WEDNESDAY 11 OCTOBER

### SESSION 9: Consumer Related Outcome Measures (CROM)

Chair: Stacey McCAFFREY

Yucatán Room

| | | |
|---|---|---|
| 14:00-14:20 | **Report** | **Task Force Consumer Reported Outcome Measures Consortium (CROM)**<br>CHREA C.(1); CAHOURS X.(2)<br>*(1) Philip Morris Products S.A., Quai Jeanrenaud 3, CH-2000 Neuchâtel, Switzerland*<br>*(2) Imperial Tobacco – SEITA, 216 rue Raymond Losserand, 75014 Paris, France* |

### CROM SYMPOSIUM
### Science Starts with Measurement: Essential Measurement Science for Self-Report in Tobacco and Nicotine Product Research

*(please see page 145 for full programme and abstracts)*

Starting at 14:20 — Yucatán Room

| No. | Time | Titles | Lead, Affiliation |
|---|---|---|---|
| **CROM 01** | 20 min. | **Overview of the psychometric CROM guidelines** | Stacey McCAFFREY<br>*Juul Labs, Inc.* |
| **CROM 02** | 20 min. | **Overview of psychometrics: the science of measurement** | Stacey McCAFFREY<br>*Juul Labs, Inc.* |
| **BREAK – 5 minutes** | | | |
| **CROM Guest** | 20 min. | **Risk perceptions in tobacco regulatory science** | Alexander PERSOSKIE<br>*FDA Center for Tobacco Products* |
| **CROM 03** | 20 min. | **Evaluating the psychometric properties of a CROM for use with a different product for which it was developed: the modified e-cigarette evaluation questionnaire (MCEQ) as a case study** | Meghan MOREAN<br>*Yale School of Medicine* |
| **CROM 04** | 20 min. | **Further validation of the ABOUT-Dependence measure: Extending assessment of perceived dependence on tobacco and nicotine products to users of a heated tobacco product (*IQOS*)** | Esther AFOLALU<br>*Philip Morris International* |
| **CROM 05** | 20 min. | **An illustration that one size may not fit all: assessing invariance of the WISDM scale in PATH across youth and young adult cohorts** | Ryan BLACK<br>*Juul Labs, Inc.* |
| **CROM 06** | 20 min. | **Can individuals with limited health numeracy use quantitative scales to make ratings of risk perceptions?** | Saul SHIFFMAN<br>*Pinney Associates* |
| **BREAK – 10 minutes** | | | |
| | 20 min. | **Discussion and Q&A** | Mohamadi SARKAR<br>*Altria Client Services*<br>All speakers |
| **Total: 195 minutes** | | | |

## THURSDAY 12 OCTOBER

### SESSION 10: Product Use Behaviour

Chair: Sarah BAXTER-WRIGHT

Yucatán Room

| | | |
|---|---|---|
| 8:30-8:50 | **Report** | **Sub-Group Product Use Behaviour (PUB)**<br>PRASAD K.<br>*BAT Investments Limited, Group R&D, Regents Park Road, Millbrook, Southampton SO15 8TL, U.K.* |
| 8:50-9:10 | **ST 29** | **Longitudinal analysis of sustained cigarette smoking abstinence and relapse behaviors**<br>WEI Lai; HE Yisha; MUHAMMAD-KAH R.; RICHTER N.; LARGO E.; SARKAR M.<br>*Center for Research & Technology, Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 9:10-9:30 | **ST 30** | **Perception of harm and addictive potential of the RELX Infinity electronic nicotine delivery system (ENDS) and future product use intentions of adult smokers switching from combustible cigarettes over eight weeks**<br>GRAFF D.W.(1); FEARON I.M.(2)<br>*(1) Cheerain HK Limited, Hong Kong, China*<br>*(2) whatIF? Consulting Limited, Harwell, U.K.* |
| 9:30-9:50 | **ST 31** | **Emotion analysis of consumer comments on cigarette products by integrating multi-level features**<br>WANG Rui; WANG Di; ZONG Guohao; WANG Yongsheng; HU Bin; FENG Weihua<br>*Zhengzhou Tobacco Research Institute of CNTC, No.2, Fengyang Street, High-tech District, Zhengzhou 450001, Henan, China* |
| 9:50-10:10 | **ST 34** | **Assessing the potential impact of e-cigarette policies on smoking prevalence: a European Union study**<br>CAHOURS X.(1); VERRON T.(1); NAHDE T.(2); PARKER R.(1)<br>*(1) Imperial Tobacco – SEITA, 216 rue Raymond Losserand, 75014 Paris, France*<br>*(2) Reemtsma Cigarettenfabriken GmbH (an Imperial Brands PLC Company), Max-Born-Straße 4, 22761 Hamburg, Germany* |
| 10:10-11:10 | | **COFFEE** |

*[**ST 32, ST 33** – Withdrawn]*

## THURSDAY 12 OCTOBER

### SESSION 11: HTP – Aerosol Assessment

Chair: Jana JEFFERY

Yucatán Room

| | | |
|---|---|---|
| 11:10-11:30 | **Report** | **Sub-Group Heated Tobacco Products (HTP)**<br>DIGARD H.<br>*BAT Investments Limited, R&D, Regents Park Road, Millbrook, Southampton SO15 8TL, U.K.* |
| 11:30-11:50 | **ST35** | **Heating vs. burning: a non-targeted analytical characterization of compounds with higher abundance in the aerosol of a heated tobacco product than in mainstream cigarette smoke**<br>LANG G.; ALMSTETTER M.; ARNDT D.; HENAO C.; GOUJON-GINGLINGER C.<br>*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland* |
| 11:50-12:10 | **ST 36** | **Numerical simulation of evaporation characteristics for heated tobacco products**<br>WANG Wie; ZHANG Bo; SUN Zhiwei; WEI Weiwei; WANG Zhiguo; DU Wen<br>*Technology Center, China Tobacco Hunan Industrial Co., Ltd., Changsha 410007, Hunan, China* |
| 12:10-12:30 | **ST 37** | **Quantitation of selected elements in the aerosol of electrically heated tobacco products (eHTPs) by inductively coupled plasma mass spectrometry (ICP-MS)**<br>O'REILLY C.<br>*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland* |
| 12:30-14:00 | | **LUNCH** |

# THURSDAY 12 OCTOBER

## SESSION 12: Tobacco Processes

Chair: Bin HU

Yucatán Room

| | | |
|---|---|---|
| 14:00-14:20 | **Report** | **Sub-Group Cigar Smoking Methods (CSM)**<br>LINDEGAARD T.<br>*Scandinavian Tobacco Group A/S, Sydmarken 42, SE-2860 Soborg, Denmark* |
| 14:20-14:40 | **ST 39** | **Analysis of tobacco stem shaping effect based on compression-set**<br>LIU Ze(1); QIU Changgui(2); ZUO Wen(3); LI Siyuan(3); YANG Ji(1); LI Zhenjie(1); HE Banghua(1); FU Liang(1); YANG Qianxu(1); ZHOU Yuanzhen(3); YANG Hongming(1); ZHOU Guofu(1)<br>*(1) China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, China*<br>*(2) Yunnan Comtestor Co., Ltd., Kunming 650106, China*<br>*(3) HongyunHonghe Tobacco (Group) Co., Ltd., Kunming 650231, China* |
| 14:40-15:00 | **ST 40** | **Removal of nicotine, flavors, and tobacco constituents from machine wash water**<br>BUSSEY R.O.<br>*Reynolds American, 950 Reynolds Boulevard, Winston Salem, NC 27105, U.S.A.* |
| 15:00-15:20 | **Report** | **Sub-Group Physical Test Methods (PTM)**<br>EITZINGER B.<br>*delfort AG, Fabrikstraße 20, A-4050 Traun, Austria* |
| | | |
| 15:20-16:00 | | **TEA** |

*[**ST 38** – Withdrawn]*

# THURSDAY 12 OCTOBER

## SESSION 13: Cigarette & Filter Design

Chair: Bernhard EITZINGER

Yucatán Room

| | | |
|---|---|---|
| 16:00-16:20 | **ST 43** | **Degradation and toxicity evaluation of cigarette butts**<br>DE JONGH S.; RAVERDY-LAMBERT D.<br>*SWM LTR Industries, Usine Le Mans, 72702 Allonnes, France* |
| 16:20-16:40 | **Report** | **Sub-Group Smoke Analysis (SA)**<br>YAMAZAKI H.<br>*Japan Tobacco Inc., 1-17-7, Yokokawa, Sumida-ku, Tokyo 130-8603, Japan* |
| 16:40-17:00 | **ST 41** | **Study on the combustion coupling-matching mechanism of cigarette ash integration during smoking**<br>WANG Xiaofeng(1); ZHANG Jin(1); WANG Peng(1); ZHANG Xiaoyu(1); ZHANG Yaping(1); ZHANG Qi(2); CAO Yun(1); GUAN Mingjing(1); WANG Le(2); GUO Dongfeng(1); TIAN Huijuan(1); LI Yanyan(1); LI Bin(2); ZHOU Shun(1)<br>*(1) Key Laboratory of Combustion & Pyrolysis Study of CNTC, Anhui Tobacco Industrial Co., Ltd., Hefei 230088, China*<br>*(2) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* |
| 17:00-17:20 | **ST 42** | **Effect and influence of perforation methods for tipping paper on the control of the thermal energy of smoke from tobacco products**<br>LINDNER M.(1); RUFENER C.(2); BENSE T.(2)<br>*(1) TANN HOLDING GmbH, Fabrikstrasse 48a, A-4050 Traun, Austria*<br>*(2) C.I.T. MONTEPAZ S.A., San Ramon 716, UY-11800 Montevideo, Uruguay* |
| 17:20-17:40 | **ST 45** | **Filtration characteristics of polycyclic aromatic hydrocarbons in mainstream cigarette smoke by tobacco rod**<br>SUN Xuehui(1); FENG Weiwei(1); QIN Yaqiong(1); GUO Jizhao(1); CAO Yi(2); SUN Peijian(1); WANG Yipeng(1); ZHU Huaiyuan(2); XIE Fuwei(1); NIE Cong(1)<br>*(1) Zhengzhou Tobacco Research Institute of CNTC, No. 2, Fengyang Street, Hi-Tech District, Zhengzhou 450001, Henan, China*<br>*(2) China Tobacco Jiangsu Industrial Co., Ltd., No. 29, Xinglong Street, Jianye District, Nanjing 210019, China* |

*[**ST 44** – Moved to Monday 9 Oct, Session 4a]*

**CORESTA SSPT2023 CONFERENCE**

# SMOKE SCIENCE and PRODUCT TECHNOLOGY

# ABSTRACTS

## ORAL PRESENTATIONS

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*Presenter's name is underlined when the main author (listed first) is not presenting the paper*

# IG 01

## CORESTA Tobacco Harm Reduction Workshop Overview

STEVENS R.(1); FLORA J.(2)

*(1) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.*
*(2) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

In June 2022, CORESTA held a Science Day in Paris, France. Presenters included experts in tobacco harm reduction (THR); Karl Fagerström, Riccardo Polosa, Clive Bates, Luca Rossi, and Lea Scott. Topics covered included the role of nicotine in harm reduction, misperceptions on the harm of nicotine, the challenges and opportunities of harm reduction substantiation, innovation and harm reduction of alternative products, and supply chain integrity. Through guidance from the CORESTA Board and with what we learned from Science Day, a workshop was developed and executed in April 2023, in Antibes, France, to develop insights into how CORESTA can advance the science related to THR. A diverse group of participants were selected by the CORESTA delegates (approximately 70 participants attended), and the workshop was designed to address the following objectives: 1) Identify key areas where CORESTA can advance the science related to THR; 2) Create actionable objectives to advance the key areas of science related to THR; and 3) Develop a recommendation for the infrastructure by which to accomplish the actionable objectives. In this presentation, we will provide an overview of the outcomes of the THR Workshop, progress to date, and next steps.

**Notes**

# IG 02

## Potential ultra-low nicotine limit in tobacco – can we meet it?

FISHER A.M.(1); FISHER C.R.(1); YANG S.(2); PATRA B.(1); SLONE S.(3); JI H.(1); KINNEY J.(1)

*(1) Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.*
*(2) United States Department of Agriculture, 1616 Albrecht Blvd N, Fargo, ND 58102, U.S.A.*
*(3) Dr. Bing Zhang Department of Statistics, University of Kentucky, 725 Rose Street, Lexington, KY 40536, U.S.A.*

The FDA has sought comments on a possible nicotine limit of 0.3-0.5 mg/g in the filler of cigarettes. Over the years, we have tested the conventional low alkaloid (LA) nic1nic2 mutants, agronomic practices known to lower alkaloids, and a combination of LA lines and agronomic practices. Neither the LA lines nor the agronomic practices alone lower nicotine to anywhere near this limit. In the trial combining LA varieties and agronomic practices, even in a very wet year, none of the varieties met this limit in all stalk positions, although several varieties did meet it in some stalk positions. In a dry year, the lowest level measured was 1.4 mg/g nicotine+nornicotine. We are now testing two gene-edited ultra-low alkaloid lines and two F4 lines combining a novel low alkaloid gene and the nic1nic2 mutants. We used the Burley 21 (Bu21) alkaloid series as checks. The nicotine+nornicotine range across stalk positions for Bu21 was 33.2-46.3 mg/g and for LA Bu21 was 2.60-3.42 mg/g). For the two F4 lines, the range was 2.19-2.80 and 1.80-2.71: significantly lower than LA Bu21 only in the primings, but consistently lower in the other stalk positions. For the two gene-edited lines, the range was 0.513-0.679 and 0.546-0.703, significantly lower than LA Bu21 in all stalk positions. Only the two gene-edited lines had significantly lower yields (2,774 and 2,794 kg/ha) than the Bu21 check (3,602 kg/ha). The poorest quality was in the two F4 lines (grade indices of 35 and 36), consistently but not significantly lower than LA Bu21 (53), which was significantly lower than the check, Bu21 (82). The two gene-edited lines had grade indices consistently but not significantly higher than the F4 lines and lower than LA Bu21. We conclude that we cannot consistently meet a 0.5 mg/g nicotine limit, not at this time, not with our current knowledge; although nicotine can be reduced to a level lower than the LA lines, albeit with yield and quality penalties.

**Notes**

## ST 01

### Workflow assessment of potential leachables in aerosol from ENDS systems

CARTER K.M.; SMITH C.R.; CHEVVA H.; AYALA-FIERRO F.; COOK D.K.; LYNDON M.; GILLMAN I.G.

*Juul Labs, Inc., 1000 F Street NW, Washington, DC, 20004, U.S.A.*

Leachables are substances that have the potential to transfer to a product from its container closure system. For Electronic Nicotine Delivery Systems (ENDS), leachables may transfer from all materials that contact the e-liquid. Leachable substances from materials in contact with e-liquid may subsequently transfer to the aerosol and be inhaled by the user. There is no specific guidance for conducting leachable studies on ENDS, there is general guidance for inhaled drug products (*United States Pharmacopeia*, Chapters 1663 & 1664; ISO-10993-18, Product Quality Research Institute - Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, etc.). Herein, we present a workflow for the analysis of leachable compounds in ENDS. To illustrate the workflow's applicability, we present a case study using the JUUL2 pod-based ENDS system. First, leachable compounds were assessed for closed JUUL2 pods filled with non-commercial base e-liquid formulation (propylene glycol/glycerin/nicotine/benzoic acid). Results for the initial analyses performed using GC-MS and LC-MS (+/- mode) provided a list of 29 organic compounds including potential leachables and nicotine related compounds. After long-term 12-month ambient storage of JUUL2 pods, e-liquid formulation was re-analyzed to investigate the migration of substances over time which provided a list of 12 previously and 24 newly identified compounds. Finally, to better understand potential exposure to leachable compounds, aerosol was collected from the aged pods using non-intense and intense puffing. Results indicated 11/18 of the organic compounds identified in the GC-MS analysis of the e-liquid transferred to aerosol while only 5/30 of the organic compounds identified in the LC-MS analysis transferred. Under GC/MS evaluation, non-nicotine related compounds transferred to aerosol, whereas LC/MS aerosol transfer was exclusively nicotine related compounds. This work supports the observation that larger compounds have limited transfer efficiencies into the aerosol.

**Notes**

## ST 02

### Analytical investigation of data deficient simulated leachables in ENDS products: case study

SMITH C.R.; LYNDON M.; JEONG L.; LEHMAN D.; JAMESON J.B.; CHEEVA H.; AYALA-FIERRO F.; CARTER K.M.; COOK D.K.; GILLMAN I.G.

*Juul Labs, Inc., 1000 F Street NW, Washington, DC, 20004, U.S.A.*

Leachable investigations are routinely undertaken across a range of sectors, including Electronic Nicotine Delivery Systems (ENDS), to determine if any chemicals from the container closure system can migrate or leach into a product. In general, leachable study conditions and analytical protocols are fairly straightforward, however, risk assessment of the analytical results can be challenging and may require additional data. Herein, we present a case study of the analytical investigation of two leachable compounds with little, if any, toxicological information (data deficient) that were found in simulated leachable studies using JUULpods filled with flavorless base formulation (PG/VG/nicotine/benzoic acid). Because no commercial reference standards for the two data deficient leachable compounds were available, nor any reasonable synthetic route possible due to the compounds' molecular size and structural complexity, an analytical approach was needed to determine whether these two data deficient leachable compounds identified in aged e-liquid from JUULpods transfer to the aerosol. LC-MS/MS analysis using ESI negative mode confirmed that molecular mass and fragmentation patterns for each leachable compound in e-liquid were consistent with proposed structures and compound rationalizations reported in simulated leachable studies. Upon e-liquid confirmation, aerosol was collected from aged JUULpods in order to determine the transfer efficiency of leachable compounds from e-liquid to aerosol. Aerosol collected from aged JUULpods did not contain any detectable levels of either leachable compound, and transfer efficiency from e-liquid to aerosol was experimentally determined to be < 2 %. The novel analytical approach used in this case study provided experimentally determined exposure estimates on two data deficient leachable compounds to support risk assessment and the observation that larger compounds have limited transfer efficiencies into the aerosol.

**Notes**

## ST 03

### Assessment of $SiO_2$ particle in aerosol generated from e-cigarette by single particle ICP-MS method

CHEN P.; LIN W.; LI D.

*Shenzhen First Union Technology Co., Ltd, Laboratory of Life and Health Sciences, Shenzhen, 518103, China*

The ceramic heating unit may release $SiO_2$ solid particles in the process of vaping, which possibly leads to the risk of silicosis when these particles are inhaled into the lung of consumers. It is necessary to develop a reliable analytical method to determine the concentration of $SiO_2$ solid particles, as well as the particle size distribution, in aerosols to assess the health risk of ceramic heater based e-cigarettes. Although there is no specific cut on the particle size, it is typically considered that particles with a size within the range of a few hundred nanometers to five micrometers have the highest tendency to be deposited in the deep lung.

In our study, the aerosol of an e-cig was enriched by two impingers connected in series (each filled with 20 mL $H_2O$) following CRM No. 81 smoking regime. The Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) technique was used for the measurement of $SiO_2$ particles in sample solution. Both particle size distribution and mass concentration are calculated. Based on the preliminary data, the amount of $SiO_2$ solid particles in aerosol was less than 20 ng/pod (the lower limit of method detection). Therefore, the risk of solid particle exposure is low, referring to the EPA limit (National Ambient Air Quality Standards for PM 2.5).

**Notes**

## ST 04

### A universal smoking machine adaptor for emissions testing with smoking/vaping machines: development, validation, and benchmarking

BRINKMAN M.C.(1,2); WATSON C.(3); HUANG M.(3); TRAN H.(3); EL HELLANI A.(1,2); WILSON C.W.(1,2); FLESHMAN C.C.(1,2); PETITTI R.(4); PANCAKE M.H.(4); BENNETT C.(5); ALLREAD G.(6); KELLER-HAMILTON B.(2); JONES J.(7); McGUIGAN M.(3); BRAVO R.(3); WAGENER T.L.(2)

*(1) College of Public Health, The Ohio State University, Columbus, Ohio, U.S.A.*
*(2) Center for Tobacco Research, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, U.S.A.*
*(3) Tobacco Products Laboratory, Centers for Disease Control and Prevention, Atlanta, Georgia, U.S.A.*
*(4) Center for Design and Manufacturing Excellence, The Ohio State University, Columbus, Ohio, U.S.A.*
*(5) Medicinal Chemistry Shared Resource, Drug Development Institute, The Ohio State University, Columbus, Ohio, U.S.A.*
*(6) Ergonomics, Spine Research Institute, The Ohio State University, Columbus, Ohio, U.S.A.*
*(7) Produced Better, Alpharetta, Georgia, U.S.A.*

Tobacco product innovation has surpassed our ability to conduct non-clinical, standardized machine testing with commercial, benchmarked apparatus. Existing smoking and vaping machines, designed to hold rods of a certain diameter and mass, require a leak-free seal between the machine and the product mouthpiece.

Objective: Design and test a smoking machine adaptor that seals well with the variety of newer tobacco product geometries and sizes.

A prototype universal smoking machine adaptor (USMA) that interfaces with existing cigarette, cigar, and e-cigarette smoking/vaping machines was designed and fabricated. Using the USMA, emissions were generated from e-cigarettes (n=7 brands), cigars (n=2), cigarillos (n=2), and a heated tobacco product, encompassing a variety of product geometries and weights for the most popularly used US products. To benchmark and validate results, reference tobacco products for cigars, cigarillos, and cigarettes were tested alongside the commercial products. Three comparison adaptors, the standard cigarette, Cerulean cigar, and CDC's JUUL adaptor, were also used to generate emissions. Mainstream nicotine and total particulate/aerosol matter (TPM/TAM) were quantified. Product variability was compared across adaptors using non-parametric Wilcoxon Rank Sum testing.

For mainstream nicotine and TPM, the USMA had greater precision and accuracy than the standard cigarette adaptor when testing a certified reference cigarette (1R6F). Replicate data (n=10 per brand) indicate repeatability across all products tested generally meets or exceeds that for the comparison adaptors and extant data.

The USMA seals well with a variety of e-cigarette, cigar, cigarillo and heated tobacco product geometries. Variability among replicates for product consumption, mainstream nicotine and TPM/TAM was similar or smaller when machine smoking/vaping with the USMA vs the other adaptors. Precision and accuracy when testing the 1R6F with the USMA were excellent. The USMA is more user friendly, has fewer parts, and takes less time to assemble/ disassemble than existing commercial adaptors.

## ST 05

### A quantitative risk assessment approach to compare toxic emissions of electronic cigarettes with combustible cigarettes

LIU C.(1); BERNAL A.J.(2); HUANG Yilang(3); LIU Chuan(4); GRAFF D.W.(1); JIAN Xingtao(3)

*(1) RiskWise Solution LLC, Princeton, NJ, U.S.A.*
*(2) ToxCreative LLC, 22171 Bianco, Laguna Hills, CA 92653, U.S.A.*
*(3) RELX Technology, RELX International, Boton Technology Park, 1044 Chaguang Road, Tower B, 7/F, Shenzhen, China*
*(4) Pin$^{ev}$ale Ltd, 1st Floor Chilworth Point, 1 Chilworth Road, Southampton, SO16 7JQ, U.K.*

Electronic cigarettes (e-cigarettes) heat a nicotine-containing e-liquid that could significantly reduce the number and levels of inhaled toxicants compared to combustible cigarette smoke. A framework for analytical evaluation of the e-cigarette aerosol and assessment of potential health risk can support regulatory evaluation and inform regulatory decisions of whether an e-cigarette product meets U.S. and international regulatory agency requirements. This quantitative risk assessment (QRA) approach evaluated relative cancer and noncancer health risks associated with the use of RELX Infinity Tobacco compared with U.S. Kentucky 1R6F reference cigarettes and Vuse Alto Golden Tobacco. The e-cigarette aerosol samples were analyzed for the U.S. Food and Drug Administration recommended harmful and potentially harmful constituents (HPHCs) and target constituents under both non-intense and intense puffing regimens. Machine-generated HPHC yields for 1R6F reference cigarette mainstream smoke were obtained from the literature. Toxicity reference values for HPHCs and target constituents were obtained from regulatory and public health agency databases. Exposure concentrations were estimated under “typical “ and “heavy” scenarios by utilizing exposure parameters specific to adult tobacco product consumers and standard default exposure parameters for human health risk assessment. The potential health risks associated with inhalation of machine generated HPHC and target constituent emissions from e-cigarettes and combustible cigarettes for adult consumers were assessed and compared by integrating the exposure assessment and the dose-response assessment into quantitative estimates of noncancer health hazards and excess lifetime cancer risk. The QRA results indicated that estimated risks of vaping RELX and comparator e-cigarette products for cancer and respiratory, cardiovascular, reproductive and developmental toxicity were at least 97 % lower than those of cigarette smoking. This study demonstrates that QRA is a practical tool for evaluating relative health risks of e-cigarettes to support regulatory submissions and inform regulatory decisions. In addition, this study adds to the body of evidence that e-cigarettes have the potential for substantial reduction in toxicant exposure and associated health risk compared to combustible cigarettes.

**Notes**

## ST 06

### Simultaneous determination of four sweeteners in e-liquids by ultra performance liquid chromatography

WANG Di; YANG Wenbin; DING Cheng; LIN Guifeng; MO Dongni; LI Weibo

*Shenzhen Yupeng Technology Co., Ltd., Building A2, Niujiaolong Industrial Zone, 320 Wuhe Avenue, Longhua District, Shenzhen, Guangdong, China*

Sweeteners play an irreplaceable role in daily life and are also common additives in e-liquids. Study of the composition and monitoring of the content of sweeteners is significant for the product quality control of e-liquids. In this study a new method has been developed to detect four artificial sweeteners (aspartame, advantame, neotame and saccharin) in e-liquids simultaneously by ultra performance liquid chromatography (UPLC) combined with diode array detector (DAD). The separation effect and determination sensitivity of target compounds in this method were improved by exploring different chromatographic conditions, including the type of mobile phases, pH, and temperature. Under the condition of ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 µm), the conditions for analysis and detection have been optimized. Mobile phase is acetonitrile-20 mmol/L ammonium acetate aqueous solution, with the pH of 4.5 and temperature of 35 °C. The linearity coefficients of target compounds were > 0.999. The LOQs of analytes ranged between 0.0268 and 0.2127 mg/g and the mean recoveries were in the range from 92.5 to 108.3 %. After validation, the method was applied to detect the artificial sweeteners in 10 kinds of e-liquids with fruit, tobacco, and mint flavour characteristics. Sweeteners were found in most of the e-liquids except the tobacco flavour one. And the most detected target compound was neotame. The results have shown the good performance of the method because of its sensitivity, accuracy, effectivity and convenience.

**Notes**

## ST 07

### Study on the transfer rate of five sweeteners in disposable e-cigarettes with mesh coil

LI Weibo; WANG Di; YANG Wenbin; DING Cheng; LIU Shaohua; YU Hanmou

*R&D Department, Shenzhen Yupeng Technology Co., Ltd, Shenzhen 518110, China*

The widespread use of e-cigarettes has raised concerns about their ingredients, especially the addition of sweeteners. Disposable cotton core e-cigarette with mesh coil is a kind of popular e-cigarette device, and the research on the transfer rate of sweetener in it is still relatively limited. These research results are of great significance to guide the reasonable addition amount. This study aims to investigate the transfer rate of five commonly used sweeteners (neotame, advantame, mogroside V, rebaudioside A, and NHDC) in disposable e-cigarettes with mesh coil e-cigarettes, in order to provide a reasonable guidance on how much to add. The transfer rate of five sweeteners in the cotton core was determined by experiment. First, a predetermined concentration of simulated e-liquid containing sweeteners is injected into the cotton of disposable e-cigarettes, the smoking conditions of e-cigarettes are simulated on a vaping machine, and aerosols containing sweeteners are collected with Cambridge filters. Then, the sweeteners in the samples were extracted using methanol. Finally, the extract was analyzed by HPLC to determine the sweetener content and calculate the transfer rate. The research results show that the transfer rate of neotame is 54.66 %, advantame is 28.67 %, mogroside V is 42.20 %, rebaudioside A is 48.54 %, and NHDC is 44.02 %. According to the results of this study, the sweeteners in cotton core e-cigarettes will be transferred to a certain extent during the puffing process. Therefore, in order to ensure the safety and user experience of e-cigarette products, manufacturers should carefully add sweeteners and control their usage. In addition, regulators should also pay attention to the use of sweeteners to ensure the quality of e-cigarette products and the health of users. Further studies could explore more specific transfer mechanisms and reasonable ranges of addition to provide more accurate guidance.

**Notes**

## ST 08

### Study on the storage stability of three flavours in e-liquid

XIAO Junfei; LIU Shaohua; LI Weibo; DING Cheng; YANG Wenbin; ZHANG Zhonghu; ZHONG Qiaoxia; YU Hanmou

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Acetic acid, octanal, and raspberry ketone are common flavour additives in e-liquid. This study mainly focused on the storage stability of the three flavour components under different temperatures and illumination conditions. The study was carried on in simulated e-liquid , in which the concentrations of acetic acid, octanal and raspberry ketone were all 500 ppm, percentage concentration of nicotine was 5 % and the mass ratio of PG: VG was 1:1. According to the guideline of ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use), three kinds of storage stability study conditions were set as 25 °C with light, 25 °C in darkness, and 40 °C in darkness, and the sampling times were 0d, 5d, 10d, 15d, 1M, 3M, and 6M. GC-FID and GC-MS were applied as quantitative detection methods for the three target compounds. The contents of 3 analytes under 25 °C-light had no significant differences compared with those under the condition of 25 °C-darkness, while decreased by 11.62 %, 99.19 %, and 56.46 %, respectively after 6 months of storage at 40 °C-darkness. The results of linear regression analysis at 25 °C-darkness condition showed that the stable period of octanal and raspberry ketone was 30 days and 42 days, respectively, while the content of acetic acid remained at the relatively stable level after six months of storage. The illumination had no effect on the stability of the three flavours in the simulated e-liquid but the high-temperature could accelerate the process of esterification, acetalization and ketalization reaction. The storage stability of flavour substances in e-liquid has been explored preliminary in this study, which provided a theoretical basis and guidance for further research on the e-liquid shelf life.

**Notes**

## ST 09

### Study on the thermal stability and interaction of three kinds of cooling agents in simulated e-liquid system

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A cooling agent is a food additive which can make the human body feel a cooling effect. Currently, there are three kinds of cooling agents: N,2,3-trimethyl-2-propan-2-ylbutanamide (WS-23), N,2-diethyl-3-methyl-2-propan-2-ylbutanamide (WS-27) and N-ethyl-5-methyl-2-propan-2-ylcyclohexane-1-carboxamide (WS-3) used in e-liquids. During the heating process, the aerosols with cooling agents make people feel cool and refreshed. Among them, the thermal stability and interactions of cooling agents are of more interest to researchers. This study mainly focused on the pyrolysis kinetics and interactions of WS-23, WS-27 and WS-3 under the simulated e-liquid system (the main components of the e-liquid system are 50 % propylene glycol, 50 % glycerol, 3 % nicotine salt, and no other flavour substances), by a pyrolysis instrument-gas chromatography-mass spectrometry (Py-GC-MS) simulating the heating environment (aerobic environment) of the e-liquid smoking process. The data obtained by GC-MS and Py-GC-MS were compared and analysed by the area normalization method. The results demonstrated that the thermal stability of the three cooling agents in the simulated e-liquid system is good, and no interaction with propylene glycol, glycerol, and nicotine salt in the simulated e-liquid was found, and neither were any degradation products found.

**Notes**

## ST 10

### Innovative design and application: a home-built undiluted system for particle size characterization based on laser diffraction principle

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The characterization of particle size distribution of atomized aerosols is important to both product quality control and fundamental research in aerosol inhalation. A big challenge is that most existing commercial instruments are not suitable for the electronic nicotine delivery systems (ENDS) industry generating dense and volatile aerosols, either contaminating instruments or modifying particle size through dilution. In this study, an undiluted aerosol size system was invented to measure the ENDS aerosols based on laser diffraction principle. This system consists of a customized commercial laser diffraction analyzer, a home-made puffing machine, a purposely designed sampling cavity and low-cost exhaust collectors. Featured with (1) immediate inlet to avoid evaporation and coagulation of aerosols, (2) thinner aerosol layer guild by innovative sampling cavity across laser beam to minimize multiple-scattering of detection, this system enables aerosol to be detected directly after its generation from the atomizer without dilution. The fast online and *in-situ* measurement via this system can capture variations of aerosol size distribution and relative concentration at high time and size resolution, providing essential information of aerosol delivering rate, consistency and malfunction within a short time interval (highest time resolution: 5 milliseconds) or among different inter-comparisons of products. Several interesting application cases indicating product performance will be shown in this study. While more fundamental research related to accurate data interpretation is under process, this system already demonstrated strong capabilities in industrial scenarios as a powerful tool to assist R&D and quality control.

**Notes**

## ST 11

### Comparison of the particle size distribution and vapor phase of two electronic nicotine delivery systems using two Impactors

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Electronic nicotine delivery systems (ENDS) contain numerous volatile aerosol constituents (e.g., nicotine, propylene glycol, flavors, etc.). Past work clearly indicates that the temporal and chemical dynamics of ENDS aerosol requires consideration of these volatile constituents when measuring the particle size distribution. An MSP-135-8 Mini MOUDI™ and Electrical Low Pressure Impactor (ELPI®+) were used to measure the particle size distribution of two JUUL ENDS products. Volatile chemicals were measured from each impactor's exhaust airflow to assess their effect on collection efficiency. Similar mass median aerodynamic diameters were obtained for both ENDS products by both impactors, however the geometric standard deviation from the ELPI®+ measurements were larger for both products than measurements using the Mini MOUDI™ impactor. Although the measured mass of volatile chemicals was greater with the Mini MOUDI™ impactor, a greater variety of volatile chemicals were found with the ELPI®+. The greater variety of volatile chemicals correlated with more room air sampling by the ELPI®+. The measured volatile chemicals improved the recovery efficiency of the Mini MOUDI impactor by 2.9-7.5 % with the average recovery efficiency exceeding 82 % for the two JUUL ENDS Products. In comparison, the measured volatile constituents increased the recovery efficiency of the ELPI®+ impactor by 0.4 % or less, which did not narrow the recovery efficiency range that consistently exceed the measured mass loss from both JUUL ENDS products.

**Notes**

## ST 12

### Differential analysis of aroma components of different tobacco types using GC×GC-TOF-MS combined with chemometrics

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The quality of cigarettes is determined by the tobacco aroma components, which are influenced by the characteristics of tobacco varieties, thereby creating complexity and distinctiveness. The conventional technical method for detecting tobacco aroma components is GC-MS, but its low resolution and overlapping peaks restrict the comprehensiveness of tobacco aroma components, leading to significant inaccuracies in identifying the characteristic aroma of various tobacco types. This study employed GC×GC-TOF-MS combined with chemometrics, to analyze and screen the aroma components of three various tobacco types. The process of simultaneous distillation extraction was employed to extract aroma components from three distinct tobacco varieties, namely flue-cured tobacco, sun-cured tobacco, and cigar tobacco. The qualitative analysis of aroma components was conducted using a self-constructed database and the NIST database, while the internal standard method was utilized for quantification. The differential aroma components were identified through the application of orthogonal partial least squares discriminant analysis (OPLS-DA) and variable importance for the projection (VIP). The findings indicate that the three types of tobacco contained a total of 1593 aroma components, with 357 of them being common across all three. The OPLS-DA analysis demonstrated the stability of the three discriminant models, with a prediction index exceeding 0.5. The VIP analysis demonstrated that flue-cured tobacco contained 32 primary differential aroma components, including alcohols, aldehydes, and ketones. Likewise, sun-cured tobacco was found to possess 35 differential aroma components, with a predominance of alcohols, hydrocarbons, and aldehydes, which significantly contributed to the model classification. In contrast, cigar tobacco was distinguished by 32 significant aroma components, primarily alcohols and ketones, which were instrumental in differentiating it from the other two tobacco varieties. This study utilized high resolution mass spectrometry in conjunction with chemometrics to screen the aroma components present in various types of tobacco. The outcomes of this investigation may serve as a benchmark for evaluating the aromatic characteristics of distinct tobacco varieties.

**Notes**

## ST 13

### Quantitative analysis of mycotoxins in sub-ppb levels in tobacco products by use of two-dimensional liquid chromatography coupled to high resolution mass spectrometry

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Mycotoxins can be formed by moulds when they grow on vegetables and other organic material. IARC (International Agency for Research on Cancer) classify aflatoxin as a human carcinogen and ochratoxin A as a possible human carcinogen. The Swedish National Food Agency has set a limit for total aflatoxins in snus (< 5.0 µg/kg) and there are EU-wide limits for aflatoxins in certain foodstuffs. Total aflatoxin and ochratoxin has had a GOTHIATEK limit since 2015 and 2016, respectively. Thus, there is a need of good and reliable analytical methods. Traditional way of mycotoxins analysis for tobacco related products is dependent on expensive immuno-affinity solid-phase extraction (SPE) for clean-up extract before sample analysis on liquid chromatography (LC) coupled to mass spectrometry, which is not time- or cost-effective. The aim of this study was to develop a low-cost, simple, fast, and accurate method for analyzing five mycotoxins including aflatoxin B1, B2, G1, G2 and ochratoxin A with low limit of quantification (LOQ) using two-dimensional (2D) liquid chromatography coupled to high resolution mass spectrometry (HRMS). An amount of 5 grams tobacco related products is extracted with a mixture of 1 % acetic acid in methanol. The extract is then evaporated to dryness under gentle nitrogen stream before reconstituted with mixture of 2 % acetic acid in methanol:water 60:40. Reconstituted extract is filtered through 0.2 µm filter prior to injection to 2D-LC-HRMS using two analytical columns and one trap-column. LOQs of mycotoxins ranged from 0.01 to 0.02 µg/kg with recovery ranging from 68.1 to 90.3 % depending on the type of product. Yearly cost reduction for this method is estimated to 100,000 US dollars compared to the traditional method applied previously in the laboratory (based on 5000 samples). In conclusion, the developed and validated method in this study showed similar or better LOQs compared to the traditional method that uses SPE clean-up. It allows a lower cost and simpler and faster sample preparation.

**Notes**

## ST 14

### Development of certified reference cigars representing three product categories

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The Center for Tobacco Reference Products (CTRP) has provided tobacco reference products for research since 1968. In 2016, CTRP was awarded the first of three cooperative agreements with the U.S. Food and Drug Administration (FDA) to produce certified reference tobacco products to expand the scientific knowledge within the tobacco research community. Certified reference tobacco products manufactured under the first two cooperative agreements include the 1R6F certified reference cigarette and the four certified reference smokeless tobacco products: 1S4 Swedish Style Snus, 1S5 Snus, 3S1 Loose Leaf Chewing Tobacco, and 3S3 Moist Snuff. The introduction of certified reference tobacco products is a significant advancement beyond previous reference tobacco products and is an important tool for developing the science of tobacco regulation. All current certified reference tobacco products have been used in the proficiency testing program under the A2LA accreditation of CTRP. The current FDA cooperative agreement is for the development and characterization of three machine-made certified reference cigar products (large cigars, cigarillos, and filtered cigars). Commercially available cigars within each product category were tested to define the design parameters for manufacturing these reference cigar products. All three reference products have been produced, and characterization of each product is currently underway at three ISO 17025 accredited laboratories. The results of the characterization will be published in the Certificate of Analysis for each product, and the fully characterized products will be made available to tobacco researchers and incorporated into the proficiency testing program coordinated by CTRP. The targeted design parameters, results of characterization, and future uses for the newly produced certified reference cigars will be presented.

**Notes**

## ST 15

### Nicotine pharmacokinetics assessments of two types of e-cigarettes compared to conventional cigarettes: two randomised, crossover studies

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The nicotine pharmacokinetics (PK) of non-combustible tobacco products, including e-cigarettes, have been widely assessed, with lower or similar nicotine exposure reported for most products when compared to conventional cigarettes (CC).

We conducted two clinical studies to evaluate the nicotine PK in U.S. cigarette smokers who used two types of e-cigarettes with several flavour variants.

Study 1 was a single-centre, randomized, open-label, controlled, 6-period crossover study conducted in healthy adult smokers. The primary objective of the study was to evaluate the nicotine PK following a single *ad libitum* use of cig-a-like e-cigarette (eDNC1.0a) with three flavour variants in comparison to CC, an FDA-approved nicotine gum, and a comparator e-cigarette. A total of 52 subjects were randomized to one of 6 product use sequences, with 52 subjects completing the study. The plasma PK data demonstrated that nicotine exposure using eDNC1.0a was lower than CC and somewhat similar to the comparator e-cigarette and the nicotine gum.

Study 2 was a single-centre, randomized, open-label, controlled, 7-period crossover study conducted in healthy adult smokers. The primary objective of the study was to evaluate the nicotine PK following a single *ad libitum* use of closed tank e-cigarette (eDNC2.0a) with four flavour variants in comparison to CC, an FDA-approved nicotine oral inhaler, and a comparator e-cigarette. A total of 55 subjects were randomized to one of 14 product use sequences, with 51 subjects completing the study. The plasma PK data demonstrated that nicotine exposure using eDNC2.0a was lower than CC, somewhat lower than the comparator e-cigarette, and higher than the nicotine inhaler.

**Notes**

## ST 16

### An approach to review pharmacokinetic studies of tobacco-free oral nicotine delivery products to enable product comparison

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One of the most recent nicotine delivery product innovations is tobacco-free oral nicotine pouches (OND), which are available in an increasing number of countries as a potentially less harmful alternative to continued cigarettes smoking. These products provide a smoke-free and tobacco-free alternative for responsible nicotine consumption, reducing the exposure to harmful chemicals that are produced by burning tobacco.

However, for OND products to reach their harm reduction potential, they need to be accepted by adult smokers. Nicotine delivery is key for some smokers; thus it is important for alternatives to cigarettes to achieve satisfactory levels of nicotine delivery. This is where pharmacokinetic (PK) studies play a vital role. These studies measure the amount of nicotine absorbed by consumers during product use, providing valuable information on the bioavailability of nicotine with such products.

In PK studies, a higher maximum concentration of nicotine in the bloodstream ($C_{max}$) and a shorter time to reach this maximum ($T_{max}$) after product use generally indicate faster absorption and higher bioavailability. However, to compare PK studies of different OND products, the results should be examined using the same clinical protocol. For example, the length of time individual consumers keep pouches in their mouths during use could influence the PK parameters. To address this issue, we propose a simple approach to compare PK parameters from different clinical studies that use different ONDs and different use protocols.

This proposed approach can help researchers and manufacturers better understand and compare the bioavailability of different OND products and develop products that deliver satisfactory levels of nicotine uptake for adult smokers.

**Notes**

## ST 18

### An adverse outcome pathway for oxidative stress in plaque formation

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Adverse outcome pathways (AOPs) have been developed as risk assessment tool for regulatory applications. These AOPs describe a logical mechanistic sequence of events, starting with a Molecular Initiating Event (MIE), and ultimately leading to a disease outcome via a series of Key Events (KE). The AOP framework provides a system to make predictions and assessments while reducing the need for *in vivo* assessment. In the absence of epidemiological evidence, assessment of the health effects of a product, chemical, or therapy on the progression of atherosclerosis would necessitate long-term animal exposure studies such as the use of the Apolipoprotein E deficient mouse. We followed Organisation for Economic Co-operation and Development (OECD) guidelines to formulate and propose an AOP for atherosclerotic plaque progression, collating the evidence by which cigarette smoke-induced oxidative stress forms a MIE. The downstream pathway includes multiple KEs including the upregulation of proinflammatory mediators, nitric oxide depletion, and endothelial dysfunction. Alterations in these KEs can lead to plaque formation and progression in cardiovascular disease and increase the risk of morbidity and mortality. Identifying preclinical endpoints and clinical biomarkers associated with these KEs provides a framework for *in vitro* and clinical data, supporting a mechanistic narrative for regulatory assessment. The application of this pathway provides a powerful alternative to animal models by developing preclinical assays and biomarkers for the assessment of atherosclerosis progression risk.

**Notes**

## ST 19

### High-sensitive exposure system in Ames test using bacteria suspension for fresh smoke and aerosol - showcasing by spiking experiment

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The bacterial reverse mutation test (Ames test) is an established regulatory *in vitro* assay recommended by CORESTA to test for potential mutagenicity.

This presentation will provide an overview of methods used to generate and test whole smoke/aerosol using the Ames test. We shall also highlight the relevance of gas vapour phase (GVP) sensitivity in mutagenicity testing.

Proper standardization is essential for correct testing. While smoke generation is standardised between laboratories, trapping of smoke and exposure to cells are conducted differently depending on the methodology used. The differences between Air Agar Interface and direct bubbling of bacterial suspension using the Air Liquid Transfer (ALT) method will be presented. The ALT is used by the Imperial Brands laboratories due to its extreme sensitivity to GVP.

The ALT method is ISO17025 accredited and validated with all five OECD recommended *S. typhimurium* strains for smoke and aerosol testing. Positive responses to whole smoke from 1R6F cigarette was detected with TA98, TA100, TA102, and TA1537 strains. GVP mutagenicity contributed up to 37 % of total mutagenicity in TA100 and TA102. E-cigarette aerosol did not exhibit a mutagenic effect in this test. To force an effect, formaldehyde (FA) was added to base e-liquid. Significant mutagenic effects were subsequently detected in TA98, TA100 and TA102 both for the whole fresh aerosol and GVP. Around 50 % of the mutagenicity was detected in the GVP. The ALT method can detect mutagenicity when FA is added to e-liquids at a concentration equivalent to 30 % of a cigarette with strain TA100 being the most sensitive.

The strain TA100 was proved to be the most sensitive strain to whole smoke/aerosol and GVP. Significant differences in mutagenicity per puff were found between cigarette smoke, aerosol from heated tobacco products and e-cigarettes.

**Notes**

## ST 20

### *In vitro* cytotoxicity assessment of arecoline benzoate aerosol by using an air-liquid interface exposure system

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Chewing areca nuts are considered the world's fourth most commonly used substance after tobacco, alcohol and caffeine, which have been shown to induce oral submucous fibrosis (OSF). Arecoline, the main ingredient extracted from chewing areca nuts, exhibits some pharmacological activities. Pulmonary delivery of arecoline provides several benefits over chewing, which may represent an optimal route of delivery to reduce the oral cancer risks of areca nut chewing. This study developed a purposely-designed aerosol generator and the CULTEX®RFC aerosol exposure system to evaluate acute *in vitro* toxicity of arecoline benzoate (ABA) aerosol using A549 cell model at an air liquid interface (ALI). The exposure time was optimized to 300 puffs, with the CORESTA recommended method No. 81 puffing regime (55 ml volume, 3 s duration and 30 s frequency). Different amounts of ABA dissolved in the carrier solvents propylene glycerol (PG) and vegetable glycerin (VG) were used to generate aerosol by the atomizer, which were used for A549 cell exposure at the ALI. The undiluted aerosol and the filtered air (1L/min) diluted aerosol were directly delivered to the ALIs containing the cells. The dynamic particle size alterations of the aerosol under these conditions were observed. The undiluted aerosol deposited in the exposure chamber in a dose-dependent manner, leading to a dose-dependent cytotoxicity, was followed by morphological alterations of the A549 cells, whereas the phenomenon was not observed at diluted condition. These results indicated that the undiluted exposure system was more appropriate for the evaluation of ABA aerosol *in vitro* inhalation toxicity.

**Notes**

## ST 21

### Evaluation of mixtures of flavor ingredients in 90-day nose-only inhalation exposures

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One of the challenges to using some flavor chemicals in aerosol products is the lack of route of administration specific data. Flavor chemicals (88) were divided into four different flavor mixtures based upon chemical compatibility and evaluated in two-week range-finding and subsequent 90-day nose-only rodent inhalation studies (Organization for Economic Co-operation and Development Guideline 413 and Good Laboratory Practices compliant). Sprague-Dawley rats were exposed to vehicle control or one of three increasing concentrations of each flavor mixture. In the range-finding studies, only exposure to flavor mixture 4 resulted in notable findings which included reduced body mass and adverse nasal histopathology in female rats at the high dose, resulting in this flavor formulation not being further evaluated. In the 90-day studies, food consumption, body weights, respiratory physiology, serum chemistry, hematology, coagulation, urinalysis, bronchoalveolar lavage fluid analysis and terminal organ weights were unaffected by exposure to flavor mixtures 1, 2 or 3 compared to vehicle control. All histopathology findings were observed in both vehicle control and flavor mixture exposed animals, with similar incidences and/or severities, and therefore were not considered flavor mixture related. Based on the absence of adverse effects, the no-observed-adverse-effect concentration for each of the three flavor mixtures evaluated in the 90-day inhalation studies was determined to be 2.5 mg/L of the aerosolized high dose formulation.

**Notes**

## ST 22

### Cytotoxicity evaluation of vanillin in electronic cigarette liquids on human aortic artery endothelial cells

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Concerns have raised about the integrity of some scientific research due to the impossibility of reproducing the claimed results of some experiments. This phenomenon has been described as 'reproducibility crisis' and affects various fields of sciences. In this context, the REPLICA project aims to replicate *in vitro* studies on toxicity of cigarette smoke and e-cigarette vapour in order to verify the robustness of the data. The safety of chemical flavours in the liquids of e-cigarettes (e-liquids) is a hot topic. There is a strong lack of research on this issue that must be filled. Many studies on flavours in e-cigarettes have serious methodological gaps. For our study we investigated the effects of vanillin, widespread in e-liquids formulations. We prepared e-liquids (PG/VG) with vanillin at high concentration. In the first step of our study we evaluated the cytotoxicity induced by vapour with vanillin on human aortic arteries endothelial cells (HAECs). The evaluation was performed with three different methods and in four different research centres scattered around the world, following a harmonized protocol. The preliminary results seem to indicate a slight or zero toxicity, except at the highest concentration of vanillin, but which is mostly attributable to the solvent (ethanol) used to capture vanillin from the vapour generated with an e-cigarette.

**Notes**

## ST 23

### Replication study to evaluate the *in vitro* toxicity profile of the myblu™ electronic cigarette compared to tobacco smoke: the REPLICA project

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In the context of Tobacco Harm Reduction, the Center of Excellence for the Acceleration of HArm Reduction (CoEHAR) was established within the University of Catania (Italy) and the multi-center project, created under its leadership, the REPLICA project, which aims to replicate *in vitro* studies originally conducted by tobacco and e-cigarette manufacturers, in order to verify the robustness and replicability of the data.

In this work the REPLICA Team replicated part of the work published by Rudd and colleagues in 2020, which aims to establish the aerosol-induced cytotoxicity, mutagenesis and genotoxicity of a pod system e-cigarette aerosol compared to tobacco cigarette smoke. As in the original paper, we performed Neutral Red Test (NRU) for the evaluation of cytotoxicity, AMES test for the evaluation of mutagenesis and *In Vitro* Micronuclei (IVM) assay for the evaluation of genotoxicity on cells treated with cigarette smoke or e-cigarette aerosol. The results obtained showed high cytotoxicity, mutagenicity and genotoxicity induced by cigarette smoke, but slight or no cytotoxic, mutagenic and genotoxic effects induced by the e-cigarette aerosol. The data obtained support those previously presented by Rudd and colleagues, although we have highlighted some methodological flaws of their work. Overall, we can affirm that the results obtained by Rudd and colleagues have been established and our data also confirm the idea that e-cigarette aerosol is much safer and less harmful than e-cigarette smoking, making it a useful device in smoking harm reduction.

**Notes**

## ST 24

### Comparing adult smokers who switched to JUUL vs continuing smokers: biomarkers of exposure, biomarkers of potential harm and respiratory symptoms

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Assessment of biomarkers of exposure (BOEs) and biomarkers of potential harm (BOPHs) is an important way to inform the likely health impact of ENDS on users' health. Observations in long-term users in real-world contexts are particularly important, as are data on actual clinical symptoms. This cross-sectional, observational study assessed adults who had smoked ≥ 10 cigarettes/day for ≥ 10 years. Analyses compared (1) 124 continuing cigarette smokers (Smokers) and (2) 140 former smokers who switched to JUUL-brand ENDS exclusively for at least 6 months (Switchers). Switchers averaged 3 years of JUUL use. Comparisons adjusted for a range of demographic and lifestyle factors. Nicotine levels were significantly higher in Switchers, who were unusually heavy users of JUUL (e.g., > 2.5 times more likely to consume at least 20 pods/month than a more general JUUL-user sample). All other BOEs, including NNAL and HPMA3 (primary endpoints), were significantly lower in Switchers than Smokers. Even after adjustment for demographic and lifestyle factors, most BOPHs (sICAM-1 [primary], and e.g., white blood cell count, MCP1, HbA1c, 8-epi-PGF2α) were significantly lower in Switchers than Smokers; HDL was significantly higher. Further, Switchers' respiratory symptom scores were significantly lower than Smokers', and their dependence on JUUL was lower than Smokers' dependence on cigarettes. Thus, compared to continuing smokers, smokers who switched to JUUL and were using JUUL heavily had substantially lower exposures to multiple toxicants, favorable differences in markers of inflammation, endothelial function, oxidative stress, and cardiovascular risk, and less respiratory symptoms. These findings suggest that switching from cigarettes to JUUL likely reduces smokers' health risks.

**Notes**

## ST 25

### Switching exclusively from smoking to using glo results in significant, substantial reductions in exposure to cigarette smoke toxicants

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For those who would not otherwise quit smoking, a proposed tobacco harm reduction approach relies on the proposition that the health burden of smoking can be reduced by encouraging smokers to switch to alternative products that, while not being risk free, would reduce or eliminate exposure to toxicants and potentially reduce smoking-related harms associated with conventional cigarettes. Heated Tobacco Products (HTP) deliver nicotine in an inhalable aerosol, but with lower or immeasurable aerosol levels of toxicants associated with combusting tobacco.

The purpose of this study was to assess whether selected biomarkers of exposure (BoE) to cigarette smoke toxicants are reduced in smokers switched to a HTP for three months, compared to those who continue to smoke combustible cigarettes.

Participants in this randomised, ambulatory study were healthy smokers assigned either to continue smoking or switch exclusively to one of five variants of the glo HTP; a group of smokers who abstained from cigarette smoking; and never-smokers. Various BoE to carcinogens and respiratory, cardiovascular, and reproductive toxicants included in FDA's established list of HPHCs were assessed at baseline and three months. Compliance with cigarette smoking restrictions was assessed via daily SMS questionnaires and haemoglobin levels of N-(2-cyanoethyl)valine at three months.

In the glo HTP switching groups, compared to the continued smoking group, significant and substantial reductions from baseline levels were observed at three months in the primary endpoints: BoE to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 1,3-butadiene, acrolein, benzene, and carbon monoxide.

These findings, alongside chemical and toxicological studies undertaken on the glo HTP used in this study, add to the weight of evidence of our belief that smokers who would otherwise continue to smoke and instead switch entirely to the use of the glo HTP, will reduce their exposure to tobacco smoke toxicants linked with smoking-related diseases compared to those continuing to smoke.

**Notes**

## ST 26

### Non-targeted analysis of exhaled breath to distinguish different nicotine product user groups

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Over the past decade, the tobacco landscape has changed dramatically with several new emerging nicotine-delivery products. Particularly for longitudinal cohort studies, subjects' compliance must be ensured by appropriate biomarkers to generate valid exposure data. Since volatile compounds in exhaled breath (EB) are postulated to be in equilibrium with systemic circulation, their analysis in combination with other biological matrices may reveal suitable novel biomarkers specific to a certain nicotine-delivery product. Therefore, a controlled clinical study with 10 sole users of different nicotine products was conducted: smokers of combustible cigarettes, electronic cigarettes, heated tobacco products, users of oral nicotine products, nicotine replacement therapy, and non-users. BioVOC-2™ and thermal desorption tubes were used for EB collection. For analysis, a non-targeted method was implemented using thermal desorption coupled to GC-ToF-MS, followed by a data evaluation workflow with spectral library search in NIST20/Wiley Registry (12$^{th}$ ed.). More than 50 compounds were identified being elevated and therefore specific to a product user group. Identified compounds can be classified into distinct groups such as flavours, VOCs, alcohols, aromatic compounds, heterocyclic compounds, and hydrocarbons. This demonstrates the successful development of an analytical method and assessment strategy to evaluate EB samples to distinguish between different nicotine product user groups.

**Notes**

## ST 27

### Analysis of the smoke components in smokers' oral fluids by large volume injection-in-column evaporation concentration

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The study of the smoke components in smokers' oral fluids plays an important role in elucidating the sensory material basis of smoke from tobacco products. To analyze the trace components in complex matrices, such as oral fluids and urine, a novel large volume injection-in-column evaporation concentration system was developed through adding supplementary constant flow carrier gas at the injection port and adopting a special backflush module at the column outlet. By optimizing pre-treatment and instrument conditions, the large volume injection-in-column evaporation concentration-GC-MS analysis of oral fluid samples collected from smokers of various types of tobacco products, including cigarettes, cigars, heated tobacco products, and e-cigarettes, was performed after reverse extraction with dichloromethane. The results showed that: 1) The pre-treatment process was simple, efficient, and suitable for high throughput sample analysis. 2) The method was sensitive and accurate. The RSDs of 121 smoke components analyzed were all less than 10 %, with 99 components having RSDs less than 5 %, which met the requirements for the rapid, comprehensive, and accurate analysis of trace smoke components in smokers' oral fluids. 3) There were significant differences in smoke components between the tested oral fluid samples from smokers of various types of tobacco products. The contents of pyridines, pyrazines, pyrroles, alkaloids, and cyclopentanones were relatively higher in samples from cigar smokers, and the contents of cyclopentenones, furans, and phenols were relatively higher in samples from cigarette smokers. The total content of smoke components, except for nicotine, was lower in samples from smokers of heated tobacco products and e-cigarettes. This method provides a reference for the research on the sensory material basis and smoking behaviour of consumers.

**Notes**

## ST 28

### Revealing the urinary exposome of smokers, vapers, HTP users and pouch consumers by high-resolution LC-MS/MS – an important step in the identification of use-specific biomarkers of exposure

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Over the past decade, the tobacco landscape has changed drastically with several emerging product categories available such as e-cigarettes (ECs), heated tobacco products (HTPs) and nicotine pouch (NP) products and their potential risks and benefits have been controversially discussed ever since.

Usually, longitudinal and large population-representative studies are used to address key questions like the harm reduction potential and cessation efficacy of these products. Herein, nicotine product use relies solely on self-reports. Especially under uncontrolled settings this may lead to misclassification and misinterpretation of the results. Use-specific biomarkers would be desirable to verify the subjects' compliance. Moreover, the exposure assessment today focuses on the comparison to smoking and the reduction in smoking-related harmful constituents. A comprehensive approach comprises a holistic view at the exposure profile in users of each of the new tobacco/nicotine products.

Hence, we conducted a clinical study with 10 sole users of combustible cigarettes, ECs, HTPs, oral tobacco/NP, nicotine replacement therapy (NRT), and non-users. We developed a non-targeted method by means of high-resolution LC-MS/MS to decipher the urinary exposome of the different nicotine product user groups. The method was able to detect differences in the exposure profile between groups down to the low ng/mL concentrations as demonstrated during method validation.

As expected, smokers showed by far the highest burden with 171 significantly elevated compounds while only few compounds were identified as specific to one of the other product categories. Moreover, we were able to identify propylene glycol as a specific biomarker of exposure for EC vaping which was verified by quantitative analysis.

Our non-targeted approach also revealed compounds specific to the other use categories, HTP, OT, and NRT. Suitable candidates to distinguish each of the product categories, for instance, flavor metabolites for NRT use or VOC metabolites in HTP consumers will be discussed in this presentation.

**Notes**

## ST 29

### Longitudinal analysis of sustained cigarette smoking abstinence and relapse behaviors

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Observations regarding smoking cessation patterns among adults who smoke cigarettes (AS) are dated and most rely on cross-sectional surveys. Longitudinal assessments are needed to better understand the complex cessation and relapse behaviors. We present longitudinal data analysis using the Population Assessment of Tobacco and Health (PATH) Study to investigate cigarette smoking abstinence and relapse among AS.

In this study, AS in Wave 3 (October 2015 to October 2016) were followed up in Wave 4 (December 2016 to January 2018) and Wave 5 (December 2018 to November 2019) to obtain sustained cigarette cessation rates and relapse rates. We further studied the association between sustained cigarette abstinence/relapse and factors such as intention to quit and use of smoke-free tobacco products.

The results showed that among overall Wave 3 AS (n=6,686), 35.07 % had intention to quit smoking, with mean quit attempts being 4.59 times, and 18.93 % used smoke-free tobacco products. In Wave 4, 7.82 % (95 % CI 7.03-8.68) of AS in Wave 3 reported past 30-day smoking abstinence. In Wave 5, 4.96 % (95 % CI 4.22-5.82) of AS in Wave 3 maintained smoking abstinence in both Waves 4 and 5, indicating a 36.56 % (95 % CI 30.85-42.66) relapse rate. Relatively higher sustained cessation with lower relapse rates were observed among those who have reported trying to quit, having made quit attempts, or having used NRT or smoke-free products. For example, those who used smoke-free products in Wave 3 tended to have higher past 30-day smoking abstinence (10.01 %, 95 % CI 8.47-11.79) in Wave 4 with a greater sustained abstinence rate (6.71 %, 95 % CI 5.36-8.36) in Wave 5, reflecting a relatively lower relapse rate (32.97 %, 95 % CI 24.78-42.34).

Our longitudinal analyses of PATH dataset provide unique insights regarding contemporary cigarette smoking cessation patterns. The trends in differential cessation and relapse rates suggest a multi-pronged approach may be necessary to accelerate cessation and maintain sustained smoking abstinence.

**Notes**

## ST 30

### Perception of harm and addictive potential of the RELX Infinity electronic nicotine delivery system (ENDS) and future product use intentions of adult smokers switching from combustible cigarettes over eight weeks

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Evidence supports that switching from cigarettes to ENDS reduces toxicant exposure; however, misperceptions of the relative harm of ENDS may discourage their use for smoking reduction/cessation. We assessed perceptions of harm and addictiveness of cigarettes and ENDS and future intent to use each before and after adult smokers switched from cigarettes to the Infinity ENDS. All subjects smoked their usual cigarettes through baseline and were randomized to use an Infinity ENDS with a tobacco or menthol flavoured e-liquid based on their own-brand combustible cigarette flavour, to use both e-liquids, or to continue smoking. Subjects responded to questions regarding absolute and relative harm and addictiveness of cigarettes and ENDS at baseline and of cigarettes and the Infinity ENDS after eight weeks. Intentions to smoke and use the Infinity ENDS or other ENDS were also assessed, along with other endpoints. At baseline, 45 % of the 150 subjects in the switching cohorts perceived ENDS to be less harmful than cigarettes, while 39 % perceived ENDS to be less addictive. Following eight weeks of use, 59 % and 61 % rated the Infinity ENDS as less harmful and less addictive, respectively, compared to cigarettes. Furthermore, at week eight more of the switching subjects indicated a negative intention to continue smoking after the study (45 %) than at baseline (15 %), with 77 % indicating that they would “probably” or “definitely” switch to the Infinity ENDS to reduce their health risk. Additionally, 88 % and 69 % of subjects indicated that they would use the Infinity ENDS to reduce the number of cigarettes they smoked or to quit smoking, respectively. Switching to the Infinity ENDS for eight weeks resulted in a reduction in perceived harmfulness and addictiveness of ENDS compared to cigarettes and a lower future intention to smoke. These changes may enhance the public health potential of the Infinity ENDS by encouraging prolonged switching or smoking cessation.

**Notes**

## ST 31

### Emotion analysis of consumer comments on cigarette products by integrating multi-level features

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Text emotion analysis uses natural language processing techniques to extract important features and determine the emotional colour of text, which is important for research in social opinion analysis and economic activity prediction. In order to obtain feedback from consumers and find out the causes of consumption behaviour, a large-scale multi-emotional consumer comment dataset was constructed by collecting data from the Internet, and a consumer emotion analysis model, EHCRNN, was proposed by integrating multi-level features. This model uses a pre-trained model, ERNIE, to generate dynamic word vectors with contextual information, and extracts local and global features through Convolutional Neural Network (CNN) and Bi-directional Long Short-Term Memory (BiLSTM). A hierarchical attention network is employed to extract key features for emotion judgment at both the word and sentence level. Experimental results on the cigarette consumer comment dataset showed that the overall classification accuracy of the model was 85.29 %. The *F1*-scores for positive, neutral and negative emotion classification were 90.51 %, 67.96 %, and 86.21 %, respectively. The model outperformed the other nine machine learning and deep learning baseline methods on all metrics, and improved on the best performing baseline method, ERNIE, by 4.77 %, 5.32 %, 2.02 %, and 3.28 % on each metric. On the basis of the above achievements, we have developed an intelligent mining system for the typical opinions of cigarette consumers, which performs functions such as cigarette product portraits and emotion analysis result comparison. This system can help companies quickly understand consumer attitudes toward cigarette products and support product development.

**Notes**

## ST 34

### Assessing the potential impact of e-cigarette policies on smoking prevalence: a European Union study

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The impact of tobacco control policies on smoking prevalence in European Union countries has been heavily studied, with higher levels of policy implementation linked to decreased smoking prevalence. However, there has been a lack of research on the impact of e-cigarette control policies on smoking prevalence in the EU. This is an important gap in the literature, given the wide variation in e-cigarette and tobacco control policies across European countries.

To address this gap, we have developed a model to predict future smoking prevalence using e-cigarette and tobacco policy scores and past smoking prevalence. The model allowed for the quantification of the potential impact of e-cigarette control policies on tobacco smoking prevalence.

The findings indicate that less restrictive e-cigarette policies, i.e., lower e-cigarette scores, have the potential to decrease smoking prevalence. Conversely, increased regulatory pressure on e-cigarettes may discourage smokers from switching to a less harmful alternative, potentially leading to an increase in tobacco smoking prevalence.

The study underscores the significance of taking e-cigarette control policies into account and highlights their potential to alter the prevalence of cigarette smoking. Consequently, policymakers must exercise caution in considering e-cigarette regulations during the formulation of tobacco control strategies, aiming to ensure their effectiveness and minimize unintended consequences.

**Notes**

## ST 35

### Heating vs. burning: a non-targeted analytical characterization of compounds with higher abundance in the aerosol of a heated tobacco product than in mainstream cigarette smoke

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Heated tobacco product (HTP) aerosols have been shown to contain—on average—lower levels of known harmful or potentially harmful constituents (HPHCs) than smoke from conventional cigarettes. However, few studies have investigated the chemical composition of HTP aerosols beyond the routinely analyzed HPHCs (e.g., WHO-9, FDA-93). This study aimed to identify all constituents exclusive to or more abundant in an HTP aerosol produced by the Tobacco Heating System 2.2 (THS) than in smoke of a comparator cigarette (CC). To focus exclusively on chemical differences due to heating vs. burning tobacco, confounding factors were minimized by using the same tobacco in both test items without added flavorants. Untargeted analysis of whole aerosol and smoke samples using seven comprehensive liquid chromatography- and gas chromatography-mass spectrometry methods revealed that 92 % of analytical features were unique or significantly higher in CC smoke compared to 4.2 % in THS aerosol. A total of 31 distinct compounds were significantly more abundant in THS aerosol than CC smoke, and 29 of their chemical structures were assigned and confirmed using reference standards. A notable fraction of these compounds can be classified as glycerol reaction products. The only compound present exclusively in THS aerosol likely leached from a paper adhesive. These results support the hypothesis that heating tobacco to temperatures typical of HTPs does not produce compounds that are absent in CC smoke. Characterizing the compounds with intrinsically higher abundance in THS aerosol may provide valuable information to guide the chemical and toxicological evaluation of other types of HTP aerosols.

**Notes**

## ST 36

### Numerical simulation of evaporation characteristics for heated tobacco products

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Electrically heated tobacco products have been rapidly developed for their advantage of having less harmful substances due to evaporation at low temperature. To investigate the evaporation mechanism in the porous tobacco segment, an evaporation heat transfer computational model based on the Eulerian-VOF method was established, and the effects of capillary forces and diffusion of different components were studied by coupling the species transport model with the Skjaeveland capillary model. Based on established model, the numerical simulation for the evaporation process of binary mixtures (water-glycerol) in the porous domain of the tobacco section of heated tobacco product was performed. The variation of temperature and velocity distribution under different porosity and heating power were compared. The results showed that, with the heating process, the surface temperature in the single-phase region first increased linearly, then the slope increased sharply after exceeding the saturation temperature, and then gradually cooled to reach a stable temperature. With the increase of porosity, the heating rate of the porous surface in the single-phase region slowed down, and the onset of boiling was delayed. In addition, the maximum temperature and stable temperature increased with the increase in porosity. The heating power is dominant in the early stage of evaporation, while the later stage of evaporation is more influenced by the parameters of the porous structure. Therefore, with the increase of heating power, the heating rate of surface in the single-phase region is accelerated and the maximum temperature is also increased. However, the stable temperature and the final liquid-vapor interface basically follow the same trend.

**Notes**

## ST 37

### Quantitation of selected elements in the aerosol of electrically heated tobacco products (eHTPs) by inductively coupled plasma mass spectrometry (ICP-MS)

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Metals are among the most ubiquitous environmental contaminants and can enter the tobacco plant through various sources including soil adsorption and fertilizer application. Additional sources of trace levels of metals present in tobacco can be due to the manufacturing process.

The US Food and Drug Administration (FDA) issued guidance on reporting harmful and potentially harmful constituents (HPHCs) in tobacco products and listed several metals (including arsenic [As], cadmium [Cd], chromium [Cr], nickel [Ni], and lead [Pb]) as compounds for reporting. However, no guidance is provided on recommended analytical techniques or testing methodologies.

The objective of this study was to validate a method for the quantitation of selected metals under the Health Canada Intensive (HCI) smoking regime for an electrically heated tobacco product (eHTP). A commercially available linear smoking machine equipped with an electrostatic precipitator unit was used to trap the metals followed by a sample extraction and mineralization before analysis by inductively coupled plasma mass spectrometry (ICP-MS).

The CORESTA Technical Guide N°28 was used to set method detection limits, and lower quantification limits (LOQs) of 0.4 ng/item for Cd and 5.3 ng/item for Ni were reported. The recovery of the method calculated from spiked aerosol extracts was 92-108 %, and the coefficients of variation of repeatability (CVr) ranged from 2-11 % for the majority of elements.

A number of specific sample handling techniques were applied throughout the aerosol collection and analytical process to minimize any potential source of metal contamination from the laboratory environment. The method was validated according to ICH Q2 (R1) Validation of Analytical Procedures and was demonstrated to be selective, precise, accurate, and linear over the intended working range.

**Notes**

## ST 39

### Analysis of tobacco stem shaping effect based on compression-set

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In order to investigate the technical basis of the heavy rolling process for tobacco stems, the relationships between compression-set of tobacco stems and several influencing factors including stem structure, moisture content and roller clearance were studied. Compression-set is a professional term which means the thickness change of the material before and after compression. In this study, a compression-set-characterization method was developed to evaluate the shaping effect of tobacco stems, and the influence of stem rolling on the structure of cut rolled stems was analyzed. The results showed that: 1) The coefficient of variation and relative error of the compression-set-characterization method were 3.43 % and 0.77 %, respectively. 2) A high compression-set resulted in an improved stem shaping effect after rolling. Notably, the stem compression-set occurred mainly in the parenchymal tissue layer with large pore size and loose pore structure, while the duct structure was not significantly affected. 3) The compression-set of stems was significantly negatively linearly correlated with the roller clearance, and positively linearly correlated with stem diameter and moisture content. Stem length had no significant effect on stem compression-set. 4) In the rolled stem cutting process, a higher value of stem compression-set resulted in a smaller width, a lower integral stem rate, and a higher broken stem rate in the cut rolled stem. While focusing on optimizing the filamentous effect of the stems, attention should also be paid to the lower cut stem structure and the higher cost due to the higher breakage rate during the cutting process. During the evaluation of stem shaping effect, the compression-set method has shown its high precision and clear physical principles, and provided a reference for maintaining roller clearance and consistent stem shaping effect.

**Notes**

## ST 40

### Removal of nicotine, flavors, and tobacco constituents from machine wash water

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British American Tobacco has committed to decreasing its water consumption as part of its Environmental and Social Governance (ESG) program. To help with this goal, the feasibility of recycling wastewater at our manufacturing facilities while maintaining quality standards was initiated. Recycling the wastewater should help towards BAT's goal of decreasing water consumption. This study investigated the removal of nicotine and flavor components from machine wash water at our manufacturing facilities for traditional moist snuff tobacco products. Simulated wastewater batches were each made with one of four different SKUs from our facilities. This water was then run through activated carbon columns to remove the nicotine, flavors, and other tobacco components. Aliquots of the water were taken at regular intervals while using the column. These water samples along with the original simulated wastewater underwent partitioning with chloroform to extract the nicotine and the flavors. The chloroform partition was analysed by gas chromatography mass spectrometry and the remaining water partition was analysed by liquid chromatography mass spectrometry. For all the SKUs used to create simulated wastewater, the data showed a 99.7 % decrease in nicotine and an equivalent decrease in flavors when comparing chromatograms of before and after using the carbon column. Activated carbon was successfully used to remove nicotine, flavors, and other tobacco components from simulated wastewater.

**Notes**

## ST 41

### Study on the combustion coupling-matching mechanism of cigarette ash integration during smoking

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Cigarette paper and cut filler, as direct participants in cigarette combustion, couple with each other during cigarette smouldering, making their combustion interdependent. This combustion coupling-matching phenomenon directly affects the smoke release and ash integration in the smoking process. The purpose of this study was to establish an effective method for the quantitative evaluation of the combustion coupling-matching of cigarette and to clarify the combustion coupling-matching mechanism of cigarette ash integration. Combustion coupling degree, defined as the ratio of the combustion rate of cigarette paper to that of cut filler, was adopted to quantitatively evaluate the combustion coupling-matching phenomenon between cigarette paper and cut filler, with static combustion coupling degree ($R_s$), puffing coupling degree ($R_p$) and total coupling degree ($R_t$) as the main indicators. And a synchronous testing method for the instantaneous combustion rates of cigarette paper and cut filler was established using the image method and the characteristic temperature method, respectively. During the cycle of static combustion and puffing of the cigarette, the cut filler and the cigarette paper burned alternately, and the combustion coupling between them was in an alternating transformation between relatively matched state and unmatched state. When $R_s$ was less than 1, $R_p$ was greater than 1, $R_t$ was less than 1 or slightly greater than 1, and the difference between $R_p$ and $R_s$ was about in the range of 0.3-0.7, there was better ash integration of the burning cigarette. It was found that the ash integration of a burning cigarette can be effectively improved by adjusting the combustion coupling degree within a reasonable range.

**Notes**

## ST 42

### Effect and influence of perforation methods for tipping paper on the control of the thermal energy of smoke from tobacco products

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Perforation of tipping paper is mainly relevant for adjusting the ventilation level of a combustible filter cigarette to achieve specific smoke deliveries. Moreover, the dynamic smoke flow underneath the tipping paper and inside the filter plug undergoes essential interaction with the stream of ambient air penetrating through the perforation area of the paper thus generating significant impact of diluted cigarette smoke on the human sense of taste. With the successful market launch of next generation products, which include all kinds of e-vapor items as well as heated tobacco products (HTPs), particular physical properties of smoke moved closer into the focus of scientific research activities. Hereby, the temperature of the aerosol generated by HTPs represents a typical example of a parameter which requires special attention in terms of its control and optimization. The purpose of the present study is to determine experimentally the temperature of the mainstream smoke of combustible filter cigarettes made with tipping paper comprising different perforation methods (electrostatic, laser and plasma perforation) and selected permeability levels. Physical and geometrical aspects of these individual perforation types serve as basis for a numerically derived dynamic smoke flow simulation model that is applied to evaluate and confirm the correlation between the measured mainstream smoke temperature and the smoke flow characteristics. With the gained results, possible options will be outlined for projecting the conclusions onto HTPs in order to lower the thermal energy of the created aerosol through the effect of filter ventilation.

**Notes**

## ST 43

### Degradation and toxicity evaluation of cigarette butts

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Cigarette butts (CBs) are the most common litter found all over the world. The European Union Single Use Plastic (SUP) directive sets Extended Producer Responsibility's (EPR) obligations for plastic base CBs, not for paper filter CBs. Nevertheless, there is a need to assess the actual environmental impacts of CBs whatever the nature of the filtering material.

The objectives of the study that will be presented were:

- To observe the disintegration and biodegradation capacity of post-consumer paper and cellulose acetate CBs in marine water.
- To gain some preliminary insights on their acute and chronic toxicity on the model freshwater organism, *Daphnia magna*.
- To monitor the presence of selected harmful and potentially harmful compounds (HPHCs) into water.

Commercial cigarettes with paper and cellulose acetate filters at similar smoke deliveries were smoked according to the ISO smoking regime. CBs were tested for disintegration and biodegradation following an adapted ASTM D6691 standard, and the *Daphnia magna* acute ecotoxicity tests were done following an adapted OECD 202 standard.

Paper based CBs disintegrate and biodegrade rapidly reaching 81 % disintegration and 76 % biodegradation after 8 weeks, versus 7 % and 27 % for cellulose acetate. *Daphnia* acute survival rate was higher for the paper-based CBs than the cellulose acetate CBs. After 6 weeks CB incubation, no *Daphnia* toxicity was observed.

Nicotine, phenol, and benzo(a)pyrene were occasionally detected in water after 48 h, but rapidly became undetectable after one week, suggesting a rapid degradation.

These first results show the importance of sampling and testing standardization to have representative and reproducible results allowing a fact-based comparison of the environmental impact of different CBs.

**Notes**

## ST 44

### Determination of tar filter efficiency using spectrophotometric method UV-VIS

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When smoked, a fraction of tar is retained in the cigarette filter plug. To evaluate nicotine filtration efficiency there is already prior knowledge and established method, while for the tar generated during smoking, there are currently no methods available. Since the information of the efficiency of the filtration is important for developing new products, the objective of this study was to develop a method to determine tar filtration efficiency.

The proposed method starts from the procedures described in ISO 3308 and ISO 4387; and for Intense Canadian Regime in ISO 20778 and ISO 20779. For nicotine and water quantification, the method follows ISO 10315, ISO 22253 and ISO 10362-1. The method also considers parameters described in ISO 20773, used for tar determination in side stream smoke. After the smoking run, plug filters are detached from the tobacco rod. The filter pad and filter plug (without wrappers) follow to extraction. An aliquot of both extracts is diluted and follow to UV-Vis. An aliquot of the filter pad extract follows to water and nicotine quantification in GC-FID. The tar retained in the cigarette filter plug is calculated by the correlation with the tar determined in the filter pad. Tar filtration efficiency is determined by the percentage of total tar (both cigarette filter plug and filter pad) retained in the cigarette filter plug.

Selectivity study evaluated interferences from different matrices and was ensured by the blank discount. The linearity (1.5 to 14 mg/cig) matched the linear regression model. The accuracy studied the recovery in relation to the tar target in the filter pad, reaching a range between 90 and 110 %. The parameters evaluated guarantee the method robustness, allowing more information about the filters used in cigarettes.

**Notes**

## ST 45

### Filtration characteristics of polycyclic aromatic hydrocarbons in mainstream cigarette smoke by tobacco rod

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Tobacco rod filtration plays an important role in the formation and delivery of cigarette smoke. To investigate the filtration properties of smoke constituents in tobacco rod during smoking, a method was established for the quantitative analysis of polycyclic aromatic hydrocarbons (PAHs) retained in tobacco and released in mainstream smoke. The distribution of 21 PAHs in tobacco rod with different parameters was studied. A cigarette with the filter removed was smoked for one puff under defined machine-smoking conditions and then the combustion cone was extinguished with liquid nitrogen. The smoked cigarette was cut transversely into several 5 mm long sections using an automatic cutting device and extracted in cyclohexane. The contents of PAHs retained in these tobacco sections were determined by GC-MS/MS, and the longitudinal distribution patterns of PAHs were analyzed. The effects of puff volume, tobacco filling density, and cigarette circumference on the filtration properties of PAHs were investigated. The results showed that: 1) The filtration efficiencies of tobacco sections for the 21 PAHs ranged from 25.2 % to 65.5 %, with 19 PAHs being above 40 %, indicating strong filtration of PAHs in tobacco rod. Furthermore, the filtration efficiency increased as the boiling points of PAHs generally increased. 2) The retained PAHs were concentrated in the first 25 mm of the tobacco rod adjacent to the combustion cone and decreased toward the rear. 3) The filtration efficiencies of PAHs decreased with increasing puff volume from 20 to 70 mL. 4) When the tobacco filling density was increased from 223 to 281 mg/cm$^3$, the filtration of PAHs was improved. 5) The filtration efficiencies of the 24.2 mm circumference cigarette for PAHs were higher than those of the 17.0 mm cigarette. Therefore, the filtration of PAHs by tobacco rod is influenced to varying degrees by puff volume, tobacco filling density, and cigarette circumference.

**Notes**

**CORESTA SSPT2023 CONFERENCE**

# SMOKE SCIENCE and PRODUCT TECHNOLOGY

# ABSTRACTS

## POSTER PRESENTATIONS

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*Presenter's name is underlined when the main author (listed first) is not presenting the paper*

# IGPOST 01

## CORESTA strategy, cooperation and achievements

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The mission of CORESTA is to promote and facilitate international cooperation and best practices in scientific research relative to tobacco and its derived products. Solidly based governance rules are defined and published to optimally fulfil this mission, and responsibilities are delegated by a General Assembly to a Board and a Scientific Commission.

The vision of CORESTA is to be recognised as an authoritative source of publicly available, credible science and best practices. In 2023, hundreds of scientists from 168 organisations and 43 countries are cooperating in working groups to develop consensual methods, tools and guidelines, and to conduct collaborative studies.

A Strategy House composed of four strategic areas, eight strategic subjects and 16 workstreams has been implemented with the objective to efficiently identify and drive key activities. A circular process of cooperation has also been deployed to prioritize, monitor and report progress on projects, to debate and align contributors' views, and to inform stakeholders. Such a framework leads to the production and publication of an impressive number of quality materials on a wide range of topics.

This poster will describe and illustrate the Strategy House, the collaborative framework, the 5-year plan and 2-year expected deliverables, and the main achievements over the last three years.

**Notes**

## STPOST 01

### Statistical derivation of a fold-change cut-off for non-targeted differential screening studies based on analytical method variability

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Chemical characterization of smoke-free products relies on both validated quantitative methods for specific analytes and screening methods such as non-targeted differential screening (NTDS). The latter approach identifies compositional differences between complex samples without prior knowledge of the differential compounds. However, for a fraction of compounds with variable concentrations between two samples in an NTDS study, the observed differences are due in part to intrinsic variability in the aerosol collection and analytical methods. As a result, a subset of the reported compounds—particularly those with low fold changes—are specious and not reproducible between studies. The goal of this work was to derive an appropriate fold-change cut-off value to limit false-positive discoveries to a reasonable number.

A dataset was acquired by applying seven liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry methods to aerosol samples produced by the Tobacco Heating System (THS) 3.0., and this dataset was augmented with simulated data points. A fold-change cut-off value of 1.25 was calculated, such that if a THS product was subjected to a comparison to itself, only 2.5 % of all analytical features would be above the cut-off value.

Considering only compounds increased by > 25 % in the sample vs. the comparator improves the statistical and scientific relevance of NTDS results and shifts the focus to compounds with differences that have greater toxicological relevance.

**Notes**

## STPOST 03

### Methods for analysis of sweeteners and flavor components in nicotine pouches and saliva samples

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Modern oral nicotine products are becoming increasingly popular alternatives to smoking. However, the palatability is largely dependent on added flavorants which may have high chemical diversity with regard to analytical methods development. Furthermore, in clinical studies the measurement of remnant flavors in used products and in saliva during use are often critical for understanding the bioavailability of product components and the overall expected usage timeframe. In this study, a comprehensive strategy was developed and validated to quantify two non-volatile artificial sweeteners plus fourteen volatile flavor components in unused and used smokeless pouch products as well as in saliva for application in support of clinical trials. Analysis of artificial sweeteners required two independent LC-ESI-MS/MS injections using reversed phase and HILIC separation prepared from a single aqueous extract. The volatile flavor components were analyzed by GC-MS using an ethanol-based extract. Each method developed in this study demonstrates analytical characteristics well suited for use in clinical trials in both matrices including low saliva volumes required, low or sub ppm limits of quantification (0.025 µg/mL for sweeteners and < 3 µg/mL for flavorants). The ranges of applicability for the methods are on the order of 100-fold for sweeteners and 400-fold for flavorants (i.e., up to 2.5 µg/mL and 750 µg/mL per saliva sample, respectively). Linearity ($R^2 \geq 0.996$ for sweeteners and $\geq 0.998$ for flavorants), accuracy, precision, and specificity all met validation criteria. The development process, methodology, and exemplary validation results will be discussed.

**Notes**

## STPOST 04

### Quantitative analysis for metabolic profiling of aroma compounds by near infrared spectroscopy (NIR)

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It is well-known that the types and content of aroma components in tobacco is an important focus of the cigarette industry. Different types and contents of aroma components are the reaction of different metabolic profiling. This study proposed quantitative analysis for metabolic profiling of K326 tobacco aroma compounds' PLS-DA model by NIR, which had been planted in three typical ecological environments (Henan, Guizhou and Yunnan Provinces of China). Firstly, the GC-MS method was used to detect the kinds and content of aroma components of K326 flue-cured tobacco. A partial least squares discriminant analysis (PLS-DA) model was built to develop the feature parameters, the principal component score, of the metabolic profiling of the aroma components. Secondly, using the partial least squares (PLS) method, the model between the near infrared spectrum data and aroma components of metabolic features of relevant parameters was fitted. Thirdly, the internal parameters that include the correlation coefficient ($R^2$), the appropriate number of principal components (k), the standard error of calibration (SEC) and root mean square error of cross validation (RMSECV) were calculated to evaluate the models; and the external parameters that include average error verification, standard error of validation (SEV), standard deviation of validation error (SDV), the t distribution of paired t test value and probability P were also calculated to evaluate the models. Results show that (1) The PLS-DA model can clearly develop the characteristics of metabolic profiling of aroma compounds; (2) The score of component 1 correction model of internal evaluation parameters: $R^2$, k, SEC and RMSECV is 0.970, 9, 0.829 and 0.976, respectively; the external evaluation parameters: average error verification, SEV, SDV, the t distribution of paired t test value and probability P is 0.00291, 0.09960, 0.10083, 0.09960 and 0.856, respectively. The score of component 2 correction model of internal evaluation parameters: $R^2$, k, SEC and is 0.908, 7, 1.13 and 1.22, respectively; the external evaluation parameters: average error verification, SEV, SDV, the t distribution of paired t test value and probability P is 0.02111, 0.12606, 0.12586, 0.12606 and 0.295, respectively. Thus, the established model is of good stability and prediction accuracy. The establishment of the method for using near infrared spectroscopy technology combined with chemometrics to rapidly quantitatively predict metabolic profiling of K326 tobacco aroma compounds is a good and significant practice.

**Notes**

## STPOST 05

### Identification of a novel mode of action of vanillin derivative compound veratraldehyde

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Compounds that test positive in *in vitro* micronucleus tests (MNTs) need to be followed up with *in vivo* MNTs. However, it is common for these compounds to test negative in *in vivo* MNTs, known as "misleading positives." To reduce *in vivo* follow-up tests, we developed an *in vitro* mode of action (MoA) analysing platform which consists of ToxTracker assay and high-content imaging analysis (HCA) to classify MN-inducing compounds according to their MoA: clastogenic, aneugenic, or reactive oxygen species–generating. To demonstrate the utility of our platform, we analysed the MoA of a flavouring compound veratraldehyde (VER), a derivative of vanillin in which the hydroxyl group of vanillin is replaced with a methoxy group. Both VER and vanillin are known to be misleading positives in *in vitro* MNTs. To reveal the MoA of these compounds, we employed the ToxTracker assay which uses flow cytometer to analyse six reporters. The results showed that VER activated one of the two reporters associated with DNA damage, characteristic of aneuploidy, whereas vanillin did not activate any of these six reporters. A subsequent ToxTracker ACE (Aneugen Clastogen Evaluation) assay showed that VER induced cell cycle defects, indicative of aneugenicity. HCA combined with nucleus staining and γ-H2AX immunostaining revealed that VER also induced an abnormal nuclear morphology but minimal DNA damage. Additionally, staining microtubules with fluorescent dye resulted in a decrease in the fluorescence signals, indicating modulation of microtubule dynamics. From a perspective of chemical structure, we considered that VER interacts with the colchicine binding pocket of tubulin to modify its polymerization because VER has adjacent methoxy groups, similar to colchicine. A novel MoA by which VER induces micronuclei *in vitro* was revealed through the collective results of this study, thus demonstrating the utility of our platform that combines ToxTracker assays with HCA.

**Notes**

## STPOST 06

### Validation of extraction method for tobacco-free nicotine pouches and Swedish snus pouch products - towards standardization of *in vitro* testing

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Tobacco-free nicotine pouches (NP) and tobacco-containing Swedish snus pouches (Swedish snus) are two types of nicotine-delivery products for oral use. A standard method to generate an extract of these products for *in vitro* testing has not been established. Here, we describe the development and validation of an extraction method for NP. Four NPs with various flavours and nicotine contents were tested using five commonly used reference solvents: phosphate-buffered saline, Dulbecco's Modified Eagle's Medium, RPMI-1640 Media, McCoy's 5A Glutamax Media, and artificial saliva. Extractions were performed at 37 °C to mimic the human oral cavity. Pouches were cut in half and infused in the solvents at a 1/5 w/v dilution under gentle shaking (400 $min^{-1}$) for 1 h. The extracts were centrifuged, and the supernatant was filtered through 0.45 µm and 0.22 µm pore filters.

Extraction selectivity was confirmed by quantifying nicotine and menthol levels and qualifying the presence or absence of other target flavour-related ingredients. The coefficient of variation (CV) between runs reached a maximum of 9 % for menthol (as a representative flavour) and 2 % for nicotine quantification, confirming good intermediate precision (i.e., the extraction method was not impacted by variables such as the instrument, day of analysis, or operator). Nicotine extraction recovery was ~100 % (CV 5 %) across all solvents and pouches. The effect of time on extraction efficiency was evaluated by extracting the pouches for 1 h ± 20 %. A maximum difference of 6 % compared to reference timing was measured, indicating the variation was linked to the method itself rather than the impact of the infusion timing. Stability was also evaluated for two months at -80 °C storage and indicated no loss of nicotine in the trapped solvents. In conclusion, we successfully developed and validated an extraction method for NP that can be used as a standard for future *in vitro* assessment.

**Notes**

## STPOST 07

### *In vitro* cytotoxicity and mechanistic insight from MucilAir™ and SmallAir™ tissues exposed to cigarette smoke and next-generation products

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There is a current push to investigate emerging Next Generation Products (NGPs) including Electronic Nicotine Delivery System (ENDS) and Heated Tobacco Products (HTPs). 3D airway tissue models are a relevant method for analysis of toxicity for these aerosol products. These tissues have increased relevance over monolayer cell cultures as they have many attributes which are generally only seen *in vivo*; characteristics such as metabolic activity, and increased physiological relevance.

MucilAir™ and SmallAir™ (1R6F only) (Epithelix Sarl, Switzerland) tissues were exposed using a Vitrocell® VC10® smoking robot; to 1R6F Kentucky Reference cigarettes (smoked to ISO 20778 for 64 minutes), a commercially available HTP (puffed to modified ISO 20778 for 180 minutes) and a commercially available ENDS (puffed to ISO 20768 for 180 minutes). Aerosol was diluted at varying concentrations with flowing air; 10, 8, 4 and 1 L/min for the 1R6F, and 2, 1, 0.5 L/min and an undiluted airflow for HTP and ENDS. Liquid traps were placed at the air-liquid interface (ALI) and analysed for nicotine, which was used as a marker for delivered dose.

Following exposure cytotoxicity was measured via WST-8 and Lactate dehydrogenase (LDH) assays. Additionally, TEER, cytokine, histological and RNA analysis of tissues were performed to provide molecular insights.

Exposure of MucilAir™ and SmallAir™ to whole aerosol resulted in different levels of viability and cytotoxicity with significantly different $IC_{50}$ values.

This study was a proof of concept suggesting that MucilAir™ tissues can be used to differentiate between combustible cigarettes or HTP, and ENDS products with regards to cytotoxicity assessment. These tissues are also useful in elucidating pathways of cytotoxicity. Difference in response between MucilAir™ and SmallAir™ can be useful in determining appropriate tissue model to use depending on particle size of test aerosol.

**Notes**

## STPOST 08

### Aerosol generation and exposure atmosphere characterization of VITROCELL Air Liquid Interface (ALI) exposure system using ENDS products

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Electronic nicotine delivery systems (ENDS) are frequently tested to evaluate their potential as reduced-risk alternatives to combustible cigarettes. This involves a variety of tests including *in vivo* and *in vitro* inhalation toxicity evaluations. A major challenge in comparing *in vitro* to *in vivo* models is the difference in properties of aerosol atmosphere to which the test system is exposed during these assessments. Here, we produced and characterized ENDS aerosol for *in vitro* air-liquid-interface (ALI) cell culture exposures to establish that the aerosol properties were stable and consistent with *in vivo* exposures.

CORESTA CRM 81 puffing regimen (55 ml puff volume, 3 seconds puff duration and a 30 second puffing interval) was used to produce aerosol from a commercially available ENDS product. Twelve ENDS were loaded simultaneously on the puff ports of the top bar of a linear smoking machine (LM24E). The master controller automatically adjusts the sequence of puffing to produce continuous exhaust from the machine, thus enabling generation of consistent and stable aerosol atmosphere. A mixing bulb installed at the output of smoking machine enabled proper mixing of the aerosol and addition of dilution air flow. The aerosol was then introduced to a VITROCELL 24/48 exposure system. The VITROCELL dilution manifold was set to achieve three target concentrations – Maximum-Achievable-Concentration (MAC) based on gravimetric measurements, mid dose of ~50 % of MAC and a low dose of ~20 % of MAC.

Aerosol characterization showed a MAC of 3.9 mg/L was achievable in the exposure system. The mid and low doses were ~56 % and ~23 % of MAC. Aerosol particle size measurements showed a MMAD of ~1.0 µm with a GSD of ~1.6 across all groups. The temporal variability of aerosol concentration was <10 %.

In summary, methods were developed to generate stable and consistent ENDS aerosol in the ALI exposure system.

**Notes**

## STPOST 09

### Examination of automated growth inhibition classification in Ames test by machine learning

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The Ames test is a commonly used bacterial bioassay to assess the mutagenicity of chemicals and has been applied to evaluate the mutagenicity of tobacco products. In Ames test, a statistically significant dose-related increase in revertant colonies suggests the mutagenicity of the test substance. However, when the test substance also induces severe cytotoxicity, bacterial growth may be inhibited, and the mutagenicity may not be assessed correctly. Therefore, Organization for Economic Co-operation and Development (OECD) guideline 471 requires information on growth inhibition (GI) as well as the number of revertant colonies. GI is determined based on a reduction in the number of microcolonies (background lawn) and is observed microscopically. This process places a high burden on researchers, and periodic checks are required to prevent differences in classification criteria between individual researchers. Hence, it is desirable to improve the efficiency of the process and to ensure objectivity in the classification of GI.

In this study, we employed machine learning for the classification of five types of GI. *Salmonella Typhimurium* TA100 was used without metabolic activation, and 1,856 images of GI were obtained to build models to classify types of GI using DataRobot, an automated machine learning platform providing more than 40 models including Auto-tuned K-Nearest Neighbors Regressor, Keras Deep Residual Neural Network Regressor, and Ridge Regressor. Among the provided models, we selected Auto-tuned K-Nearest Neighbors Regressor as the best model based on root mean square error (= 0.1458), and employed this model for prediction of the GI type of images obtained in three independent trials of Ames test. This model achieved average accuracy of 97.6 % for GI level classification of all data in approximately 10 minutes. This approach has the potential to improve the efficiency and objectivity of GI classification in Ames test.

**Notes**

## STPOST 10

### *In vitro* micronucleus in V79 cells using Microflow – treatment at the air liquid interface with aerosol from three different nicotine containing products

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The *in vitro* micronucleus assay is a standard genetic toxicology test. Historically this has been run with a slide scoring endpoint which is labour intensive and low throughput for testing multiple products. Flow cytometer analysis can be performed, with the use of additional stains, to measure micronucleus induction.

This process was used for analysis of samples treated at the air-liquid interface (ALI) with whole aerosols. A Vitrocell® VC10® smoking robot and high throughput dilution system was utilised to generate aerosols from a 1R6F Kentucky Reference cigarettes (ISO 20768 for 12 min), commercially available heated tobacco product (HTP) (modified ISO 20768 for 42 min) and electronic nicotine delivery system (ENDS) (ISO 20778 for 180 min). Whole aerosol was tested at varying concentrations and diluted with the addition of clean air.

V79 cells grown on 24 mm Transwells™ were placed at the ALI within the Vitrocell High Throughput system, where they were treated with these aerosols. Liquid traps were placed in each airflow concentration and analysed for carbonyls and nicotine.

Endpoints were measured using the Litron™ MicroFlow kit. Cytotoxicity was measured by RPD, additionally the use of Cell Sorting Set-up Beads (Invitrogen™) during flow cytometry assessment was performed.

Results showed increases in micronucleus (MN) induction across aerosol concentrations for combustible cigarette. HTP and ENDS products showed low MN induction up until the increase of cytotoxicity where the MN induction increased.

This study demonstrated that micronuclei can be detected at the ALI using V79 cells treated with varying aerosols.

**Notes**

## STPOST 11

### Validation of the Vitrocell® HTP 2.0+ 12 well mammalian module for assessing cigarettes, ENDS, and eHTPs: evaluation of dilution airflow, dose resolution, and dose repeatability

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The present study aimed to validate the performance of the Vitrocell® HTP 2.0+ 12 well mammalian module for assessing three different test articles: cigarettes, electronic nicotine delivery systems (ENDS), and electronic tobacco heated products (eHTPs). Specifically, we investigated the variation within dilution airflow, dose resolution, and dose repeatability to ensure the reliability of the experimental setup.

To evaluate dilution airflow, measurements were conducted across all test articles, revealing consistently low variation, except for the lowest dilution rates where higher variability was observed. Overall, the dilution airflow showed satisfactory performance, indicating its suitability for use in the experimental system.

Dose resolution, a critical parameter for accurate assessment, was assessed for all test articles. The results demonstrated good dose resolution in general, although ENDS exhibited the lowest resolving power among the three tested products. This finding emphasizes the need for careful consideration when evaluating ENDS to ensure accurate dosing and subsequent analysis.

Furthermore, the experimental dose repeatability was examined. Notably, the repeatability was highest on test articles that required multiple products per dose, cigarettes and eHTPs, indicating the importance of accounting for pod-to-pod variability when planning exposures using ENDS.

In conclusion, the validation of the Vitrocell® HTP 2.0+ 12 well mammalian module demonstrated reliable performance for assessing cigarettes, ENDS, and eHTPs. The study findings revealed low variation within dilution airflow, overall good dose resolution, and highlighted the significance of considering pod-to-pod variability for ENDS exposures. These findings contribute to the accurate evaluation of aerosol exposures and support the use of this module in relevant toxicological investigations and risk assessments.

**Notes**

## STPOST 12

### Rapid *in vitro* toxicological screening using ToxTracker to determine the effect of repeated freeze/thaw of combustible cigarette extract

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Combustible cigarette extracts (CCE) are stored at -80 °C to preserve sample integrity. Sample shipping and repeated freeze/thaw cycles prior to testing may lead to changes in the chemical composition through the formation of ice-water interfaces, phase separation, pH induced changes and chemical degradation may produce inaccurate results in chemistry and in *in vitro* toxicological assays. We sought to determine the impact of repeated freeze/thaw on CCE at the DNA level using the stem cell based ToxTracker© reporter assay.

Combustible reference cigarettes (1R6F) (n=3) were smoked (Health Canada standard regime). Particulate phase (PP), gas-vapor phase (GVP) and the 1:1 combined phase (PP+GVP)] were collected and evaluated in the ToxTracker assay. Samples were tested within 1 hr of sample generation or after 3 freeze/thaw cycles. Cells were incubated for 24 hr (both +/- S9). Green-fluorescence protein (GFP) reporter gene induction and cytotoxicity were assessed using flow cytometry.

Fresh PP (-S9) samples induced a 35-fold increase in Srxn1 (indicative of oxidative stress) and a 10-fold increase in Ddit3 (indicative of protein damage) at 200 µg/mL; addition of S9 decreased Srxn1 to 12-fold GFP-induction in fresh PP. GFP-induction was not observed in fresh GVP samples. Fresh PP+GVP (+S9), induced Srxn1 and Blvrb (indicative of oxidative stress) 10 and 4-fold, respectively at 200 µg/mL and no effect on Ddit3. Sample freeze and thaw decreased Srxn1 from 35 to 20-fold while Ddit3 increased from 10 to 21-fold (-S9). No significant difference between fresh (+S9) and freeze/thaw (+S9) for PP or PP+GVP samples was observed.

This data indicates that sample freeze/thawing can alter *in vitro* toxicity results in the ToxTracker assay. A “fresh” sample should be used for the ToxTracker assay to eliminate unintended potential sample manipulation effects. This allows for the test system to be treated with a sample that more closely resembles the chemical profile produced immediately after sample collection and representative of normal smoking behavior.

**Notes**

## STPOST 13

### Application of rapid *in vitro* toxicological screening using ToxTracker to determine the effect of flavors in snus products.

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Snus is an oral tobacco derived nicotine product that is sold in flavored pre-packages. Snus is traditionally sold as tobacco flavored. The potential impact of flavors on toxicity is unknown. Regulatory assays such as Ames, neutral red uptake (NRU), and *in vitro* micronucleus (ivMN) have not been traditionally used to differentiate the possible toxicological impact of the different flavor systems. ToxTracker is a stem cell-based reporter assay that provides mechanistic insights into the mode-of-action of products.

The objective of this study was to utilise ToxTracker to determine if the toxicity of different snus flavors can be differentiated and if they have different reporter gene induction profiles. The CORESTA snus reference product CRP 2.1 and 3 different flavored (traditional, menthol, and wintergreen from the same brand) commercially available snus products were extracted with DMSO, and concentrations (up to 1.6 µg/mL) applied to each of the six reporter cell lines (+/- S9) in the ToxTracker assay. Reporter gene (GFP) induction and cytotoxicity were assessed by flow cytometry following a 24-hr incubation. The induction profile for all products tested, including CRP 2.1, showed a 3 to 4-fold induction of the Ddit3 reporter gene in the absence of S9 indicating that the products could potentially damage proteins. Among flavor assessment, a 2-fold increase in the Srxn1 (indicator for oxidative stress) reporter gene was observed only with the wintergreen snus product in the absence of metabolic activation. However, there was no reporter gene induction in the presence of metabolic activation. None of the concentrations tested has an impact on cellular cytotoxicity indicating that the doses were not toxic, and the concentration range could be increased.

This preliminary work suggests that under the tested experimental conditions, ToxTracker is capable of distinguishing the effect of wintergreen flavor compared to traditional or menthol flavor.

**Notes**

## STPOST 14

### Comparison of urinary biomarkers of tobacco smoke exposure among different smokers

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Biomarkers of exposure (BoE) in human urine can reflect the smoke constituent exposure level and risk of smokers. To compare the changes in urinary metabolic markers of nicotine, tobacco specific nitrosamines (TSNAs), and other volatile organic chemicals (VOCs) in consumers who smoked conventional cigarettes and heated tobacco products (HTPs) of different flavour types, a total of 200 volunteers were recruited from four Chinese cities, Shanghai, Suzhou, Hefei, and Guiyang, with 50 participants each randomly divided into three groups. The three groups in each city smoked conventional cigarettes, regular HTPs, and mint-flavoured HTPs for one week each in alternate order. Urine samples were collected from all participants on days 8, 15, and 22, and the levels of 17 smoke exposure biomarkers in the samples were determined using UPLC-MS/MS. The results showed that: 1) Compared with conventional cigarette smokers, HTP smokers had lower urinary metabolic markers of nicotine, TSNA, and VOCs by approximately 33 %, 28 %, and 35 %, respectively. 2) Total BoE levels in urine samples were higher in males than in females. There was no significant difference in total BoE levels between participants who smoked different flavours of HTP. 3) No significant difference in total BoE levels was observed between participants from different cities. However, the levels of nicotine metabolites in urine samples of participants smoking regular HTPs in GY were significantly higher than those in the other three cities. This study provides data support and theoretical reference for the comparison of exposure risk assessment between HTPs and conventional cigarettes.

**Notes**

## STPOST 15

### Effect of cigarette smoke on interfacial chemical properties of pulmonary surfactant

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Cigarette smoke is the major source of indoor air pollution, and the lungs are the primary target organ affected by it. After entering the lungs, cigarette smoke first comes into contact with the pulmonary surfactant (PS) in the alveoli. Understanding the interfacial chemical mechanism between cigarette smoke and PS is important for assessing the potential health effects of cigarette smoke. This article focuses on the water-soluble solution of cigarette smoke, selecting the natural pulmonary surfactant PS as a model to study the adsorption of active components (phospholipids and proteins) in PS by cigarette smoke, as well as the effects of cigarette smoke on the interfacial chemical properties such as surface tension, foaming properties, and the surface pressure-area isotherm of PS. It was found that exposure to cigarette smoke caused an increase in the surface tension and a decrease in the foaming ability of PS, which was related to the adsorption of cigarette smoke on phospholipids and protein components in PS. In addition, cigarette smoke also caused the outward expansion of the pressure-surface area isotherm curve of the PS film, which was related to the embedding of particles in the interstices of the PS film in cigarette smoke, and the organic components in cigarette smoke act as film-forming components. Thus, it is clear that exposure to cigarette smoke alters the initial composition and interfacial chemical properties of the PS system and has the potential to interfere with normal lung function.

**Notes**

## STPOST 16

### Biomarkers assessment in healthy adult smokers who switched from conventional cigarettes to two types of non-combustible tobacco products: a randomized, controlled study

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There has been a growing interest in the non-combustible tobacco products, including e-cigarettes and heated tobacco products. Reductions in biomarkers of exposure associated with selected harmful and potentially harmful constituents (HPHCs) have been reported in adult smokers when switching from conventional cigarettes (CC) to non-combustible tobacco products.

We conducted a clinical study with two types of non-combustible tobacco products. Test products were a cig-a-like e-cigarette (eDNC1.0a) with three different flavour variants and a novel heated tobacco product (IT2.0b) with three different flavour variants. This study was a multi-centre, randomized, open-label, controlled, 8-arm parallel-group study conducted in U.S. healthy adult smokers who either 1) continued to smoke CC, 2) switched to each flavour variant of eDNC1.0a, 3) switched to each flavour variant of IT2.0b, or 4) stopped smoking for 60 days. The objectives of the study were to investigate the reduction in exposure to selected HPHCs when smokers switched to the various eDNC1.0a and IT2.0b flavour variants and to characterize nicotine uptake relative to CC over a 60-day period with 5-day confinement and 55-day ambulatory conditions.

485 subjects were randomized to the study and used the assigned products or stopped smoking. 361 subjects completed the study. Following 60-day switching, statistically significantly lower values for most measured BoE were observed for the eDNC1.0a and IT2.0b groups compared to the CC group. No marked differences were noted for most BoE measured as compared to the cessation group.

**Notes**

## STPOST 17

### Biomarkers assessment in healthy adult smokers who switched from conventional cigarettes to closed tank e-cigarette: a randomized, controlled study

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There has been a growing interest on the non-combustible tobacco products, including e-cigarettes and heated tobacco products. Reductions in biomarkers of exposure associated with selected harmful and potentially harmful constituents (HPHCs) have been reported in adult smokers when switching from conventional cigarettes (CC) to non-combustible tobacco products.

We conducted a clinical study with a closed tank e-cigarette (eDNC2.0a) with four different flavour variants. This study was a multi-centre, randomized, open-label, controlled, 5-arm parallel-group study conducted in U.S. healthy adult smokers who either 1) continued to smoke CC or 2) switched to each flavour variant of eDNC2.0a for 60 days. The objectives of the study were to investigate the reduction in exposure to selected HPHCs when smokers switched to the four flavour variants of eDNC2.0a and to characterize nicotine uptake relative to CC over a 60-day period. In this study, half of each group was allocated to a 5-day confinement and 55-day ambulatory condition, and the other half to a 60-day ambulatory condition.

319 subjects were randomized to the study and used the assigned products. 241 subjects completed the study. Following 60-day switching, statistically significantly lower values for most measured BoE were observed for the eDNC2.0a groups compared to the CC group.

**Notes**

## STPOST 18

### Comparison of biomarkers of exposure in users of various tobacco/nicotine products

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In the last two decades, various types of tobacco and nicotine products have been developed and marketed, which avoided combustion of tobacco and thus released much lower amounts of toxicants. These toxicants are almost exclusively combustion products, including polycyclic aromatic hydrocarbons (PAHs), aldehydes, volatile organic compounds (VOCs), nitrosamines, aromatic amines, and many others. The products include electronic cigarettes (ECs), heated tobacco products (HTPs), and oral tobacco (OT) such as snus and nicotine pouches. Although still controversially discussed in the public health and tobacco control communities, these products might potentially serve as tobacco harm reduction (THR) products. The objective of this study was the identification of biomarkers and biomarker patterns in body fluids, to distinguish between combustibles, heated tobacco products, electronic cigarettes, oral tobacco and oral/dermal nicotine products used for nicotine replacement therapy (NRT), as well as a control group of non-users. Therefore, a controlled, single-centre clinical study was conducted with 60 healthy subjects, divided into 6 groups based on their sole use of the products of choice. The collected biofluids included urine, blood, saliva, exhaled breath and exhaled breath condensate. This poster will present the results from the quantitative assessment of several classes of biomarkers of exposure determined in urine and plasma samples for VOCs (mercapturic acids), PAHs (OH-PAHs), aromatic amines, nicotine and metabolites, propylene glycol and tobacco specific nitrosamines (TSNAs) across the 6 different study groups.

**Notes**

## STPOST 19

### Analysis of cannabidiol and its three major metabolites in human plasma by online extraction LC-MS/MS

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The main active ingredients of *Cannabis sativa* are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). CBD as the main non-psychoactive constituent has been marketed in a growing number of products for recreational use such as CBD oils and vapes. Pharmacokinetics (PK) studies are needed to determine the absorption, distribution and metabolism of CBD after administration, which is largely affected by the formulation of the product in analogy to nicotine-containing products.

Hence, we developed a quantitative bioanalytical method for the determination of CBD and its three major metabolites – 6-hydroxy-CBD, 7-hydroxy-CBD, and 7-carboxy-CBD – in plasma by an online-LC-MS/MS for this purpose. The method is characterized by an online sample preparation procedure, in order to achieve high sample throughput needed for PK studies. 200 µL of plasma are precipitated with ZnSO4 in methanol and subsequently injected onto the LC-MS/MS system (Shimadzu Nexera X2 uHPLC coupled to Sciex QTrap6500+ MS/MS). Online extraction is achieved by means of a Zorbax C8 column (Agilent) followed by gradient elution on an Amid UHPLC column (Merck).

The method was fully validated according to FDA guidelines for bioanalytical method validation showing excellent results in terms of precision, accuracy and sensitivity.

The LLOQ of 0.39 ng/mL and broad linear range of up to 200 ng/mL for CBD and the three major metabolites in combination with the straightforward sample preparation demonstrate the suitability of this method for its application in CBD PK studies.

**Notes**

## STPOST 20

### White blood cell count is a biomarker of a potential harm in short-term smoking abstinence and product switching to electronic nicotine delivery systems

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Smoking-related diseases develop over several decades of smoking and the adverse effects of smoking, such as chronic inflammation persist after cessation. Biomarkers of Potential Harm (BoPH) inform of interim biological responses to changes in smoking status and the potential risk of diseases. While white blood cell (WBC) levels, which are widely used markers of chronic inflammation, are higher in smokers and return to baseline values after sustained abstinence of several months, few studies have examined changes in WBC counts in short-term abstinence or exclusive use of ENDS products. First, we evaluated changes in WBC and other select hematological parameters in two weeks of smoking abstinence in two age cohorts (24-34 and 35-60 years). Significant declines in neutrophils, WBC, red blood cells (RBC), hematocrit, and hemoglobin were observed at Days 7 and 14 of abstinence compared to the baseline levels in both age groups, whereas lymphocyte counts were significantly lower only at 14 days of abstinence in the older age group. Second, we extended these assessments to those smokers who abstained, or switched to exclusive use of Vuse Solo, Vuse Ciro, or Vuse Vibe Electronic Nicotine Delivery System (ENDS) products for 7 days. Consistent and significant reductions in neutrophil, WBC and RBC counts along with reduced hemoglobin and hematocrit levels were observed in smokers who either abstained or exclusively used any of the Vuse ENDS for 7 days. Thus, rapid and reproducible reductions in WBC and neutrophils, RBC, hematocrit, and hemoglobin suggest immediate beneficial changes, indicating a potential reversal of inflammation upon smoking abstinence or switching to the Vuse ENDS products. Our work demonstrates the utility of WBC, neutrophils and the select hematological parameters as BoPH for tobacco product evaluation and replicates similar findings from larger scale clinical studies conducted over several months as found in the literature.

**Notes**

## STPOST 21

### Aromatic amines in the mainstream smoke of commercial cigars

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Aromatic amines are a class of carcinogenic compounds in tobacco smoke that are listed on the FDA list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke. Six aromatic amines yields (1-aminonaphthalene (1-AN), 2-aminonaphthalene (2-AN), 3-aminobiphenyl (3-ABP), 4-aminobiphenyl (4-ABP), ortho-toluidine (o-TOL), o-anisidine (o-ANI)) in the mainstream smoke from 23 commercial filtered cigars, 16 cigarillos, and 11 large cigars were determined with the solid-phase microextraction-coupled to gas chromatography triple quadrupole mass spectrometry (SPME headspace GC–MS/MS) method. The commercial cigars were smoked under Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Recommended Method No. 64 using a linear cigar smoke machine. The aromatic amine yields in the mainstream smoke from 50 commercial cigars show high variation within and between the products. The averages of each aromatic amine yield in the filtered cigars, cigarillos, and large cigars are 108, 371, and 623 ng/cigar for o-TOL; 6, 14, and 22 ng/cigar for o-ANI; 65, 114, and 174 ng/cigar for 1-AN; 25, 59, and 87 ng/cigar for 2-AN; 6, 17, and 27 ng/cigar for 3- ABP; 8, 11, and 17 ng/cigar for 4-ABP, respectively. The relationship between aromatic amine and total particulate matter (TPM) was evaluated. On a per cigar basis, the aromatic amines have a good linear response with TPM. On a per mass of TPM basis, the ratios of aromatic amines and TPM were consistent.

**Notes**

## STPOST 22

### The effects of characterizing flavors on mainstream cigarette smoke chemistry and *in vitro* toxicity

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The employing of flavours in tobacco products has an extensive historical background, and recently, with the emergence of novel tobacco products, the importance of flavourings is growing steadily. Nonetheless, the flavours as the tobacco ingredient have been a major subject of ongoing controversies, reflecting the diverse perspectives surrounding their role and safety. Consequently, there have been numerous toxicological studies conducted on flavour ingredients used in cigarettes; however, there is very limited research on recently developed flavour delivery systems such as capsules. Therefore, in this study, we aimed to investigate the effects of flavours applied to the tobacco as the top flavour and cigarette filter, which impart characterizing flavour, on the tobacco smoke chemistry and *in vitro* toxicity. Four prototype cigarettes with characterizing flavours and capsules were manufactured, along with four control group products that excluded all additives except for casing flavour. The NRU (Neutral Red Uptake) assay was conducted on total particulate matter (TPM) and gas vapor phase (GVP) collected from test and control cigarettes, respectively. The AMES test was performed on TPMs. TPMs from protype cigarettes showed lower cytotoxicity and genotoxicity on a per TPM basis compared to their corresponding control cigarettes, however, no significant differences were found on a per-cigarette basis. GVPs were evaluated by using NRU and comparable results were obtained to TPMs. These results were confirmed by smoke chemistry analysis. Conclusively, our findings indicate that employing characterizing flavours has no meaningful effects on the inherent *in vitro* toxicity of cigarette smoke.

**Notes**

## STPOST 23

### A pilot assessment on novel hybrid product: emission analysis of nicotine, volatile aromatic constituents and selected HPHCs

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There is a new type of emerging product which combines the e-cigarette and heat-not-burn product. Instead of adding flavours into the e-liquid, these hybrid products (HPs) contain only humectants in the e-liquid. When puffing starts the hot aerosol will flow through a section of solid tobacco materials containing flavours, and by this means aromas and nicotine are eluted into the aerosol. There is an increasing amount of studies and evidence on the traditional e-cigarette and heat-not-burn tobacco products. However, studies on this novel hybrid product are inadequate. To evaluate the performance of this novel hybrid product, a prototype HP device was developed by our collaborator and an e-liquid containing only humectants was filled into cartridge. Solid flavoured materials in granule form made of tobacco (reconstituted tobacco granules) were prepared and filled into a stick for elution purposes. Nicotine and selected aromatic compounds in reconstituted tobacco granules were determined. Emissions of HPs with elution stick were generated by a linear smoking machine. Nicotine, volatile compounds, and selected harmful and potentially harmful compounds (HPHCs) such as aldehydes in emissions were collected and quantitatively determined. Same determinations of emissions were carried out using selected commercial brands of e-cigarette and heat-not-burn tobacco products. Puff-by-puff analysis was done for the HPs to assess the delivery pattern of nicotine and aromatic constituents. Nicotine, aromatic constituents and HPHCs in the emissions of the novel HPs were determined and compared with the current e-cigarette and heat-not-burn products. Puff-by-puff analysis was carried out to assess the delivery pattern and stability of HPs during puffing. This study can be regarded as a pilot assessment of this novel type of hybrid next generation product.

**Notes**

## STPOST 24

### Humectant ability and bilateral humectant method construction for cigarette components

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In order to study the humectant ability and bilateral humectant effect of cigarette components, the desorption curve of cut tobacco and cut stem and the area of various water activities were quantified by GAB model integral, and a humectant direction index model was constructed to accurately quantify the desorption curve and adapt to different water activities. The effects of different cigarette components and different cut stem ratio on humectant ability and bilateral humectant effect were analyzed. The results showed that: (1) Under different water activity conditions, the W value of cut tobacco was significantly lower than that of cut stem sample, and the cut tobacco had high humectant effect. (2) There were common differences in the bilateral humectant index H of cut tobacco and cut stem, which showed less water loss and poor moisture resistance, and the bilateral humectant index H of cut tobacco was less than that of cut stem. (3) Increasing the proportion of cut stem in cigarette formula, the moisturizing ability of tobacco decreased, the moisturizing directional index increased, and the moisture-proof effect became worse. This method is suitable for the analysis of humectant effect of different cigarette components and the development of humectant ability.

**Notes**

## STPOST 25

### Nicotine pharmacokinetics and subjective effects of novel heated tobacco product compared to cigarettes: a randomised, crossover study

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The nicotine pharmacokinetics (PK) and subjective effects of non-combustible tobacco products, including heated tobacco products, have been widely assessed, with a lower or similar nicotine exposure noted for many products when compared to conventional cigarettes (CC).

The novel heated tobacco product (IT2.0b) creates a vapor by heating an e-liquid that is nicotine-free. This vapor passes through capsules containing flavoured tobacco granules prior to being inhaled by the user.

We conducted a clinical study with an IT2.0b, using three flavour variants, to evaluate the nicotine PK and subjective effects following a single *ad libitum* use of IT2.0b compared to CC and FDA-approved nicotine gum. This study was a single-centre, randomized, open-label, controlled, 5-period, 10-sequence, crossover study conducted in U.S. healthy adult cigarette smokers.

A total of 61 subjects were randomized to one of 10 product use sequences, with 50 subjects completing the study. Three flavour variants of IT2.0b were characterized by later $T_{max}$ and lower $C_{max}$ and $AUC_{0\text{-}last}$ values compared to CC and faster $T_{max}$ and lower $C_{max}$ and $AUC_{0\text{-}last}$ values compared to the nicotine gum. Overall, for subjective effects questionnaire, scores obtained for the three flavour variants of IT2.0b were less than those obtained for CC and were similar to those obtained for nicotine gum.

**Notes**

## STPOST 26

### Characterization of nicotine pharmacokinetics from use of a novel heated tobacco capsule prototype in adults who smoke

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Heated tobacco products (HTPs) may offer a potentially reduced-risk alternative to adults 21+ who smoke cigarettes (AS) and are unable or unwilling to quit. In this study, we characterized the nicotine pharmacokinetics (PK) and subjective measures of a novel heated tobacco capsule (HTC) prototype in AS. The HTC prototype consists of a hand-held battery-operated device and a disposable tobacco-containing capsule that is inserted into the device. We conducted a randomized, crossover study to characterize nicotine PK of prototype capsules containing menthol and nonmenthol tobacco blend variants, relative to the subject's usual brand cigarette (UBC) in 20 menthol and 24 non-menthol AS. To facilitate acclimation with the novel HTC prototype, subjects were allowed to use up to five capsules for each prototype tobacco blend variant during a product trial, and up to two capsules for each prototype the day prior to the PK session. The PK assessments were conducted during a single use session of the HTC prototype or UBC. Subjects also completed questionnaires on "urges to smoke", "craving cigarettes", and rated the products for "pleasant" and "satisfying" using visual analog scales (VAS). The non-baseline adjusted average plasma nicotine $C_{max}$ values ranged from ~13-18 ng/ml for the HTC prototypes and were ~24 and ~19 ng/ml for menthol and non-menthol UBCs, respectively. The "pleasant" and "satisfying" VAS scores for the prototype non-menthol HTCs used in this study were statistically significantly lower compared to the UBC. Reductions in "craving a cigarette" were significantly lower for the prototype menthol HTC compared to UBC. Overall, the nicotine uptake during use of the HTC prototypes suggests they have the potential to be acceptable substitutes for cigarettes.

**Notes**

## STPOST 27

### Monitoring puff-by-puff aerosol generation and delivery when vaping viscous liquids by using in-line pressure drop measurement

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Vape devices containing highly viscous liquids can be problematic in delivering a consistent aerosol over the lifetime of a vape experiment due to liquid mobility, with the user potentially being unaware of reduced deliveries. To mitigate this problem a methodology was developed that visualised potentially changing aerosol delivery rates on a puff-by-puff basis.

A bluetooth in-line pressure drop (PD) transducer recording both the PD and visually showing the puff profile in 'real' time was developed to allow the user to observe the puff profile shape/PD change on a puff-by-puff basis. This technique can alert the user to increased PD, indicating volatilisation is dropping during puffing due to e-liquid fluidity (viscosity) restricting transport to the heater.

Multiple vape devices (nominally termed low, medium and high viscosity liquid depending on contents) were puffed on a vaping machine using the 55/3/30/square regime, on a puff-by-puff basis using various puffs blocks up to the lifetime of each vape device. The PD during puffing and block deliveries were measured.

Changes in delivery were evidenced in some of the vapes by device PD changing from 120 mmWg to 375 mmWG over a short period of time (< 10 puffs) whilst others showed no change in PD. Where high viscosity liquids were used with change to high PD, there was an 88 % drop in volatilisation over a 120 puff series of puff blocks as measured by mass recovered.

To mitigate the low aerosol collected mass (ACM) delivery rates the addition of a heater jacket to encase the vape pen devices, maintained at a steady temperature of 50 °C, showed the vape liquid can be kept sufficiently fluid to produce a constant PD and a steady stream of aerosol (0.76 mg/puff SD 0.0121, high viscosity device: 1.325 mg/puff SD 0.0258 medium viscosity device: 200 puffs), without producing potential thermal degradation products.

**Notes**

## STPOST 28

### Determination of nicotine, propylene glycol, menthol, glycerol and water by GC-FID & TCD using nitrogen as the carrier gas

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The CORESTA E-Vapour Sub-Group has developed a Recommended Method No. 84 (CRM 84) for nicotine, propylene glycol, glycerol and water in e-vapour aerosols by gas chromatography (GC). We also have developed an analytical method for those components in aerosols of heated tobacco products (HTPs) based on CRM 84 (CORESTA Congress, Kunming, 2018, Smoke Science/Product Technology Groups, STPOST 35). In these GC-FID (flame ionization detector) and GC-TCD (thermal conductivity detector) methods, helium is used as the carrier gas. However, recent global helium shortages, supply instability and price increases have led to growing interest in the use of alternative carrier gases (hydrogen and nitrogen) for GC analysis. The objective of this study was to develop a simultaneous GC-FID & TCD method for the analysis of nicotine, propylene glycol, menthol, glycerol and water using nitrogen, but not hydrogen, as an alternative carrier gas to helium. For this objective, we tried to adjust several GC parameters such as oven temperature and linear velocity based on our conventional method using helium. As a result, we confirmed that the total run time (approximately 5 minutes), accuracy, sensitivity and stability were equivalent to those of conventional methods even when nitrogen was used as the carrier gas. Regarding water analysis, although hydrogen is generally recommended as an alternative carrier gas from the viewpoint of thermal conductivity, it was confirmed that nitrogen also can be used for water analysis by GC-TCD. Based on the above, we developed a new GC-FID & TCD analysis method using nitrogen as the carrier gas for these analytes in HTP aerosol.

**Notes**

## STPOST 29

### Formation of small organic acids during ends aerosol collection

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To reduce the risks associated with combustible nicotine products, efforts have focused on the production of non-combustible alternatives such as electronic nicotine delivery systems (ENDS). At the 2022 CORESTA Conference, we presented our findings into potential ketene generation through thermal degradation of ester flavorants during ENDS aerosol generation. As part of this, we observed the creation of products formed from reactive species generated by propylene glycol (PG) and glycerol (VG), speculating that these species may have been small organic acids (SOA). Here we present our findings in a study looking at SOA generation from PG, VG, and select ester flavorants.

Model e-liquids were vaped under regular-to-moderate experimental conditions (0.7–1.8 Ω coils, 12–75 W power setting, 55/3/30 puff regime) using the CORESTA recommended device (Aspire Nautilus™ tank system with an EvolvTM Reference Mod DNA 75 Color battery), collected in puff blocks to dryness. Generally, the amount of SOA produced was dependent on the power setting and collection duration/puff block. PG and VG both contributed to the formation of acetic acid (0.112–8.07 µg/puff and 0.207–86.3 µg/puff, respectively) and formic acid (0.122–0.713 µg/puff and 0.229–5.43 µg/puff respectively). VG was shown to be primarily responsible for the formation of propionic acid (ND–11.2 µg/puff). At higher power settings, acrylic acid was also observed at levels up to 2.46 µg/puff along with conversion of ester flavorants to their corresponding acid to varying degrees. Though not on the FDA's published HPHC list, these acids have associated hazards that warrant further investigation into their production and effects of long-term exposure.

**Notes**

## STPOST 30

### Comparison of material surface staining following exposure to heated tobacco and e-vapour aerosols versus cigarette smoke

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Cigarette smoke deposition on indoor surfaces leads to discoloration, particularly for light-coloured materials. The less complex aerosols emitted by heated tobacco products (HTPs) and e-vapor products (EVPs) contain ≥ 90 % water and aerosol formers and are therefore supposed to induce less staining than cigarette smoke. This study compared indoor material staining caused by cigarette smoke versus HTP and EVP aerosols in a test chamber based on a model of a standard residential setting. Four typical indoor materials (wallpaper, cotton fabric, placemats, and tablecloths) were exposed to cigarette smoke (200 puffs of Marlboro Gold® corresponding to 1 pack of cigarettes), HTP aerosol (240 puffs of IQOS Iluma® corresponding to 1 pack of HTP), or EVP aerosol (240 puffs of MESH® 2.0). Colour changes were assessed using the Commission Internationale de l'Eclairage L*a*b* colour space. The colour readings were performed on non-exposed samples, immediately after exposure, and after a 28-day aging period. The calculated colour changes were correlated with the visual perception of a standard observer (average person with normal colour perception). The results in freshly exposed materials showed that cigarette smoke caused the materials to turn darker and yellowish, while HTP and EVP aerosols induced minimal or no colour change. Aging only affected cotton fabric, which turned yellow, particularly when exposed to cigarette smoke. Overall, colour changes following HTP and EVP aerosol exposure were significantly lower (by at least 95 %) than those caused by cigarette smoke. An exception was the colour change of aged cotton fabric exposed to HTP aerosol, which had a reduction of 90 % compared to cigarette smoke. These results suggest that HTP and EVP aerosols are less likely to discolour indoor materials and corroborate previous findings indicating that these aerosols cause less tooth discoloration than cigarette smoke.

**Notes**

## STPOST 31

### Simultaneous multisensor physical characterisation of heated tobacco product aerosol and the selection of a reference system

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As a measurement of consistent aerosol delivery, simultaneous multi-sensor analysis of physical characteristics of electrically heated tobacco product (eHTP) aerosol reveals the inherent difficulties of utilising commercially available, or specially selected “near commercially available” eHTP systems, as a reference for collaborative or proficiency studies.

Five commercially available systems were explored using a custom aerosol analysis chamber fitted with sensors measuring physical properties such as temperature (in “mouth” and at “lip”), pressure drop and humidity. Data capture was synchronised in a post processing operation. System A and System B used the same consumable sticks and alternate blade heating devices whilst system C, D and E used different heating principle.

System to system variability, as indicated by the COV of a measurement, is illustrated, although each physical measurement shows a different variability hierarchy. For PD COV D>>A>E>B>C; for humidity B>E>D=C>A and for mouth temperature B>C>E=A>D.

Typical variation for the same stick and different device types (A/B) is demonstrated: Heater type A, first puff PD mean of 94.8 mmWg, COV of 0.16, Heater type B PD mean 107 mmWg COV of 0.08. A more consistent aerosol formation was observed with one of the alternate heating systems (Heater type C) where first puff PD was measured at 79 mmWG with COV 0.04. Similarly mouth temperature first puff Heater A, mean 26.4 °C, SD0.86, Heater B mean 25.15° SD1.15.

Consistent aerosol delivery might be improved by strict control of the temperature of aerosol creation with improved uniformity of heating of a standard substrate using an “oven” system. A simple commercial system operating at 182 °C and 215 °C loaded with 0.2 g of cast leaf was investigated. The advantages/disadvantages of this approach and the merits of adopting such an approach for a reference is discussed.

**Notes**

## STPOST 32

### Simultaneous determination of six furans in aerosol of heated tobacco products by gas chromatography/mass spectrometry

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Due to the absence of high-temperature pyrolysis and combustion, the releases of most harmful components from heated tobacco products (HTPs) are much lower than those from conventional cigarettes (CCs). However, some harmful components were reported to be released at higher levels than CCs, including furans. Furans, which are harmful or potentially harmful to humans, are an important class of baked aroma components in cigarette smoke. To investigate the release characteristics of furans in the aerosol of HTPs, a gas chromatography/mass spectrometry (GC/MS) method was established. The releases and distributions of six furans in the aerosols of eight HTPs and three CCs were investigated under ISO and HCI regimes. The effects of different heating temperatures on the release efficiencies of six furans were also investigated. The results showed that: 1) The calibration curves showed good linearity for six furans and the correlation coefficients ($r^2$) were 0.9989-0.9999. The limits of detection (LODs) and limits of quantification (LOQs) were 0.01-0.12 μg/cig and 0.02-0.39 μg/cig, respectively. Recoveries were in the range of 95.33 % to 108.66 % and the relative standard deviations (RSDs) were 1.33 % to 8.51 %. 2) Furfural, 5-methylfurfural, 2-acetylfuran and furanmethanol were distributed in the gas phase and particle phase, and the proportions in the gas phase under HCI regime were higher than those under ISO regime; while 5-methyl-2-furanmethanol, and 5-hydroxymethylfurfural were mainly distributed in the particle phase. 3) The main furans in the aerosol of HTPs were furanmethanol and furfural, and the release amounts of furanmethanol, furfural, and 5-methylfurfural in the aerosol of some HTPs were higher than those of CCs with ratios ranging from 1.73 to 5.65. 4) Under low-temperature heating conditions, the release amounts of six furans in the aerosol of HTPs increased with increasing heating temperature. The proposed method is simple, sensitive and suitable for the determination of six furans in the aerosol of HTPs, and provides technical support for the product design and quality control of HTPs.

**Notes**

## STPOST 33

### Comparison of HPHCs in aerosol generated from a novel heated tobacco capsule prototype to HPHCs in conventional cigarette smoke

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Heated tobacco products (HTP) contain a tobacco substrate that is heated to low temperatures (below 350 °C), resulting in an inhalable nicotine containing aerosol. The objective of this work is to compare concentrations of select harmful and potentially harmful constituents (HPHCs) in the aerosol generated by a novel heated tobacco capsule (HTC) prototype to their corresponding concentrations in smoke generated from conventional cigarettes (University of Kentucky 1R6F Certified Reference Cigarette). The HTC prototype consists of a hand-held battery-operated device (BVR 3.2) and a disposable tobacco-containing capsule that is inserted into the device. The selection criteria employed to identify the 50+ HPHCs, compared across the HTC prototype and conventional cigarettes, will be discussed. Comparisons of the HPHC data will be presented on a per unit and normalized per nicotine basis. Data presented will demonstrate significant reductions (80-99 %) for most HPHC chemical classes such as carbonyls, volatile organic compounds, aromatic amines, carbon monoxide, and polycyclic aromatic hydrocarbons in the aerosols generated from the HTC prototype compared to cigarette smoke. The significant reduction in HPHCs substantiates that heating tobacco in the HTC prototype prevents high temperature pyrolysis and combustion reactions that occur when tobacco is burned in a conventional cigarette. The data indicates that Adults 21+ who smoke (AS) should significantly reduce their exposure to HPHCs by switching to use of the HTC prototype.

**Notes**

## STPOST 34

### Research on the distribution of nicotine retained by filters of heated tobacco products based on visualization

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In order to quickly compare the filtration efficiency of filter rods with different materials and structures of heated tobacco products (HTPs) for nicotine, visualization technologies such as fluorescence tomography technology and 3D image reconstruction are used to obtain the fluorescence images of nicotine distribution in five combined filtering forms. The practical nicotine distribution in the filters was investigated. The results showed that: 1) Nicotine fluorescence imaging had a high degree of similarity to the internal channel structure of the filter, and this detection technology was universal across different filter materials. 2) The total fluorescence intensity of nicotine increased as the number of puffs increased. There was a positive correlation between the total fluorescence intensity and the nicotine retention. 3) Nicotine was not uniformly distributed across the radial section of the filters and was influenced by many factors, including the internal channel structure. 4) The structural combination of the heated tobacco product influenced the nicotine filtration efficiency of the independent filtering section. 5) Most of nicotine was retained by the filter material that first came into contact with the smoke, and the distribution of nicotine along the direction of smoke flow was uneven. This detection method can be used as a qualitative or semi-quantitative detection method for nicotine retention and for the quick comparison of nicotine filtration efficiency between various filters.

**Notes**

## STPOST 35

### Determination of aerosol mass and five primary constituents in aerosols generated from heated tobacco products

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In 2021, CORESTA conducted a proficiency study for the determination of aerosol mass, propylene glycol, glycerin, and nicotine in aerosols generated from heated tobacco products (HTPs) to determine and recommend a suitable method to measure these components. Due to most laboratories using similar methodology based on ISO 24199 (CORESTA Recommended Method No. 84) and ISO 20778 (intense puffing regime), the study was considered to be a proficiency and a collaborative study. However, the study did not include the determination of two additional primary HTP aerosol constituents, water and menthol. Our efforts focused on expanding the method scope to include the two additional constituents. The sample trap was modified to include a Cambridge pad followed by an impinger to capture the breakthrough of water and menthol, which ranged from 15 % to 30 %. The modified method was validated using a novel heated tobacco capsule (HTC) prototype, which consists of a durable hand-held battery-operated device and a disposable tobacco-containing capsule that is inserted into the device. Method modifications and validation results for the determination of propylene glycol, glycerin, nicotine, water, and menthol from the HTC prototypes will be presented. The mean sample recoveries for all analytes were 86.2 % - 103.0 %. The %RSD of repeatability for each day were = 14.6 % for all samples. The %RSD of intermediate precision over three days was = 9.0 %. These method validation elements all met their pre-determined acceptance criteria. This validation data demonstrates that the modified method is suitable and reliable for measuring aerosol mass and the five primary constituents in aerosols generated by HTPs.

**Notes**

## STPOST 36

### A comparison of HPHCs in on!® PLUS nicotine pouches to HPHCs in cigarettes, smokeless tobacco including snus and an oral NRT product

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on!® PLUS nicotine pouches (on!® PLUS NPs) are smoke-free tobacco products intended for adults aged 21 years or older who use smokeless tobacco (ST) or who dual Use ST and cigarettes. On!® PLUS NPs are a new type of oral tobacco derived nicotine (OTDN) product that has a soft-feel and does not contain cut, ground, powdered or leaf tobacco. To receive market authorization in the United States, the U.S. Food and Drug Administration (FDA) must determine if on!® PLUS NPs are appropriate for the protection of public health (APPH). To inform an APPH determination, we characterized the levels of harmful and potentially harmful constituents (HPHCs) for on!® PLUS NPs.

In this study, we measured HPHCs in on!® PLUS NPs from the FDA ST abbreviated list, recognizing that these products do not meet the statutory definition of a ST product. This approach was considered appropriate as there is no FDA specific guidance for reporting HPHCs for OTDN products. The HPHCs on FDA's ST abbreviated list include nicotine, NNN, NNK, B[a]P, acetaldehyde, formaldehyde, crotonaldehyde, cadmium and arsenic. In this work we compared the ST HPHCs in on!® PLUS NPs to commercially available tobacco products including cigarettes, smokeless tobacco, OTDN pouches and a nicotine replacement therapy product. Nicotine, formaldehyde and acetaldehyde were the only HPHCs that have quantifiable values in on!® PLUS NPs. Except for nicotine, the HPHCs measured in on!® PLUS NPs demonstrated a 91 % reduction compared to traditional smokeless tobacco and 99 % reduction compared to cigarettes. Additionally, a toxicological risk assessment of the HPHCs present at measurable levels in on!® PLUS NPs were below levels of toxicological concern.

**Notes**

## STPOST 37

### Determination of unprotonated nicotine in aqueous extract of snus and nicotine pouches by $^{1}$H NMR spectroscopy

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Under the Comprehensive Smokeless Tobacco Health Education Act, the U.S. Centers for Disease Control and Prevention (CDC) requires reporting of the total nicotine, unprotonated nicotine, pH, and moisture content of smokeless tobacco products. According to the method specified by the CDC in the Federal Register, the percentage of unprotonated nicotine is estimated by substituting the pH of an aqueous suspension and the pKa of nicotine into the Henderson–Hasselbalch equation. This method has been widely used to compare unprotonated nicotine content among various smokeless tobacco products, including nicotine pouches. On the other hand, some researchers were concerned about the effect of other ionic species present in the suspension on the estimate, considering ionic strength influences pKa. In order to investigate this effect, we have developed an accurate method to determine the percentage of unprotonated nicotine in aqueous extracts of snus and nicotine pouches by $^{1}$H NMR spectroscopy.

Unprotonated nicotine standards were prepared by adding acetic acid or sodium hydroxide to nicotine solutions, which were then analyzed by $^{1}$H NMR spectroscopy. The percentages of unprotonated nicotine were 23.4 %–24.1 %, 48.7 %–50.2 %, and 74.1 %–74.8 % for standards with molar ratios of 0.25:1, 0.5:1 and 0.75:1 acetic acid:nicotine, respectively. For smokeless tobacco reference products and commercially available nicotine pouches, aqueous suspensions were prepared according to the CDC method and analyzed after filtration. Standards were individually prepared by adding acetic acid or sodium hydroxide to the extract. A small difference in the percentage of unprotonated nicotine was found between the two methods for each product. That indicated the presence of bias, seemingly caused by using the pKa of nicotine that does not take into account the ionic strength of the extract, however, it had less effect on the estimate than inter-laboratory precision of pH measurements.

**Notes**

## STPOST 38

### Establishment of analytical method for mycotoxins in smokeless tobacco products

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Smokeless tobacco (SLT) such as snus, snuff and chewing tobacco has been considered as a less harmful alternative compared to conventional cigarettes, used as a substitute for cigarettes. SLT was consumed by 350 million people in 133 countries and accounted for approximately $12 billion in the global cigarette market ($785 billion, 2017). However, consumption of SLT has been reported to be associated with many diseases such as oral cancer, pancreatic cancer and heart disease. Aflatoxin B1, frequently contaminate agricultural products, was included as a carcinogen on the list of Harmful and Potentially Harmful Constituents (HPHCs) in smokeless tobacco products by the Food and Drug Administration (FDA). We have developed a LC-MS/MS method for the simultaneous analysing of aflatoxins (B1, B2, G1 and G2) and ochratoxin A by immunoaffinity column chromatography in smokeless tobacco products. The method was validated as linearity, limits of detection, limits of quantification, precision, accuracy and recovery, and applied to CORESTA Reference Product (CRP) and diverse samples of the smokeless tobacco products sold in on-line malls. The established method could be suitable for the management of mycotoxins in smokeless tobacco products.

**Notes**

## STPOST 39

### Non-target analysis survey of tobacco-free nicotine pouches

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In the continuing quest for Reduced Harm Nicotine Delivery products, there has been a dramatic increase in the types of products available to the consumer. Many of these products are not regulated and there is little information available to evaluate the reduced harm potential.

One of these product classes is Tobacco-Free Nicotine Pouches (TFNP) or Modern Oral Nicotine Pouches (MONP). In 2022 we presented results of target compound analysis of a selection of these products. In this work, we have expanded the original scope to include Non-Targeted Analysis of a cross section of flavoured products readily available on the market.

Products of varying flavour descriptions were extracted into ethanol and analysed using GCMS full scan with Mass Hunter deconvolution, followed by spectral matching using the NIST 2017 MS library.

There was not always overlap between products as far as the flavour profiles were concerned. For example, there were four cinnamon flavoured products and, although most contained high levels of cinnamaldehyde, as expected, two of the products also contained significant levels of coumarin and butylated hydroxy toluene (BHT). Furthermore, some compounds found in many of the products at low levels, were found at high levels in other products. Benzyl alcohol, and benzyl salicylate, for example, were observed in low levels across many products (< 2 µg/g) and were found at approximately 400 µg/g in at least one product. Although many of the compounds identified are considered natural flavours, they could be considered at least mild irritants when present at high levels.

Guidelines to determining best practices and product safety should be established, and screening of products in tandem with toxicological evaluation must be performed to ensure the use of these products does, in fact, reduce potential harm.

**Notes**

## STPOST 40

### Comparison of small cigar smoke yields with and without fitment of supplied plastic tip

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Machine smoking cigars is intended to produce product rankings for emissions. Although not representative of human smoking, experimental artefacts suppressing yields impacting rankings must be avoided. Some small cigars/cigarillos have plastic tips at the mouth, and are expected to be used as supplied. Obtaining a leak-tight seal around the tip for analytical machine smoking is a practical difficulty. In such cases it is common practice to remove the tip from the mouth end, potentially changing emission data.

Smoking machine cigar holders can be complex and involve multiple parts and latex sleeves. An alternative universal smoking machine adaptor (USMA) has been developed by the Ohio State University (OSU) that is suitable for a wide range of rigid and compressible tobacco products using a small number of parts and simple compression methodology. This adaptor allows the comparison of a small cigar/cigarillo with and without the presence of the tip. In this study the potential error in reported yields introduced by plastic tip removal before machine smoking small cigars/cigarillos was investigated.

A selected tipped cigarillo was machine smoked, using CORESTA methodology, with tip using the USMA and without tip using both a conventional cigar adaptor and the USMA. Butt termination was 33 mm from the mouth end according to CORESTA methodology. Mass consumed, TPM, puff count and nicotine content were compared. These show a marked difference in emissions. Typical mass of stick consumed using a standard cigar holder is 1.6 grammes whilst using the USMA 2.07 grammes, nicotine yield from the USMA is 16 % higher than the method without tip, puffs 28 % higher, and TPM 20 % greater. Repeatability is comparable for the two holders.

Consumer use without the plastic tip is not credible, removing the plastic tip for machine smoking suppresses reported yields and “rankings” may therefore be compromised.

**Notes**

## STPOST 42

### Survey investigation and methodology results of environmental exposure to heated tobacco products exhaled aerosol in Japan

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Heated tobacco products (HTP) have become increasingly popular in Japan, and reports on environmental exposure to HTP exhaled aerosol have been increasing accordingly. However, there is a limited number of studies that investigated the current status of the exposure. In addition, environmental exposure studies based on surveys analysis include several limitations. For example, respondents' imprecise memory recollection and inaccurate responses. The purpose of this study was to investigate the current status of environmental exposure to HTP exhaled aerosol in detail and to examine the methodology to improve response accuracy. This study was an Internet-based survey conducted on 30,000 non-smokers aged 20 to 69 in Japan. The status of exposure was calculated by providing the proportion of non-smoker individuals around, i.e., bystanders, who reported having the opportunity to inhale the exhaled aerosol from the HTP users, and subsequently classified by place, e.g., home, workplace. The response proportion of tobacco types (HTP, Cigarette, Unknown) to which respondents were exposed, along with the response proportion of exposure initiation time and the inconsistency response proportion for paired similar questions were examined to improve response accuracy. The results indicated that the proportion of exposure was the highest at home (1.96 %) among investigated places. The response proportion of tobacco types was the highest at home (87.7 %). At all investigated places, the response proportion of the exposure initiation time among those who could respond, tobacco types were higher than those who could not. The inconsistency proportions investigated were ranged from 3.8 % to 22.3 %. This study suggested that a survey focusing home where non-smokers can identify tobacco products users easily would be appropriate when investigating the status of environmental exposure to HTP exhaled aerosol, and that identifying inconsistent responses among similar questions would contribute to improving response accuracy.

**Notes**

## STPOST 43

### Association between heated tobacco products use and long-term health effects in Japan: Internet-based cross-sectional study

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With the popularization of heated tobacco products (HTP) in Japan, concern in the health effects of the use of HTPs is growing. However, since HTPs have been on the market for only a few years, evidence of long-term health effects is limited.

In addition, given that most HTP users were former smokers, information on past smoking status in HTP users and health status before HTP use is needed to assess the health effects of exclusive HTP use. Nevertheless, there is no epidemiological research that has obtained such information.

We conducted a cross-sectional study acquiring information on past smoking status and health status of HTP users, to investigate the association of exclusive HTP use with chronic diseases and symptoms that are reported to be associated with smoking (smoking-related diseases/symptoms).

Data were collected by distributing a questionnaire via the Internet to a large survey panel managed by Cross Marketing Inc. Current exclusive HTP users (HTP group), current exclusive cigarette smokers (smoker group), and never-smokers were randomly selected from the survey panel.

Survey responses were obtained from 17,406 participants. The incidences of each disease/symptom were observed both before and after starting HTP use. We found that most of HTP group had a long smoking history, and it particularly affected the incidences of diseases/symptoms related to respiratory system.

Unexpectedly, we noticed that the incidences of several smoking-related diseases/symptoms were higher in the never-smoker group than in the smoker group. We note that these never-smokers associated values are hardly set as endpoints in previous cross-sectional studies concerning HTP.

There results suggest that information on past smoking experience and health status before HTP use should be considered when assessing the health effects of HTP use, and that some smoking-related disease/symptoms may be not suitable as endpoints for the Internet-based cross-sectional study.

**Notes**

## STPOST 44

### Promotional materials for a novel heated tobacco capsule system increase behavioral intentions to try and use in adult smokers but not in nonusers, and do not impede quit intentions

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Heated tobacco products may offer a reduced risk alternative to adults 21+ who smoke cigarettes (AS) and are unable or unwilling to quit. We conducted an online quantitative experimental study evaluating the effect of promotional materials on behavioral intentions for a novel heated tobacco capsule system (HTC) among adults who use and do not use tobacco. The study included 4,511 U.S. adults, who were either current AS, adults who used other tobacco products (OTP), or adult nonusers (NU) who did not currently use any tobacco. The study oversampled for ages 21-24 and included a cohort of underage adults aged 18-20. Participants were randomly assigned to view either the prototype HTC concept with a portfolio of potential promotional materials (Test) or prototype concept only (Control) and completed surveys measuring behavioral intentions. Participants could re-visit the materials throughout the study. AS 21+ who viewed the promotional materials reported significantly higher intentions to try, use and switch to the HTC product than those who did not view the promotional materials. No significant impact on intentions to try and use the HTC product was observed among adults 21-24 and among underage adults who used tobacco. Both NU 21+ and underage adult NU reported low intentions to try and use the HTC product, and exposure to promotional materials did not impact intentions. The promotional materials did not impact quit intentions among AS or OTP for any age group. These results suggest that, in context of the proposed promotional materials, the novel HTC product may facilitate transition of AS 21+ to a smoke-free future, while having minimal impact on NU and underage individuals.

**Notes**

## STPOST 45

### Promotional materials for a novel heated tobacco capsule system do not alter risk perceptions

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Heated tobacco products may offer a reduced risk alternative to adults 21+ who smoke cigarettes (AS) and are unable or unwilling to quit. We conducted an online quantitative experimental study evaluating the effects of promotional materials on risk perceptions for a novel heated tobacco capsule system (HTC) among adults who use and do not use tobacco. The study included 4,511 U.S. adults, who were either current AS, adults who used other tobacco products (OTP), or adult nonusers (NU) who did not currently use any tobacco. The study oversampled for ages 21-24 and included a cohort of underage adults aged 18-20. Participants were randomly assigned to view either the prototype HTC concept with a portfolio of potential promotional materials (Test) or prototype concept only (Control) and completed surveys measuring risk perceptions. Participants could re-visit the materials throughout the study. Adults who used tobacco (AS and OTP) and NU accurately perceived using HTC as less risky than using cigarettes or dual use, and riskier than using nicotine replacement therapies or quitting all tobacco. Exposure to promotional materials did not affect relative risk perceptions of HTC (except in OTP group). Across seven health outcomes related to tobacco use, absolute risk perceptions (likelihood of risk) of the HTC ranged from 39-86 % (100 % = extremely likely) and were unaffected by exposure to promotional materials. Absolute risk perceptions were higher among NU compared to tobacco users. These results suggest that, in the context of the proposed promotional materials, tobacco users and NU appropriately perceive that the novel HTC is not risk-free. Appropriate understanding of risks may facilitate the transition of AS 21+ to HTC, leading towards a smoke-free future.

**Notes**

## STPOST 46

### Measuring tobacco product experience: CROM adapted from the mCEQ for the assessment of new tobacco products

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Originally referred to as the Smoking Effects Inventory (SEI), the self-reported measurement instrument commonly known as the modified Cigarette Evaluation Questionnaire (mCEQ) assesses the reinforcing effects of smoking cigarettes. Like other legacy instruments, the mCEQ has been employed to evaluate the subjective effects of new tobacco product (NTP) use, and further adapted per regulatory recommendations for measures of Tobacco Product Perception and Intention (TPPI) study constructs, i.e., Consumer Reported Outcome Measures (CROM). The objectives of this review were to investigate the development pathway of the mCEQ along with the reported psychometric properties of CROM adapted from the mCEQ for NTP assessment: oral nicotine products (ONP), heated tobacco products (HTP), and electronic nicotine delivery systems (ENDS). Concepts/domains, items, and measurement properties of the Product Evaluation Scale (PES), the Tobacco and Nicotine Product Experience Questionnaire (ToNiPEQ; aka the ABOUT-Product Experience), the mCEQ-C, the mCEQ-E, the mCEQ-N, and the Modified E-Cigarette Evaluation Questionnaire (MECEQ) were extracted and reviewed. Here we provide a unique historical perspective and comparative overview of the mCEQ and subsequent adapted CROM, outlining strengths and limitations inherited from this legacy instrument. The reviewed CROM offer a broad range of options to evaluate the reinforcing effects of using NTP. Key considerations for selection and utilization of optimal CROM to measure subjective effects of NTP use are discussed. In the harm reduction context, appropriate psychometric CROM have the potential to capture critical insights concerning the consumer journey (i.e., from product initiation to product adoption, transition and switching trajectories between products). Characterizing the elements that play a role in delivering a fulfilling and genuinely satisfying product experience could further contribute to evidence generation for regulatory engagement.

**Notes**

## STPOST 47

### Systematic review of the design and conduct of actual use studies of new tobacco products

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Submission of a Premarket Tobacco Product Application (PMTA) is required for manufacturers seeking a Marketing Granted Order (MGO) from the US Food & Drug Administration (FDA) Center for Tobacco Products (CTP). PMTA assessment of the public health impact of new tobacco products (NTP) must include evidence on how adult consumers actually use the products. Actual Use Studies (AUS) have been conducted to gather information on how NTP would affect the use behavior of current tobacco users in their natural environments. Given there are no detailed formal guidance or a widely accepted methodological framework for executing NTP AUS, PMTA applicants could build upon design elements and principles of actual use trial performed in support of switching prescription (Rx) to over-the-counter (OTC) drugs. A systematic review extending back to 2010 was performed to identify NTP AUS conducted by manufacturers with the main goal of delineating key design and methodological elements between studies, and outlining design parameters specific to NTP compared to the approach generally adopted for OTC drug AUS. Included studies were those of any design that captured tobacco use behavior among adult participants using Test Products and any other tobacco products *ad libitum*, under real-world conditions (e.g., at home), utilizing Consumer Reported Outcome Measures (CROM) from the same participants over time. Similarities and differences of included NTP AUS records were summarized according to methodological designs (e.g., sampling frame, study phases and duration, endpoints), operational procedures (e.g., enrolment strategy, Test Products management), data capture methods (e.g., diary, recall period), and data analysis approach. NTP manufacturers have carefully tailored AUS design parameters to study objectives towards evidence generation for scientific substantiation. Novel design elements could provide further insights concerning the consumer journey, such as the role flavors may play in a fulfilling product experience, and further support regulatory engagement.

**Notes**

## STPOST 48

### Study design of a post market surveillance pilot study on reduced risk tobacco and nicotine products

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In recent years there has been a proliferation of alternative tobacco and nicotine products that reduce consumers' exposure to harmful substances and therefore have the potential to reduce risk to health. Post-market surveillance (PMS) enables the evaluation of newly introduced tobacco and nicotine products (aka reduced risk products (RRPs)) at a population level. An epidemiological cross-sectional pilot study was designed to develop a questionnaire as a PMS tool. The main objectives of the questionnaire are to investigate tobacco and nicotine consumer demographics, use behavioural patterns, and characterise behavioural trends as transitions between RRPs and other nicotine products. These behavioural aspects, in conjunction with the intrinsic risk of the product, are essential for assessing the potential health effects and establishing a population risk assessment.

PMS data was collected using a self-administered study instrument that consists of three modules, each focused on a RRP; e-cigarettes, oral nicotine pouches and heated tobacco products.

A two phase approach was implemented; Development and a Pilot Study in Switzerland and Belgium. The development phase included concept elicitation and cognitive interviewing to identify appropriate questions and establish a robust PMS tool. Once developed, the envisaged approach was piloted. Participants must be regular users of the respective RRP to be enrolled into the study and to complete the appropriate questionnaire module. The targeted sampling size is 300 participants per module. The 3-module style questionnaire will be piloted in Switzerland and Belgium (where only the Oral Nicotine Pouches module will be piloted).

Together with the development phase and the pilot study, results and outcomes from this work ensured the development of a questionnaire that fulfilled the key PMS objectives; description of RRP behaviour, estimation of prevalence data and product-specific risk perception measurement to allow for a comprehensive assessment of the effect of introducing RRPs into a market.

**Notes**

## STPOST 49

### Design of a compact connected puffing topography instrument for real-world measurements of e-cigarette use behaviour

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Puffing topography studies are essential for understanding use behaviour and informing standard puffing regimens for the assessment of e-cigarette emissions data. Previous studies have variously used video recordings or topography analysers designed for smoking and adapted for vaping in lab-based studies. A few portable e-cigarette instruments are available, but they may affect the natural puffing behaviour of e-cigarette users. Here we report the concept and design of an e-cigarette-specific topography measurement device that incorporates a commercial pod-type e-cigarette (ePen3; Nicoventures, London, UK) within a compact 3D-printed polyamide housing. The housing contains four circuit boards and six sensors, including a miniature gas flow sensor (FLS110; Flusso™, Cambridge, UK), to record puffing parameters (duration, volume, flow rate), temperature, pressure, and humidity, among other parameters. The device automatically records these data when the user initiates a vaping session by pressing the power button. The puffing data are stored locally on an SD card and are transferred via the mobile 2G network to an IoT platform, when connected to the charger, providing device and communications management (Arm Pelion, Glasgow, UK). Subsequently, the data are transferred to the server of an analytics platforms (Zizo, Milton Keynes, UK), which provides data management and produces real-time dashboards and reports. The analytics platform also delivers questionnaires and notifications to users of JAK via a smartphone application and collates the data. This compact instrument will enable the recording of real-world use-behaviour data, which will provide accurate assessments of an e-cigarette user's exposure and may be used to inform standard puffing regimens for product regulation.

**Notes**

## STPOST 50

### Data gap analysis and proposal for environmental chemical risk assessment of cigarette butts

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The environmental impact of cigarette butts (CB) is a public concern with increasing scientific and media attention. Limited studies on the potential ecological risk have led to policy proposals for banning cigarette filters or classifying them as a microplastics source and Hazardous Waste with potential Extended Producer Responsibility and tax implications. These considerations correlate with growing concerns over chemicals in general, with > 50 % of all currently registered chemicals classified as hazardous to human health and 30 % as hazardous to the environment. This study reviews the toxic potency of CB and their environmental levels to propose a method for estimating ecological risk focusing on aquatic species (the most sensitive to chemicals in general). Regulatory values on Predicted No Effect Concentrations (PNECs) of chemicals in CB were compared with the concentrations published in fresh and sea water. When the environmental concentrations were below the PNECs, it was concluded that there was no ecological risk.

Ecotoxicological studies and data on environmental exposure scenarios of the thousands of chemicals in CB are scarce. This is in accordance with observations on chemicals by environmental regulatory agencies. However, when using well characterized PNECs (for nicotine and cotinine), an ecological risk was calculated only in a few freshwater ecosystems, with small water volume and high human population. Similar or higher risks are estimated for other common substances (e.g., caffeine, household chemicals), and much higher risks for human and veterinary pharmaceuticals.

There is a high variability and uncertainty for estimating the chemical concentrations in individual environments at local or regional levels. These chemicals may be prioritized using nicotine as a surrogate marker and considering their environmental fate. It is hypothesized that the release from CB is low when compared with other anthropogenic and natural sources. Overall, there is very limited or no ecological risk for the best characterized chemicals.

**Notes**

## STPOST 51

### Application of the human health risk assessment process for the evaluation of electronic cigarettes

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Electronic cigarettes (e-cigarettes) heat a nicotine-containing e-liquid that is aerosolized and inhaled by the consumer. Compared to the smoke produced from combustible cigarettes, e-cigarettes typically produce significantly lower levels of inhaled toxicants. The aerosol mixture delivered to the consumer from an e cigarette contains several components, such as e-liquid ingredients, thermal decomposition/reaction products, and device-derived materials. Exposure to this inhaled mixture is not without health risks that must be evaluated. The human health risk assessment process provides a systematic approach to evaluate the potential adverse effects associated with exposure to chemical compounds and can provide essential qualitative and quantitative health risk information to support a weight of evidence evaluation of tobacco products such as e-cigarettes. However, a comprehensive risk assessment framework for evaluating e-cigarettes has not previously been developed due to the complex nature of the aerosol mixture, variability of toxicological data for the inhaled ingredients (e.g., nicotine, excipients, and flavoring compounds), thermal decomposition/reaction products (e.g., harmful and potentially harmful constituents and non-targeted analytes), and device-derived materials (e.g., leachables), as well as a lack of accepted standards. The objective of this work is to propose a suitable and pragmatic risk assessment process that can be adopted to evaluate the health effects potentially caused by exposure to e-cigarette aerosol mixtures. The framework presented here considers the variability in toxicological data and toxicological prioritization for each aerosol component to incorporate appropriate analytical characterization methods, tools for hazard identification and dose-response assessment, best practices for exposure estimation, and regulatory standards for quantitative and/or qualitative risk characterization approaches. Overall, with consideration of other sources of nonclinical data, a systematic, weight-of-evidence risk assessment process is established for the whole aerosol. This comprehensive framework is the first to be presented for any tobacco product and can be utilized to support risk assessment standardization, product development, regulatory submissions, and inform regulatory decisions.

**Notes**

## STPOST 52

### Chemical, pharmacological, and toxicological assessment of 6-methylnicotine

CHEETHAM A.G.(1); PLUNKETT S.E.(1,2); MARKING S.(2); CAMPBELL P.(2); COFFA B.G.(2); GILLILAND III, S.(2); McFADDEN L.(1); SCIAN M.(1)

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*(2) Consilium Sciences, Richmond, VA, U.S.A.*

There is interest in nicotine-related alkaloids for both recreational use and pharmaceutical applications such as smoking cessation and central nervous system disorders conditions such as Parkinson's, Tourette's, and ADHD. Nicotine is one of many alkaloids produced by the tobacco plant (*Nicotiana tabacum* species) and more recently synthesized for commercial use. The compound 6 methylnicotine (CAS# 101540-79-8) has been identified as a nicotine analogue of interest based on its chemical structure, sensorial properties, and commercial availability. Chemical, pharmacological, and toxicological assessments were conducted on 6-methylnicotine and compared to pharmaceutical grade (S)-nicotine. Samples of 6-methylnicotine analysed included both freebase and salt forms, as well as in e-liquid formulations containing propylene glycol (PG) and vegetable glycerin (VG) for use in an electronic nicotine delivery system (ENDS). Chemical analysis confirmed the sample was 6 methylnicotine, racemic, and ~98 % pure utilizing $^{1}$H NMR, chiral UPLC UV, and GC-MS, respectively. The aerosol transfer efficiency of 6-methylnicotine was similar to that of nicotine (82.5 ± 2.9 % vs. 85.6 ± 0.6 % for freebase forms). The QSAR computational pharmacology of 6 methylnicotine is similar in potency and binding affinity to that of (S)-nicotine in *in vivo* and *ex vivo* models. Conventional *in vitro* toxicology testing (Neutral Red and Ames) demonstrated 6 methylnicotine salt e-liquid formulations have similar cellular cytotoxicity and mutagenicity to the analogous (S)-nicotine salt e-liquid formulation. The totality of available evidence indicates that 6 methylnicotine has comparable chemical, pharmacological, and toxicological properties to the more widely used nicotine.

**Notes**

## STPOST 53

### Effect of ion source type and extractor lens diameter on sensitivity and peak shape of semivolatile organic compounds (pyridine, quinoline, and styrene) in gas chromatography/mass spectrometry analysis using hydrogen carrier gas

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*Japan Tobacco Inc., Scientific Product Assessment Center, 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

Owing to the recent worldwide shortage and unstable supply of helium (He), the use of alternative carrier gases (hydrogen ($H_2$) and nitrogen ($N_2$)) for gas chromatography (GC) analysis has been drawing global attention. As an alternative carrier gas for GC with mass spectrometric detection (GC/MS) using electron impact ionization, $H_2$ is reported to be superior to $N_2$ in terms of sensitivity and separation; however, its use tends to cause reactions between $H_2$ and analytes in the ion source. To reduce this effect, it is recommended to use a larger diameter extractor lens in the MS ion source. The Hydroinert source, which was released by Agilent in 2022 for exclusive use with $H_2$, has a 9 mm lens by default.

The purpose of this study was to evaluate how the type of ion source and lens diameter affect the sensitivity and peak shapes in GC/MS analysis of semivolatile organic compounds such as pyridine, quinoline, and styrene when using $H_2$ as carrier gas. Data for three lens diameters (3, 6, and 9 mm) were acquired for a conventional extractor source and the Hydroinert source, and sensitivity (signal-to-noise ratio) and peak shape were evaluated. Peak tailing that was observed using the extractor source was improved by using the Hydroinert source and good peak shape was obtained. The smallest lens diameter (3 mm) showed better sensitivity than the recommended larger diameter lens.

This study showed that the Hydroinert source is effective in improving peak shape and that the sensitivity varies depending on the lens diameter. The effect of lens diameter may differ depending on the analyte.

**Notes**

## STPOST 54

### Rapid and easy removal or purification of pyridine compounds by phenylboronic acid solid-phase extraction (PBA-SPE) cartridge for compound identification for non-targeted gas chromatography/mass spectrometric analysis of tobacco products

SHIGETO A.

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In non-targeted gas chromatography (GC)/mass spectrometric analysis of tobacco products, all components in a sample can be qualitative target analytes. Although compound identification requires obtaining pure peaks and their mass spectra, separation on a GC column can be difficult, as trace components often overlap with other high-content components such as nicotine, propylene glycol, and glycerol. In such cases, sample pretreatment can be effective for removal of such high-content components and for purification of target trace components.

In this study, we present a pretreatment method to selectively remove or purify pyridines (including nicotine) and polyols using a solid-phase extraction (SPE) cartridge filled with phenylboronic acid (PBA)-functionalized silica nanoparticles. PBA is known to form stable covalent bonds to polyols; however, its adsorption and desorption of pyridines has not been fully investigated. Therefore, we evaluated the performance of PBA-SPE using 20 pyridine analogues as models.

Aprotic solvents, such as methyl t-butyl ether (MTBE), were considered appropriate washing solvents for the PBA-SPE surface because they eluted non-pyridine compounds—including other *N*-heterocycles (pyrazines and pyrroles)—and retained pyridines almost quantitatively. On the other hand, protic solvents, such as methanol, eluted most pyridines quantitatively and were considered as suitable elution solvents.

Next, MTBE condensate of 1R6F smoke was applied to PBA-SPE, which was then washed by MTBE. Nicotine, propylene glycol, and glycerol were eliminated from the original sample and some trace compounds that have overlapping peaks on a GC column were revealed. Also, the PBA-SPE retaining polyols and pyridines of 1R6F smoke was eluted by methanol, and 34 trace pyridine peaks that overlapping with those of other matrix compounds were purified.

PBA-SPE pretreatment can be an effective method for removal or purification of pyridines and polyols from a complex tobacco matrix.

**Notes**

## STPOST 55

### Assessing nicotine pharmacokinetics of new generation tobacco products and conventional cigarettes: a systematic review and meta-analysis

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New generation tobacco products (NGPs) are potential reduced-risked alternatives for smoking if they are comparable to conventional cigarettes (CCs). This study aims to evaluate nicotine pharmacokinetics (PK) of NGPs, including closed pod systems, refillable e-cigarettes (ECs), and heated tobacco products (HTPs), compared with CCs through systematic review and meta-analysis. A comprehensive search was performed in three international databases (PubMed, Embase and Web of Science) for articles published from January 1st, 2013, to April 30th, 2023. Maximum nicotine concentration ($C_{max}$), time to the peak concentration ($T_{max}$), and total nicotine exposure (area under the concentration-time curve, AUC) were extracted to assess nicotine PK. We treated NGPs as three subgroups and conducted random effects meta-analyses to calculate pooled standardized mean difference (SMD) with 95 % confidence interval (CI) to compare PK profiles between each subgroup and CCs, respectively. A total of 19 articles with 1,735 participants were included. $C_{max}$ and AUC were significantly lower for closed pod systems ($C_{max}$: SMD -1.05, 95 % CI [-1.52, -0.58]; AUC: SMD -1.32, 95 % CI [-1.77, -0.87]), refillable ECs ($C_{max}$: SMD -1.00, 95 % CI [-1.72, -0.28]; AUC: SMD -1.29, 95 % CI [-2.42, -0.16]), and HTPs ($C_{max}$: SMD -0.68, 95 % CI [-0.94, -0.41]; AUC: SMD -0.95, 95 % CI [-1.21, -0.69]), than those of CCs. $T_{max}$, however, was discovered to be statistically similar for closed pod systems (SMD 0.38, 95 % CI [-0.47, 1.24]), refillable ECs (SMD -0.00, 95 % CI [-0.61, -0.60]), and HTPs (SMD 0.02, 95 % CI [-0.48, 0.53]), to that of CCs. No difference was observed when examining PK between each type of NGPs versus CCs. All three types of NGPs delivered less nicotine than CCs but reached $T_{max}$ over a similar period of time, indicating that NGPs are likely to assist smokers in craving relief and even smoking cessation.

**Notes**

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**SMOKE SCIENCE and PRODUCT TECHNOLOGY Conference**

# SYMPOSIUM

## New Approach Methods (NAMs)-II: Applications in Tobacco Regulatory Sciences

10 October 2023

# New Approach Methods (NAMs) Symposium-II:
# Applications in Tobacco Regulatory Sciences

Dear SSPT 2023 participants,

It is a great pleasure to introduce the New Approach Methods (NAMs) Symposium-II, in a beautiful city of Cancun in Mexico. This year's Symposium is the follow-up from the first Symposium held during the SSPT2021 Conference online, titled "Advancing New Alternative Methods for Tobacco Harm Reduction"[1] , where external experts presented the latest case examples where NAMs were used to screen and even waive *in vivo* studies in regulatory applications.

The term NAM is no longer a new vocabulary to many CORESTA participants! If I offer a general definition, NAMs are *in vitro* and in silico or computational-based methodologies in toxicology that enable clinically relevant toxicological risk assessment without needing traditional animal testing. In two years since SSPT2021, the interest and need to utilize NAMs in tobacco science become ever more relevant, with many different categories of smoke-free alternative products being introduced worldwide. Albeit they are developed to be substantially less toxic than conventional cigarettes, currently no consensus is available on how to evaluate these emerging products, where we believe that some of available NAMs offer ways to expedite toxicological screening and decision making.

In this Symposium, we have invited expert toxicologists from industry to share their case examples of applying *in vitro*- and *in silico*-based NAMs to address some of immediate gaps in product screening and assessment (NAM-TODAY; toxicity screening). We then expand into long-term applications of NAMs linking *in vitro* outcomes to clinically relevant disease progression (NAM-TOMORROW; COPD as an example). We have a total of six excellent talks from NAM experts, also the panel discussion at the end, inviting active participation from all attendees.

The Symposium is endorsed by the CORESTA Scientific Commission, the 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGTX) Task Force, the Biomarkers (BMK) Sub-Group, and the *In Vitro* Toxicity Testing (IVT) Sub-Group, reflecting CORESTA's ongoing commitment to the 3Rs (reduce, replace, and refine animal testing).

Thank you and we look forward to seeing you all!

K. Monica Lee, PhD, DABT
Symposium Chair

[1] Toxics | Free Full-Text | Advancing New Approach Methodologies (NAMs) for Tobacco Harm Reduction: Synopsis from the 2021 CORESTA SSPT–NAMs Symposium (https://www.mdpi.com/2305-6304/10/12/760)

## TUESDAY 10 OCTOBER 2023

# New Approach Methods (NAMs) Symposium-II: Applications in Tobacco Regulatory Sciences

Chair: K. Monica LEE
Co-Chair: Liam SIMMS

Starting at 8:50

| No. | Time | Titles | Lead, Affiliation |
|---|---|---|---|
| **NAM 00 Intro** | 10 min. | **Introduction: New Approach Methods (NAMs): Applications in Tobacco Regulatory Sciences** | K. Monica LEE (Chair)<br>*Altria Client Services* |
| **Session I: NAM Today** | | | |
| **NAM 01** | 15 min. +<br>2 min. Q&A | **The use of NAMs in *in vitro* genotoxicity assessment of nicotine delivery products: ToxTracker assay as a case study** | Robert LEVERETTE<br>*RAI Services Company* |
| **NAM 02** | 15 min. +<br>2 min. Q&A | **Connecting exposure, dosimetry and toxicity responses in the preclinical evaluation of ingredients: case examples of flavoring chemicals in oral tobacco products** | Jingjie ZHANG<br>*Altria Client Services* |
| **NAM 03** | 15 min. +<br>2 min. Q&A | **Exposure to fresh whole smoke and aerosols: standard and novel (3D) *in vitro* models** | Liam SIMMS<br>*Imperial Brands* |
| **BREAK – 10 minutes** | | | |
| **Session II: NAM Tomorrow (NAMs without *in vivo* – clinical adverse outcomes)** | | | |
| **NAM 04** | 15 min. +<br>2 min. Q&A | **EpiAirway Nrf2 – oxidative stress model: Practical application of *in vitro* systems with clinical relevance** | Brian KEYSER<br>*RAI Services Company* |
| **NAM 05** | 15 min. +<br>2 min. Q&A | **COPD AOP-I: quantitative modeling of *in vitro* data using an adverse outcome pathway for the assessment of decreased lung function risk in humans** | Marja TALIKKA<br>*Philip Morris International* |
| **NAM 06** | 15 min. +<br>2 min. Q&A | **COPD AOP-II: *In vitro* assessment of mucus hypersecretion with quantitative AOP modeling** | Shigeaki ITO<br>*Japan Tobacco Inc.* |
| **Session III: Panel Discussion** | | | |
| | 20 min. | **NAMs – Applications in Tobacco Regulatory Sciences – Gaps and Opportunities** | Liam SIMMs (Co-Chair)<br>*Imperial Brands*<br>All speakers & panelists |
| **Total: ~140 minutes** | | | |

# NAM 00

## INTRODUCTION: New Approach Methods (NAMs): Applications in Tobacco Regulatory Sciences

LEE K.M.(1) – Chair; SIMMS L.(2) – Co-Chair

*(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
*(2) Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, U.K.*

*Dr. K. Monica Lee, Ph.D., D.A.B.T., is an Associate Fellow in Regulatory Sciences at the Altria Client Services LLC. Dr. Lee leads the Biological Science Insights group, identifying, building and utilizing innovative and pragmatic in vitro and in vivo toxicological evaluation approaches in support of product development, regulatory compliance and scientific engagement. She has extensive experiences in regulatory and foundational nonclinical studies and published numerous peer-reviewed articles on the topics of reduced-risk disease models, comparative and mechanistic toxicology, in vitro-to-in vivo extrapolation and kinetic modeling. Prior to joining ALCS in 2015, she served as the BioScience Director at the JT International and Sr. Toxicologist at Battelle Toxicology Northwest and Pacific Northwest National Laboratories. Her PhD is on Pharmacology/Toxicology from the University of Georgia. Dr. Lee builds and promotes the use of in silico and in vitro-based predictive toxicological tools in support of tobacco regulatory science, proactively engaging with CORESTA members and external partners across research community. She was the chair for the first NAM symposium during SSPT-2021, leading from the concept to execution and post-symposium peer-reviewed pubilcation.*

## NAM 01

### The use of NAMs in *in vitro* genotoxicity assessment of nicotine delivery products: ToxTracker assay as a case study

BREHENY D.(1); LEVERETTE R.(2)

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*(2) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.*

Genotoxicity and cytotoxicity assessments are an indispensable component in the safety assessment of tobacco and nicotine delivery products. They also form a cornerstone of the preclinical component of regulatory dossiers. In the case of genotoxicity, since no single test is capable of detecting all relevant genotoxic endpoints, a battery of *in vitro* testing techniques has been recommended by CORESTA. This comprises the Ames assay for gene mutation and the micronucleus (MN) assay or mouse lymphoma assay. Whilst the importance and usefulness of these assays are not in question, they are not without limitation. Traditional *in vitro* tests lack throughput, provide little mechanistic information, and have poor specificity in predicting *in vivo* genotoxicity. A number of NAMs have been developed in recent years to address these gaps in the current testing strategies. For example, the *in vitro* MN assay by flow cytometry (MicroFlow™) has greatly increased the throughput above the traditional version of the assay, also providing information on clastogenicity or aneugenicity. Other new assays such as the ToxTracker assay have emerged, that can both increase throughput and give additional insight into the mode of action of genotoxic compounds. The ToxTracker assay, which is based on a panel of 6 reporter-gene cell lines, has been shown to be predictive of both the Ames assay and *in vitro* micronucleus assay for single toxicants. Extensions of the ToxTracker assay protocol; ToxTracker ACE and ToxTracker AO, can be used to determine if a positive compound is aneugenic or clastogenic, and whether the DNA damage is direct or indirect via oxidative stress. The predictivity of the ToxTracker assay, coupled with mechanistic insights, means that it has potential to be used in a screening capacity and/or as a follow up to *in vitro* positives in traditional genotoxicity assays. Here we present a case study describing the use of the ToxTracker assay in the context of the assessment of cigarettes and heated tobacco products (HTPs) as an example. Findings from our industry will be discussed, along with recommendations of how this assay could be used in a toxicological risk assessment framework.

*Dr. Robert Leverette is currently a Master Scientist in the Modern Oral Submissions Team at RAI Services Company. In his previous role in the Non-Clinical Studies Division at RAI Services Company, he conducted regulatory toxicological studies on next generation tobacco and nicotine products for regulatory applications, and developed whole aerosol techniques and approaches. He graduated with a PhD in Biochemistry from North Carolina State University, focusing on the processing of the U14 small nucleolar RNA from the introns of the mouse cognate heat shock gene.*

## NAM 02

### Connecting exposure, dosimetry and toxicity responses in the preclinical evaluation of ingredients: case examples of flavoring chemicals in oral tobacco products

ZHANG Jingjie

*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

Flavoring ingredients that are GRAS ("generally recognized as safe") in food are commonly used in oral tobacco derived nicotine (OTDN) products. While the GRAS status is not *de facto* approval for the use in oral tobacco products, the accompanying toxicological information is relevant and useful in the safety evaluation of OTDN products, considering the similarity of how the products are consumed. In addition to available regulatory limit, a weight of evidence of all available nonclinical and clinical information is used to assess the suitability of using flavoring chemicals in OTDNs and the potential health effects of the products. For some GRAS ingredients, specific *in vitro* and *in vivo* toxicity outcomes sometimes present apparently different responses – for instance, cinnamaldehyde, a common flavor in oral consumer products is known to induce positive *in vitro* genotoxicity; however, these *in vitro* hazard findings do not lead to *in vivo* sequalae based on negative long-term carcinogenicity outcomes. In this talk, we have investigated the dosimetry basis for these apparently different *in vitro* versus *in vivo* genotoxicity and carcinogenicity outcomes using cinnamaldehyde as an example flavor in OTDN products. PBPK modelling and *in vitro* to *in vivo* extrapolation (IVIVE) approaches are used to estimate the equivalent human daily exposures (EADs) and to evaluate *in vitro* toxicity findings in the *in vivo* context. Using open-source PBPK models, we estimated the $C_{max}$ in the target organ (e.g., plasma and liver) of cinnamaldehyde under *in vivo* (rodent and human) exposure conditions and compared the estimated doses to the *in vitro* exposure ranges for cytotoxicity and genotoxicity findings. We also compared the estimated EADs from nonclinical testing to the likely use levels in human use and discuss the estimated margin of exposure in the context of known toxicological profiles of the ingredients. Using the case example, we demonstrate the relevance and opportunity of incorporating target tissue dosimetry consideration as part of nonclinical toxicity evaluation and risk assessment.

*Dr. Jingjie Zhang, Ph.D., D.A.B.T., is a principal scientist in Regulatory Sciences at the Altria Client Services LLC. Dr. Zhang has managed and lead preclinical in vivo studies since she joined the Biological Science Insights group in 2016. She also investigates applications of new approach methodologies (NAMs) to product development and regulatory complicnce for porential reduced-harm tobacco products. Prior to the current position, Dr. Zhang served as a senior scientist in the Sensomics and Analytical group at Altria Client Services LLC, and applies her knowledge and skills in aerosol science to aid in the design and characterization of inhaled products. Her PhD is on Energy, Environmental, and Chemical Engineering from Washington University of St. Louis, focusing on particle physics, aerosol instrumentation and in vivo inhalation drug delivery.*

## NAM 03

### Exposure to fresh whole smoke and aerosols: standard and novel (3D) *in vitro* models

POUR S.J.(1); SIMMS L.(2)

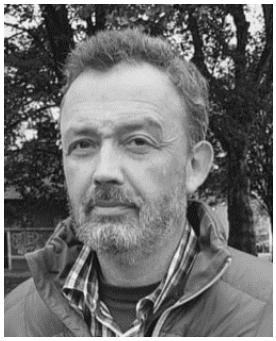
*(1) Reemtsma Cigarettenfabriken GmbH (an Imperial Brands PLC Company), Albert-Einstein-Ring 7, 22761 Hamburg, Germany*

*(2) Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, U.K.*

New Approach Methods (NAMs) describe non-animal- based methodologies that enable more human relevant toxicological risk assessments. In this talk, regulatory *in vitro* toxicology assays (CORESTA battery) are refined for NGP (Next Generation Product) testing of fresh aerosols to assess the Tobacco Harm Reduction (THR) potential of Electronic Vapour Products (EVP) and Heated Tobacco Products (HTP) compared to a reference cigarette (1R6F). Imperial's SAEIVS (Smoke and Aerosol Exposure *In Vitro* System) was used to expose cells to fresh aerosol/smoke for cytotoxicity and genotoxicity (NRU, IVM) assessment. Mutagenicity testing (Ames) was performed via bubbling of a bacterial suspension with the Vitrocell VC10 smoking robot. Using these *in vitro* systems, fresh aerosol generated by EVP and HTP were markedly less toxic than combustible cigarette smoke.

Building on these findings, *in vitro* 3D tissue models of the human upper respiratory tract (MucilAir™, Epithelix) were repeatedly exposed to whole smoke/aerosol at the air-liquid Interface (ALI) for 4 weeks, using SAEIVS. Although the EVP and HTP delivered up to 25 times more nicotine compared to the diluted cigarette smoke, minimal effects were observed for the assessed endpoints (Cytotoxicity, cilia function, barrier integrity, histology, and pro-inflammatory markers). In contrast, smoke from the reference cigarette caused significant changes across all the endpoints in a puff dependent manner. These results align with the cytotoxic and genotoxic results from the regulatory toxicology assays described above.

These results demonstrate that NAMs such as those described above can be used to provide a deeper understanding of the potentially reduced biological effects of NGP compared to cigarettes.



*Dr. Liam Simms has a PhD from Durham University in the UK and has worked for two contract Toxicology laboratories conducting toxicity studies, before joining Imperial Brands in 2002 currently in the role of Principal Toxicologist. Whilst at Imperial Dr. Simms has worked in various toxicology related roles, having lead the Product Strewardship Team for several years and working on Imperials TT21C programme to introduce new in vitro techniques with a focus on the use of human cells where possible, in the area of potential harm reduction. Dr. Simms published extensive peer-reviewed articles and currently serves as the joint Coordinator for the CORESTA In Vitro Toxicity Testing (IVT) Sub-Group and the 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGTX) Task Force.*

## NAM 04

### EpiAirway Nrf2 – oxidative stress model: Practical application of *in vitro* systems with clinical relevance

KEYSER B.M.(1); WERTMAN J.(1); HOLLINGS M.(2); JORDAN K.(1)

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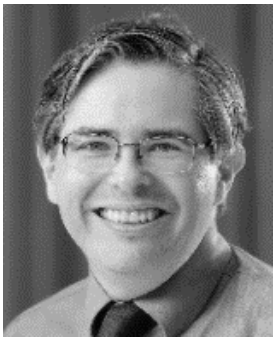
*(2) Labcorp Early Development Laboratories Ltd, Otley Road, Harrogate, Harrogate HG3 1PY, U.K.*

The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway regulates genes that contribute to the antioxidative stress response. This pathway is involved in respiratory diseases, including chronic obstructive pulmonary disease (COPD). Cigarette smoke has been shown to activate this pathway in lung. Here, we evaluated the effects of whole smoke/aerosol from two marketed combustible (nonmenthol and menthol) cigarettes (CC), two smoke-free alternative products: glo™ (heated tobacco product, HTP), and Vuse Alto® (electronic nicotine delivery system, ENDS) on cell viability and Nrf2 response in a 3D human airway model (EpiAirway™) transfected with a luciferase Nrf2 promoter. EpiAirway™ tissues were exposed at the air liquid interface to whole smoke or aerosol generated on a Vitrocell® VC10®. Whole smoke/aerosol doses were controlled using dilution airflows of 0.5 to 8 L/min for CC, and undiluted to 4 or 5 L/min for HTP or ENDS, respectively. Eighteen hours post-exposure, luciferase activity and cell viability were measured. Relative luciferase activity was expressed as fold change over the air exposed control. Post-exposure, whole smoke/aerosol deposition was quantified using chemical analysis (e.g., glycerol, nicotine, carbonyls).

Differential Nrf2 activation was observed following exposure to CC whole smoke compared to the tested HTP and the ENDS. Maximum Nrf2 fold increase occurred at the undiluted dose for the HTP and ENDS versus 3 L/min for the CC. Moreover, the minimum exposure-correlated nicotine concentration required to induce a > 2-fold increase (threshold response) in Nrf2 activation was > 30× and > 100× lower for CC than the HTPs and ENDS, respectively.

These data indicate that the tested HTP and ENDS induce significantly lower oxidative stress than the CC. Additionally, the 3D Nrf2 EpiAirway™ *in vitro* model can be used to assess and discriminate responses of a biomarker (oxidative stress) relevant to smoking-related disease pathways (e.g., respiratory and cardiovascular disease).



*Dr. Brian Keyser is currently a Senior Manager in the Global Clinical Studies Division at RAI Services Company. In his previous role in the Non-Clinical Studies Division at RAI Services Company, he developed next generation in vitro lung and oral models for potentially reduced harm products and regulatory applications. He graduated with Ph.D. in Pharmacology from Tulane University in New Orleans focusing on the role of T-type voltage gated calcium channels in diabetes mellitus.*

## NAM 05

### COPD AOP-I: quantitative modeling of *in vitro* data using an adverse outcome pathway for the assessment of decreased lung function risk in humans

TALIKKA M.; ISKANDER A.

*Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*

Adverse outcome pathways (AOPs) organize mechanistic knowledge based on empirical evidence into a sequence of cell, tissue, and organ level events that lead to an adverse outcome (AO) in humans. An AOP initiates from the interaction of a stressor with a biomolecule in a target cell, also known as molecular initiating event (MIE), progress through a series of intermediate, causally linked events termed key events (Kes) and culminate in an AO. AOP 411 is one of five AOPs connecting exposure-related oxidative stress with lung function impairment. It considers a slowing down of cilia beating frequency (CBF) following inhalation exposure and the subsequent decrease in mucociliary clearance (MCC) in the lungs.

To evaluate the potential risk of impaired lung function associated with tobacco heating system (THS) use compared with smoking, we mapped data from a series of *in vitro* studies on THS aerosol exposure, employing advanced tissue culture models. The mathematical modeling predicted a reduced risk of decreased CBF based on measurement of oxidative stress parameters and reduced risk of decreased MCC based on CBF measurement in THS aerosol- compared with cigarette smoke-exposed cultures. Finally, modeling using clinical MCC and FEV1 data predicted a 79.3 % reduced risk of decreased lung function in HTP switchers compared with smokers.

In summary, combining our *in vitro* data with publicly available clinical data allowed us to approximate the residual risk of decreased lung function in THS switchers relative to continuing smokers. Considering the causality between active smoking, reduced lung function, and COPD, the approach provides a plausible prediction of reduced COPD risk in THS switchers compared with continuing smokers.

Dr. Marja Talikka is the head of translational science at PMI. She joined the company in 2009 as Scientist exploring the conversion of biological information into computable networks, setting the foundation for the generation of a publicly available suite of network models capturing the biology of a multitude of biological processes. She also participated in a joint project with BAT and PETA to create an AOP network for decreased lung function, which is directly applicable to the assessment of smoke-free products and has since been utilized by others in the industry. More recently, Dr. Talikka has led efforts to establish an in-silico approach to the quantification of one of these AOPs to achieve meaningful risk assessment without animal experimentation. Dr. Talikka holds a PhD in biochemistry from the University of Helsinki. Her educational journey comprised research conducted at the London regional Cancer Center in Ontario, Rockefeller University in New York, and Swiss Institute for Experimental Cancer Research in Lausanne before joining PMI.

## NAM 06

### COPD AOP-II: *In vitro* assessment of mucus hypersecretion with quantitative AOP modeling

ITO S.; ERAMI K.; ICHIKAWA S.; MURATANI S.; MORI A.; YOSHINO K.

*Scientific Product Assessment Center, Japan Tobacco Inc., Yokohama, Kanagawa, Japan*

The Adverse Outcome Pathway (AOP) framework is a valuable tool for understanding the mode of action of a stressor. While traditionally used to supplement chemical risk assessment, this concept could also be useful in disease-related toxicological risk assessment. To this end, we developed *in vitro* test methods as an original version of AOP148 (AO: lung function decrease) to serve as a reference for key events. Through repeated exposure of 3D-cultured bronchial epithelial cells to whole cigarette smoke, we successfully observed goblet cell hyperplasia and mucus hypersecretion. However, since the AOP framework is based on qualitative mode of action, one needs to translate AOP into quantitative model to enable risk assessment process. To address this, we developed quantitative AOP (qAOP) models using Bayesian formalism. These models allow us to evaluate the probability of adverse outcomes occurring based on *in vitro* test results. In this talk, we will demonstrate that although data generation with next-generation-inhalable products is still ongoing, our approach holds promise for relative risk assessment among tobacco products, effectively highlighting the differences in disease risk between combustible cigarettes and NGPs. We will also discuss the importance of investigating clinical relevance, including exposure alignment between *in vitro* and *in vivo*, to enhance the reliability of our risk assessment framework.

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*Dr. Shigeaki Ito is leading a research project aimed at consolidating various biological assessment results obtained from in vitro, in vivo, and clinical studies to improve the interpretation of the Reduced-Risk (RR) potential of Next Generation Products. His primary responsibility now involves developing novel methodologies, including computational models and advanced in vitro test methods, to establish a clinically relevant approach for assessing the RR potential.*

*Dr. Shigeaki Ito obtained his Ph.D. in Agriculture from Hokkaido University in 2010, where he conducted research on anaerobic microorganisms and their characteristic enzymes. Since joining Japan Tobacco Inc. In 2010, he has consistently been in charge of conducting in vitro toxicological assessments and developing a risk assessment approach using in vitro test methods and computational modeling.*

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**SMOKE SCIENCE and PRODUCT TECHNOLOGY Conference**

# SYMPOSIUM

## Consumer Reported Outcome Measures: Science Starts with Measurement: Essential Measurement Science for Self-Report in Tobacco and Nicotine Product Research

11 October 2023

## WEDNESDAY 11 OCTOBER 2023

# Consumer Reported Outcome Measures (CROM) Symposium

## Science starts with measurement: essential measurement science for self-report in tobacco and nicotine product research

Chair: Stacey McCAFFREY
Co-Chair: Mohamadi SARKAR

Starting at 14:20

| No. | Time | Titles | Lead, Affiliation |
|---|---|---|---|
| **CROM 01** | 20 min. | **Overview of the psychometric CROM guidelines** | Stacey McCAFFREY<br>*Juul Labs, Inc.* |
| **CROM 02** | 20 min. | **Overview of psychometrics: the science of measurement** | Stacey McCAFFREY<br>*Juul Labs, Inc.* |
| **BREAK – 5 minutes** | | | |
| **CROM Guest** | 20 min. | **Risk perceptions in tobacco regulatory science** | Alexander PERSOSKIE<br>*FDA Center for Tobacco Products* |
| **CROM 03** | 20 min. | **Evaluating the psychometric properties of a CROM for use with a different product for which it was developed: the modified e-cigarette evaluation questionnaire (MCEQ) as a case study** | Meghan MOREAN<br>*Yale School of Medicine* |
| **CROM 04** | 20 min. | **Further validation of the ABOUT-Dependence measure: Extending assessment of perceived dependence on tobacco and nicotine products to users of a heated tobacco product (*IQOS*)** | Esther AFOLALU<br>*Philip Morris International* |
| **CROM 05** | 20 min. | **An illustration that one size may not fit all: assessing invariance of the WISDM scale in PATH across youth and young adult cohorts** | Ryan BLACK<br>*Juul Labs, Inc.* |
| **CROM 06** | 20 min. | **Can individuals with limited health numeracy use quantitative scales to make ratings of risk perceptions?** | Saul SHIFFMAN<br>*Pinney Associates* |
| **BREAK – 10 minutes** | | | |
| | 20 min. | **Discussion and Q&A** | Mohamadi SARKAR<br>*Altria Client Services*<br>All speakers |
| **Total: 175 minutes** | | | |

## CROM 00 – Introduction

### Science starts with measurement: essential measurement science for self-report in tobacco and nicotine product research

McCAFFREY(1); MOREAN M.(2); AFOLALU E.(3); BLACK R.(1); SHIFFMAN S.(4); SARKAR M.(5)

*(1) Juul Labs Inc., 1000 F Street NW, Washington DC 20004, U.S.A.*
*(2) Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510, U.S.A.*
*(3) Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchatel, Switzerland*
*(4) Pinney Associates, Inc., 4800 Montgomery Ln #400, Bethesda, MD 20814, U.S.A.*
*(5) Altria Client Services LLC, 601 E Jackson St, Richmond, VA 23219, U.S.A.*

Consumer reported outcome measures (CROM) are a critical component of tobacco regulatory science. Examples of CROM include measures of tobacco product dependence and risk perceptions. Like other fields of science, behavioral researchers require instruments (i.e., CROM) that are reliable and valid for measuring a given construct (e.g., risk perceptions), to ensure accuracy and replicability of measurement, and to support the validity of study conclusions. Researchers must also consider the context in which a CROM is applied, and whether a CROM valid for use in one context is valid in another. Such concerns are the focus of psychometrics, the science of behavioral measurement.

The CORESTA CROM Task Force (TF) is charged with establishing best practices and guidelines pertaining to the use of CROM in tobacco research. This symposium, organized by the CROM TF, will begin by introducing the TF's guidelines regarding the identification, development, validation, and implementation of CROM (McCaffrey). An overview of psychometrics will be provided and the importance of considering CROM psychometric functioning when used in a different context from which it was developed will be discussed (McCaffrey). To illustrate this point, two speakers (Morean and Afolalu) will present the application of CROMs to different products (e-cigarettes and IQOS) and the languages from which they were developed. Next, Black will demonstrate how invariance testing can be used to evaluate CROM functioning when used with different populations and discuss the consequences when such testing reveals non-invariance. Shiffman will present an approach for evaluating the appropriateness of numerical rating scales for individuals with limited numeracy to determine whether scores from such scales are comparable across those with limited vs. adequate numeracy. The discussant (Sarkar) will elaborate on the analogy between the methods used to establish inferences from clinical outcome measures compared to those used for self-reported measures.

# CROM 01

## Overview of the psychometric CROM guidelines

McCAFFREY S.

*Juul Labs, Inc., Washington, DC, U.S.A.*

This presentation will delve into the primary deliverable of the CORESTA Consumer Reported Outcome Measures (CROM) Working Group 02 (WG02) - guidelines and best practices with respect to Psychometric CROM for use in tobacco and nicotine product (TNP) research. After reviewing the definition of Psychometric CROM, noting differences between Psychometric and Descriptive CROM, the presentation will provide examples of Psychometric CROM commonly used in the TNP space. Next, WG02's consensus-based approach for guideline development will be described, which included literature review and consultation with subject matter experts. After providing context for the guidelines, including their purpose, scope, and intended audience, the presentation will cover the content of the guidelines. This content includes: an exercise for identifying the ideal characteristics of a Psychometric CROM within the context of the research study, recommendations for when and how to collect psychometric evidence to support a modified Psychometric CROM (based on the type and extent of CROM modifications), steps for developing and validating a new Psychometric CROM, and recommendations related to the implementation and interpretation of a Psychometric CROM. Finally, the presenter will share the status of the guidelines, including the publication/dissemination plan, and provide contact information for any audience members who are interested in learning more.

*Dr. Stacey McCaffrey is a Psychometrician at JUUL Labs, Inc. where she leads the experimental behavioral research program. Dr. McCaffrey specializes in both qualitative and quantitative psychometric research methodologies, and has developed and validated measures of behavioral intentions, risk perception, claim comprehension, and respiratory symptoms for use in research as part of tobacco product applications to the FDA.*

*Prior to her work at JUUL Labs, Dr. McCaffrey was extensively involved in NIH and industry-funded research efforts targeting the opioid epidemic through Inflexxion, Inc. These efforts included development of a computer adaptive testing version of the Addiction Severity Index, as well as brief screening tools for adults who may be at risk for opioid misuse. As a consultant at PatientsLikeMe, Inc. she also led Robert Wood Johnson Foundation funded research to better understand patient priorities in healthcare, and developed several patient-reported outcome instruments to measure global and disease-specific health-related quality of life.*

*Dr. McCaffrey is leading the CORESTA consumer reported outcome measures (CROM) working group 02 (WG02) along with Esther Afolalu (PMI). She received her PhD in Clinical Psychology from Nova Southeastern University (Florida, USA) in 2015. Dr. McCaffrey is also a licensed clinical psychologist.*

# CROM 02

## Overview of psychometrics: the science of measurement

McCAFFREY S.

*Juul Labs, Inc., Washington, DC, U.S.A.*

Consistent with other areas of science, behavioral science requires objective measurement that is precise, replicable, and measures what it is intended to measure. When measuring behavioral constructs such as dependence or risk perceptions, it is critical that the researcher has reliable and valid tools. Psychometrics is the field of science concerned with the psychometric functioning (reliability, validity) of these self-report measuring tools, which we refer to as "consumer reported outcome measures" (CROM).

The Psychometric CROM guidelines developed by CORESTA CROM Working Group 02 (WG02) present best practices for the selection, development and validation, modification, and implementation of Psychometric CROM for use in research on tobacco and nicotine products (TNPs). It is important that a behavioral researcher working in the area of TNPs has basic knowledge of foundational psychometric concepts in order to appreciate and utilize these guidelines most effectively. This presentation will review basic psychometric properties, such as types of reliability and validity, and present examples of both qualitative and quantitative methodologies for evaluating psychometric functioning of a new, modified, or existing CROM. For example, cognitive debriefing interviews will be reviewed as a qualitative strategy to evaluate and improve content validity of a CROM, and confirmatory factor analysis will be discussed as a quantitative strategy to evaluate measurement invariance across product categories or populations. Additionally, the presentation will cover psychometric considerations when identifying an appropriate CROM, such as evaluating the match between an existing CROM's psychometric functioning within a particular context of use and the psychometric properties of greatest importance within the context of the study. Finally, psychometric considerations when implementing a single or multiple CROM (i.e., when multiple CROM are combined into a survey) in a research study will be discussed.

## CROM 03

### Evaluating the psychometric properties of a CROM for use with a different product for which it was developed: the modified e-cigarette evaluation questionnaire (MCEQ) as a case study

MOREAN M.

*Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510, U.S.A.*

The Cigarette Evaluation Questionnaire, and its revised version, the Modified Cigarette Evaluation Questionnaire (MCEQ), have been used for decades to assess the subjective reinforcing and aversive effects of cigarette smoking. While not all measures are good candidates for translation for use with a different product(s), the MCEQ's items appeared relevant to e-cigarette use. Thus, we aimed to examine the psychometric properties of the Modified E-cigarette Evaluation Questionnaire (MECEQ).

In Summer 2021, 857 adults completed an anonymous online survey (52.4 % male; 40.84 [12.25] years old; 62.8 % non-Hispanic white; 22.4 % daily e-cigarette use). Analyses included exploratory/confirmatory factor analyses to confirm the original structure and/or identify alternate latent structure(s), internal consistency, measurement invariance, between-group differences, and relationships with vaping outcomes.

The results showed that the original five-factor structure and a novel four-factor structure were supported. All multi-item subscales were internally consistent. Both the five-factor and four-factor versions reached scalar invariance across multiple participant subgroups, could detect between-groups differences, and were associated with past-month vaping frequency and dependence.

The results strengthen the interpretability of previously published work using the five-factor structure and provide an alternative, psychometrically-sound, four-factor scoring approach. Future research is needed to evaluate invariance between the MCEQ and MECEQ before subjective effects of smoking and vaping can be compared directly.

*Dr. Meghan Morean completed her undergraduate training at Brown University (2004), her PhD in clinical psychology from Yale University (2011), and her postdoctoral fellowship at the Yale School of Medicine (2011-2014). She then was employed at Oberlin College (2014-2020), earning tenure as an associate professor. While at Oberlin, she maintained her affiliation with Yale (adjunct assistant professor). In Summer 2020 she returned to the Yale University School of Medicine. Dr. Morean's program of research focuses on youth and adult use of nicotine/tobacco products, cannabis, and alcohol. She has expertise in measurement development and has contributed numerous substance-relevant measures to the field.*

## CROM 04

### Further validation of the ABOUT-Dependence measure: Extending assessment of perceived dependence on tobacco and nicotine products to users of a heated tobacco product (*IQOS*)

AFOLALU E.

*Philip Morris Products S.A. (part of Philip Morris International), Quai Jeanrenaud 5, Neuchâtel, Switzerland*

The ABOUT™–Dependence is a Consumer Reported Outcomes Measure (CROM) developed to assess perceived dependence associated with tobacco and/or nicotine product (TNP) use. It was developed and validated in US English for TNP users who were either exclusive or poly users of different TNPs (including cigarettes, e-cigarettes, cigars/cigarillos, smokeless tobacco, pipes, and waterpipes) and nicotine replacement therapy (NRTs). The initial validation confirmed the psychometric performance of a 12-item version of the CROM consisting of three domains: Extent of Use (2 items); Signs and Symptoms (5 items); and Behavioral Impact (5 items).

This presentation will describe additional research conducted to assess the validity of the CROM when applied to heated tobacco products (HTPs) use (specifically, *IQOS*) and to evaluate its cross-cultural validity. The research included analyses of data collected as part of an online survey with 1320 adult TNP users in the US, Germany, Italy, Russia, and Japan who were cigarette smokers or who had switched from smoking cigarettes to using either HTPs (*IQOS*) or other TNPs (e.g., e-cigarettes) exclusively or were dual users of cigarettes and another TNP. The presentation will outline the psychometric techniques (Rasch Measurement Theory (RMT) and Classical Test Theory (CTT)) used to confirm the measurement properties of the CROM in an extended frame of reference. The findings largely aligned with the previous validation of the CROM and provided supportive evidence of the CROM's reliability and validity for the whole sample (N = 1320) and a subset of exclusive *IQOS* users (N = 263). The instrument's stability across different countries/languages was also acceptable, although differential item functioning (DIF) by country/language was observed for some items.

Overall, the research further supports the ABOUT™–Dependence as a psychometrically valid measure of perceived dependence with broad cross-cultural applicability to different types of TNPs and product use behaviors.

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*Esther F. Afolalu, PhD, is a Senior Behavioral Scientist at Philips Morris International, where she provides technical leadership for behavioral research projects, including the development, validation, and implementation of the ABOUT™ Toolbox portfolio of Consumer Reported Outcomes Measures) (CROMs) for assessment of consumer perception and behaviors related to the use of tobacco and nicotine products. She is additionally involved in the CORESTA CROM Task Force as a co-coordinator of the Psychometric CROM Working Group.*

*Her research expertise spans the fields of Behavioral Science, Clinical and Health Psychology, Population Health, Outcomes Research, and Epidemiology. She holds a PhD in Psychology (University of Warwick, UK) and her doctoral research focused on multi-methodological experimental and observational epidemiological studies on the associations of sleep disturbances with pain and health outcomes. She also has past experience in conducting pharmaceutical clinical trials, specifically in areas such as psychopharmacology.*

## CROM 05

### An illustration that one size may not fit all: assessing invariance of the WISDM scale in PATH across youth and young adult cohorts

BLACK R.A.(1); SHIFFMAN S.(2); HANNON M.J.(2)

*(1) Juul Labs, Inc., Washington, DC, U.S.A*
*(2) Pinney Associates, Inc., Pittsburgh, PA, U.S.A.*

Measuring dependence on ENDS in youth and adults is of interest, as dependence bears on continued use of such products. Dependence is an abstract, complex concept whose measurement can be highly context-specific. Typically, different scales are used to measure dependence in youth and adults. For example, 6 items from the adult Wisconsin Inventory of Smoking Dependence Motives (WISDM) scale are used to measure dependence in the PATH youth survey, while the 16-item Tobacco Dependence (TD) Index, which also includes some WISDM items, is used in the PATH adult survey. Some researchers have assessed dependence on ENDS using a single scale in a combined sample of youth and adults, without confirming that the scale is *functioning* equivalently across the age cohorts. As an illustration, we used psychometric analyses to assess the equivalence of the WISDM scale in a sample of PATH youth (15-17 years) and young adults (18-34 years). Factorial invariance of the WISDM scale was assessed across youth and young adult cohorts via confirmatory factor analysis (CFA). Data were obtained from youth (N=229) and young adult (N=761) respondents to PATH Wave 5 Youth and Adult surveys who reported current (past 30-day), exclusive ENDS use. In CFA-based invariance testing, the WISDM scale did not function equally across age cohorts, indicating that comparing or combining youth and young adult dependence scores may be problematic. The findings reinforce that one cannot *assume* that a seemingly identical scale can be used to measure dependence on ENDS in different age cohorts. A proper psychometric analysis should be undertaken to assess whether the scale is functioning equivalently. Not doing so risks drawing inaccurate conclusions regarding levels of dependence, which could lead to ill-advised public health policy.

*Dr. Ryan Black is a clinical and research psychologist with expertise in psychometrics, statistical methods and population health impact modeling. He currently works in the regulatory science department at Juul Labs, Inc., serving as VP of Clinical and Population Sciences. Dr. Black previously led the population science team in the regulatory science department at Altria. As part of his role, he led the PHIM work and presented the ALCS PHIM at various venues, including TPSAC, CORESTA, FDLI and academic institutions. Dr. Black has held the position of Director of Biostatistics and Methodology at Inflexxion, Inc., in which he served as co-PI/lead biostatistician on several NIH-funded behavioral research grants. During this time, Dr. Black developed and executed the data analytic strategy used to evaluate abuse of prescription opioid products as part of broader postmarket surveillance programs in support of regulatory engagement by various prescription opioid manufacturers. Dr. Black has published extensively in the areas of psychometric methods, substance use and tobacco regulatory research.*

## CROM 06

### Can individuals with limited health numeracy use quantitative scales to make ratings of risk perceptions?

SHIFFMAN S.(1); HANNON M.J.(1); McCAFFREY S.(2)

*(1) Pinney Associates, Inc., Pittsburgh, PA, U.S.A.*
*(2) Juul Labs, Inc., Washington, DC, U.S.A.*

Assessment of risk perceptions of lower-risk nicotine products such as electronic nicotine delivery systems (ENDS) is an important area of regulatory research. Psychometric challenges emerge when attempting to estimate and quantify their risk perceptions. The ability of individuals with limited numeracy skills to use numerical (quantitative) rating scales has been questioned. We report an online study using numerical ratings of risk perception obtained from individuals with varied numeracy. Participants were 12,557 adults including smokers, dual users, former tobacco users and never-users who viewed information about JUUL, and who were randomized to see or not see a reduced-exposure message. (Neither tobacco use status nor message exposure are part of the psychometric analyses.) Participants completed the Newest Vital Sign (NVS), which asks respondents to interpret a Food Facts label containing quantitative information, and requiring quantitative reasoning and computation. The NVS has been used to assess numeracy as well as literacy. The NVS classified 29 % of participants as having limited health numeracy ("LHN"). Using numerical scales from 0 % to 100 % harmful to health (in 10 % increments), participants rated the overall risk of harm from using JUUL, and the likelihood (0-100 %) of suffering four specific diseases (mouth cancer, lung cancer, heart diseases, respiratory disease). They also rated the risk of using JUUL using a commonly-used ordinal qualitative (descriptive) response scale (4-point scale from not at all harmful to very harmful). The psychometric properties of the numerical ratings were similar for LHN as for those with adequate health literacy (AHN). The numerical ratings of JUUL risk in the two groups showed a nearly identical orderly relationship to the descriptive perceived risk ratings, with very similar means at each qualitative level. In analyses considering the disease-specific ratings as a scale, results demonstrated equivalence between ratings from LHN and AHN individuals. The correlations among ratings of the four diseases were nearly identical for LHN as for AHN participants. Moreover, in analyses based on confirmatory factor analysis, both groups showed robust fit to a one-factor model. Tests of invariance between LHN and AHN found the scale to demonstrate configural, metric, and scalar invariance between AHN and LHN. Thus, analyses show that individuals with limited numeracy skills were able to make meaningful ratings using numeric scales, comparable to those obtained from respondents with adequate numeracy.

*Saul Shiffman, Ph.D. serves as Senior Scientific Advisor at Pinney Associates, which consults to JUUL Laboratories on e-cigarettes and tobacco harm reduction. He is also Emeritus Professor of Psychology (Clinical and Health Psychology), Psychiatry, Pharmaceutical Sciences, and Clinical Translational Science at the University of Pittsburgh.*

*Dr. Shiffman has published over 450 scientific papers on topics including smoking patterns, nicotine dependence, smoking cessation and relapse, smoking cessation treatment, e-cigarette use, and tobacco harm reduction, as well as on measurement and research methods. His papers have received over 50,000 citations in the scientific literature. Dr. Shiffman has received a number of awards, including the Society for Research on Nicotine and Tobacco's award for "ground-breaking advances in clinical research."*

*Dr. Shiffman has been involved in a number of regulatory submissions to the US Food and Drug Administration's Center for Tobacco Products, and presented at the Tobacco Products Scientific Advisory Committee, as well as other FDA Advisory Committees.*

## CROM Guest

### Risk perceptions in tobacco regulatory science

PERSOSKIE A.(1); O'BRIEN E.(2)

*(1) Supervisory Social Scientist, Division of Population Health Science, Office of Science, FDA Center for Tobacco Products, U.S.A.*
*(2) Supervisory Social Scientist, Division of Research and Evaluation, Office of Health Communication and Education, FDA Center for Tobacco Products, U.S.A.*

**CORESTA SSPT2023 CONFERENCE**

**SMOKE SCIENCE and**
**PRODUCT TECHNOLOGY**

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## CORESTA SSPT2023 CONFERENCE

# SMOKE SCIENCE and PRODUCT TECHNOLOGY

# PROGRAMME SUMMARY

### Abbreviations

**GENERAL**
**SG** ..... Sub-Group
**TF** ..... Task Force

**SMOKE SCIENCE STUDY GROUP**
**BMK** ..... SG Biomarkers
**CROM** ..... TF Consumer Reported Outcome Measures Consortium
**IVT** ..... TF *In vitro* Toxicity Testing
**NGTX** ..... TF 21st Century Toxicology for Next Generation Tobacco and Nicotine Products
**PUB** ..... SG Product Use Behaviour
**SA** ..... SG Smoke Analysis

**PRODUCT TECHNOLOGY STUDY GROUP**
**CSM** ..... SG Cigar Smoking Methods
**EVAP** ..... SG E-Vapour
**HTP** ..... SG Heated Tobacco Products
**NPSQ** ..... TF Nicotine Pouches Safety and Quality Guidance
**PTM** ..... SG Physical Test Methods
**TTPA** ..... SG Tobacco and Tobacco Products Analytes

## SMOKE SCIENCE & PRODUCT TECHNOLOGY

## SUB-GROUP / TASK FORCE MEETINGS

| | Saturday, 7 October | | | Sunday, 8 October | | | | Wed, 11 Oct | |
|---|---|---|---|---|---|---|---|---|---|
| *Meeting Rooms* | *Yucatán* | *Mérida* | | *Yucatán* | *Mérida* | *Querétaro* | | *Guadalajara* | *Meeting Rooms* |
| 8:00 | | | | | | | REGISTRATION | | 8:00 |
| 8:30-10:30 | **TF HTP** 8:30-12:30 | **SG BMK** 8:30-12:30 | | **SG TTPA** 8:30-12:30 | **TF NGTX** 8:30-12:30 | **TF CROM** 9:00-11:30 | | | 8:30-10:30 |
| **10:30-10:50** | | | | | | | | | **10:30-10:50** |
| 10:50-12:30 | | | REG-ISTRA-TION | | | | REGISTRATION | | 10:50-12:30 |
| **12:30-14:00** | LUNCH | | | LUNCH | | | | LUNCH | **12:30-14:00** |
| 14:00-15:40 | **SG EVAP** 14:00-18:00 | **SG PUB** 14:00-18:00 | | **SG SA** 14:00-18:00 | **SG CSM** 14:00-18:00 | **SG IVT** 14:00-18:00 | | **TF NPSQ** 14:00-18:00 | 14:00-15:40 |
| **15:40-16:00** | | | | | | | | | **15:40-16:00** |
| 16:00-18:00 | | | | | | | | | 16:00-18:00 |
| | | | | 19:00 -22:00 | | | | | |
| | | | | **Welcome Reception** | | | | | |

# SMOKE SCIENCE & PRODUCT TECHNOLOGY

| Mtng Room | Sat 7 Oct | Sun 8 Oct | Monday 9 October | Tuesday 10 October | Wednesday 11 October | Thursday 12 October | Mtng Room |
|---|---|---|---|---|---|---|---|
| | Guadalajara | | *Yucatán* | *Yucatán* | *Yucatán* | *Yucatán* | |
| | | | **Chair: CAHOURS** | **Chairs: LEE KM / SIMMS** | **Chair: YOSHINO** | **Chair: BAXTER-WRIGHT** | |
| (08:30 - 08:50) | | 08:00 | OPENING SESSION | **NGTX TF Report** - SIMMS | BIOLOGICAL ASSESSMENT | PRODUCT USE BEHAVIOUR | (8:30 - 8:50) |
| 8:30 - 8:50 | | | **Welcome** - CAHOURS | NAM SYMPOSIUM | **IVT SG Report** - SIMMS | **PUB SG Report** - PRASAD | 8:30 - 8:50 |
| 8:50 - 9:10 | | REGISTRATION | **Invited speaker:** **POLOSA R** - *University of Catania* | **NAM00** - LEE KM / SIMMS - *Altria / Imperial* | **ST19** - WIECZOREK - *Reemtsma* | **ST29** - WEI Lai - *Altria* | 8:50 - 9:10 |
| 9:10 - 9:30 | | | | **NAM01** - LEVERETTE - *RAIS* | **ST20** - HU Hao - *Shenzhen First Union* | **ST30** - GRAFF - *Cheerain* | 9:10 - 9:30 |
| 9:30 - 9:50 | | | **IG01** - STEVENS / FLORA - *RAIS* / Altria | **NAM02** - ZHANG Jingjie - Altria | **ST21** - OLDHAM - *Juul* | **ST31** - WANG Rui - *CNTC ZTRI* | 9:30 - 9:50 |
| 9:50 - 10:10 | | | **IG02** - FISHER AM - *Uni. Kentucky* | **NAM03** - SIMMS - *Imp. Brands* | **ST22** - CARUSO M - *Uni. Catania* | **ST34** - CAHOURS - *Imperial* | 9:50 - 10:10 |
| **10:10 - 10:30** | | | COFFEE | **NAM04** - KEYSER - *RAIS* | **ST23** - EMMA - *Uni. Catania* | COFFEE | 10:10 - 10:30 |
| | | | *Yucatán* | **NAM05** - TALIKKA - *PMI* | COFFEE | | 10:30 - 10:50 |
| | | | **Chair: JEANNET** | **NAM06** - ITO - *JT* | *Yucatán* | | **10:50 - 11:10** |
| | | | EVAP - AEROSOL ASSESSMENT | **Discussion** | **Chair: SARKAR** | *Yucatán* | |
| | | | **EVAP SG Report** - GILLMAN | | CLINICAL STUDIES 2 | **Chair: JEFFERY** | |
| 10:50 - 11:10 | | | **ST01** - CARTER - *Juul* | | **ST24** - SHIFFMAN - *Pinney Assoc.* | HTP - AEROSOL ASSESSMENT | 10:50 - 11:10 |
| 11:10 - 11:30 | | | **ST02** - SMITH C - *Juul* | | **ST25** - GALE - *BAT* | **HTP SG Report** - DIGARD | 11:10 - 11:30 |
| 11:30 - 11:50 | | | **ST03** - LI Ding - *Shenzhen First Union* | | **ST26** - SCHERER - *ABF* | **ST35** - LANG - *PMI* | 11:30 - 11:50 |
| 11:50 - 12:10 | REGISTRATION | REGISTRATION | **ST04** - BRINKMAN - *Ohio State Uni.* | | **ST27** - WU Bingyu - *CNTC Shanghai Tob.* | **ST36** - WANG Wei - *CNTC* | 11:50 - 12:10 |
| 12:10 - 12:30 | | | **ST05** - LIU Charlene - *RiskWise Solution* | | **ST28** - PLUYM - *ABF* | **ST37** - O'REILLY - *PMI* | 12:10 - 12:30 |
| **12:30 - 14:00** | | | LUNCH | LUNCH | LUNCH | LUNCH | **12:30 - 14:00** |
| | | | *Yucatán* | *Mérida & Querétaro* | *Yucatán* | *Yucatán* | |
| | | | **Chair: PANI** | | **Chair: McCAFFREY** | **Chair: HU Bin** | |
| | | | EVAP - LIQUID ASSESSMENT | | **CROM TF Report** - CAHOURS | TOBACCO PROCESSES | (14:00 - 14:20) |
| 14:00 - 14:20 | | | **ST06** - LI Weibo - *Shenzhen Yupeng* | | CROM SYMPOSIUM | **CSM SG Report** - LINDEGAARD | 14:00 - 14:20 |
| 14:20 - 14:40 | | | **ST07** - LI Weibo - *Shenzhen Yupeng* | | **CROM01** - McCAFFREY - *Juul* | **ST39** - LIU Ze - *CNTC Yunnan* | 14:20 - 14:40 |
| 14:40 - 15:00 | | | **ST08** - LIU Shaohua - *Shenzhen Yupeng* | | **CROM02** - McCAFFREY - *Juul* | **ST40** - BUSSEY - *RAIS* | 14:40 - 15:00 |
| 15:00 - 15:20 | | | **ST09** - LIU Shaohua - *Shenzhen Yupeng* | | **CROM Guest** - PERSOSKIE - *FDA* | **PTM SG Report** - EITZINGER | 15:00 - 15:20 |
| 15:20 - 15:40 | | | **ST10** - LI Ding - *Shenzhen First Union* | | **CROM03** - MOREAN - *Yale School of Med.* | TEA | **15:20 - 16:00** |
| 15:40 - 16:00 | | | **ST11** - OLDHAM - *Juul* | | **CROM04** - AFOLALU - *PMI* | *Yucatán* | |
| **16:00 - 16:20** | | | TEA | | **CROM05** - BLACK - *Juul* | **Chair: EITZINGER** | |
| | | | *Guadalajara* / *Yucatán* | | **CROM06** - SHIFFMAN - *Pinney Assoc.* | CIGARETTE & FILTER DESIGN | |
| | | | **Chair: REDEBY** / **Chair: SARKAR** | | **Discussion** | | |
| 16:00 - 16:20 | | | TOBACCO ANALYSIS / CLINICAL STUDIES 1 | | | **ST43** - RAVERDY-LAMBERT - *SWM* | 16:00 - 16:20 |
| 16:20 - 16:40 | | | **TTPA SG Report** - WAGNER / **BMK SG Report** - SARKAR | | | **SA SG Report** - YAMAZAKI | 16:20 - 16:40 |
| 16:40 - 17:00 | | | **ST12** - XIN Zhongquan - *Smoore* / **ST15** - YUKI - *JT* | | | **ST41** - WANG Xiaofeng - *CNTC Anhui* | 16:40 - 17:00 |
| 17:00 - 17:20 | | | **ST13** - PATRING - *Swedish Match* / **ST16** - VERRON - *Imperial* | | | **ST42** - LINDNER - *Tannpapier* | 17:00 - 17:20 |
| 17:20 - 17:40 | | | **ST14** - McNEES - *Uni. Kentucky* / **ST18** - MAKENA - *RAIS* | | | **ST45** - SUN Xuehui - *CNTC ZTRI* | 17:20 - 17:40 |
| 17:40 - 18:00 | | | **ST44** - FERREIRA - *BAT Brazil* | | | | 17:40 - 18:00 |
| | | 19:00-22:00 | | NETWORKING SESSION | | 20:00 - 23:00 | |
| | | Welcome Recep. | Free Evening | (17:00 - 20:00) | Free Evening | Closing Dinner | |

*Please note that to accommodated presenter schedules, the programme has had to be rearranged and some of the presentation numbers may no longer be consecutive.*

Grateful thanks to the following company
for its generous contributions: