
**SMOKE SCIENCE and PRODUCT TECHNOLOGY**

**Virtual Conference**

18 – 28 October 2021

# PROGRAMME & ABSTRACTS


Version 210919

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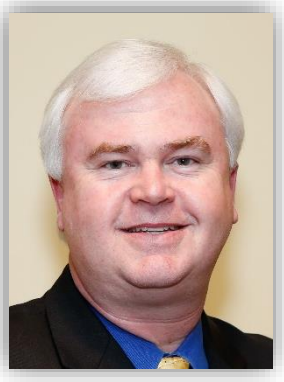
## WELCOME MESSAGE



## PROGRAMME

## ABSTRACTS

# WELCOME MESSAGE



**Rob STEVENS**
**President of the CORESTA Scientific Commission**

Dear Colleagues

Welcome to the 2021 Virtual Conference of the Smoke Science and Product Technology Study Groups of CORESTA. First and foremost, I hope you and your families are healthy and safe. Thank you so much for attending this year's conference. Despite the worldwide limitations on travel and the safety measures in place due to COVID-19, CORESTA is committed to drive forward and provide a forum to allow scientists the opportunity to openly share credible science and best practices related to tobacco and its derived products. I would like to acknowledge and congratulate Stéphane Colard, General Secretariat of CORESTA, and his amazing team for doing an incredible job to make this virtual and "live" conference a reality.

The Smoke Science & Product Technology conference is pleased to have 71 oral presentations that will occur across nine "live" on-line sessions between 18-28 October. In addition, there will be seven presentations given in the Symposium on "Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction". The technical program includes a diverse array of presentations covering numerous topics, such as perception and behavior, nicotine science, analytical methods, product chemistry, biomarkers, statistical modeling, and *in vitro* & *in vivo* toxicology. Science associated with conventional tobacco products such as combustible cigarettes and emerging tobacco products including e-vapor products, heated tobacco products (HTP), nicotine pouches, and waterpipes, will be presented. Each presentation is pre-recorded, with a "live" question and answer session after each presentation. It is our hope that this hybrid format allows participants the opportunity to interact with the presenter giving everyone time for engagement, discussion, and learning.

I am honored to serve as the President of the Scientific Commission and on behalf of the Scientific Commission, I would like to again welcome you to the Smoke Science & Product Technology Virtual Conference. We hope you find your experience both enjoyable and fulfilling.

Sincerely and respectfully

Robert D. STEVENS
RAI Services Company
Winston-Salem, NC, U.S.A.


# PROGRAMME

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

## DAY 1

## MONDAY 18 OCTOBER

### SESSION 1 - Perception and behaviour: understanding how nicotine products are perceived and used

Chair: Xavier CAHOURS

Co-Chair: Paul HARP

*CET Time Zone*

| | | |
|---|---|---|
| 13:30-13:45 | ST 01 | **Development of consumer-reported outcome measure (CROM) standards for the tobacco industry with respect to psychometric CROM using a consortium-based approach: methodology and scope**<br>McCAFFREY S.(1) on behalf of the CORESTA CROM Task Force (WG02): AFOLALU E.(2); BLACK R.(1); CHREA C.(2); CURTIN G.(3); GILES L.(4); NISHIHARA D.(4); PRASAD K.(5); SARKAR M.(6); SHETTY M.(5)<br>*(1) JUUL Labs, Inc., San Francisco, CA, U.S.A.*<br>*(2) Philip Morris Products S.A., Neuchâtel, Switzerland*<br>*(3) RAI Services Company, Winston-Salem, NC, U.S.A.*<br>*(4) JT International S.A., Geneva, Switzerland*<br>*(5) British American Tobacco, Southampton, U.K.*<br>*(6) Altria Client Services, Richmond, VA, U.S.A.* |
| 13:45-14:00 | ST 02 | **Descriptive consumer reported outcome measures and definitions in tobacco and nicotine research: a consensus approach**<br>WEI L.(1) on behalf of the CROM Task Force: CLERC E.(2); SHETTY M.(3); CHREA C.(2); PRASAD K.(3); SARKAR M.(1)<br>*(1) Altria Client Services LLC, Richmond, Virginia, U.S.A.*<br>*(2) Philip Morris International, Neuchâtel, Switzerland*<br>*(3) British American Tobacco, Southampton, U.K.* |
| 14:00-14:15 | ST 03 | **Psychometric validation of new scales expanding conceptual coverage of the ABOUT-Perceived Risk: perceived social risk and perceived practical risk**<br>CLERC E.(1); SALZBERGER T.(2); CANO S.(3); AFOLALU E.(1); CHREA C.(1)<br>*(1) PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*<br>*(2) Institute for Statistics and Mathematics, University of Economics and Business, Welthandelsplatz 1, 1020 Vienna, Austria*<br>*(3) Modus Outcomes, Spirella Building, Letchworth Garden City SG6 4ET, U.K.* |
| 14:15-14:30 | ST 04 | **Risk perceptions and likelihood of use of (tobacco-free oral) nicotine pouches among current, former and never nicotine users**<br>STONE C.; KNIGHT-WEST O.; O'CONNELL G.; TOPE A.<br>*Imperial Brands PLC., Research and Development, 121 Winterstoke Road, Bristol BS3 2LL, U.K.* |
| 14:30-14:45 | ST 05 | **Assessing the impact of next generation products on population health: a population modelling approach**<br>PHILLIPS C.V.(1); KHAKWANI A.(2); O'CONNELL G.(2); CAHOURS X.(2); TOPE A.(2)<br>*(1) epiphi Consulting Group, New Hampshire, U.S.A.*<br>*(2) Imperial Brands PLC., 121 Winterstoke Road, Bristol BS3 2LL, U.K.* |
| 14:45-15:00 | ST 06 | **A statistical methodology integrating resampling techniques to evaluate public health impact after introduction of reduced-risks products in Japan**<br>LARROQUE S.; KUBOTA T.; CHARRIÈRE M.; SONNERAT D.; KIMURA Y.<br>*JT International SA, 8 Rue Kazem Radjavi, 1202 Geneva, Switzerland*<br>*Japan Tobacco, Tokyo, Japan* |

## DAY 1

## MONDAY 18 OCTOBER

### SESSION 2 - Nicotine science: brain and body effects

Chair: Kei YOSHINO

Co-Chair: Xavier CAHOURS

*CET Time Zone*

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|---|---|---|
| 15:30-15:45 | ST 07 | **Nicotine pharmacokinetics of electronic cigarettes: an updated analysis of pooled data from the literature**<br>JACOBSON K.; MARTINEZ J.; LARROQUE S.; JONES I.; PASCHKE T.<br>*JT International SA, 8 Rue Kazem Radjavi, 1202 Geneva, Switzerland* |
| 15:45-16:00 | ST 08 | **Effects of various forms of nicotine delivery on the autonomic nervous system (ANS) and stress hormones**<br>TROFIMOV A.V.(1); BERDNIKOVA N.G.(1,2); ZAGURSKAYA A.V.(3,4); MENSHOV V.A.(1); YABLONSKAYA O.I.(1)<br>*(1) Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow 119334, Russia*<br>*(2) I.M. Sechenov First Moscow State Medical University, Moscow 119991, Russia*<br>*(3) Medical Center AVC, Moscow 127018, Russia*<br>*(4) DNKom Laboratory, Moscow 127018, Russia* |
| 16:00-16:15 | ST 09 | **Inhibitory effect of nicotine on monoamine oxidase B and its action against Parkinson's disease**<br>CAO Yun(1); ZHOU Shun(1,2); WANG Chenghu(1); WANG Peng(1); WANG Xiaofeng(1); ZHANG Yaping(1,2); ZHANG Xiaoyu(1); LI Yanyan(1); GUAN Mingjing(1)<br>*(1) Key Laboratory of Combustion & Pyrolysis Study of CNTC, China Tobacco Anhui Industrial Co., Ltd., 9 Tianda Road, Hefei 230088, China*<br>*(2) Key Laboratory for Tobacco Chemistry of Anhui Province, China Tobacco Anhui Industrial Co., Ltd., 9 Tianda Road, Hefei 230088, China* |

## DAY 2

## TUESDAY 19 OCTOBER

### SYMPOSIUM
### Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction

Chair: K. Monica LEE

Co-Chair: Shannon BELL

| *CET Time Zone* | | **PART 1** |
|---|---|---|
| 13:30-13:35 | **Welcome** | COLARD S.<br>*CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France* |
| 13:35-13:45 | **NAM 00 Intro** | **Advancing new alternative methods for tobacco harm reduction**<br>LEE K.M.(1); BELL S.(2)<br>*(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*<br>*(2) Integrated Laboratory Systems, 601 Keystone Park Drive, Suite 200, Morrisville, NC 27560, U.S.A.* |
| 13:45-14:10 | **NAM 01** | **US federal efforts to develop and implement alternatives to animal testing**<br>KLEINSTREUER N.<br>*NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), U.S.A.* |
| 14:10-14:35 | **NAM 02** | **Application of biokinetic modelling for *in vitro* to *in vivo* extrapolation (IVIVE) in chemical risk assessment**<br>PAINI A.(1); WORTH A.(2)<br>*(1) esqLABs GmbH, Hambierich 34, 26683 Saterland, Germany*<br>*(2) European Commission Joint Research Centre (JRC), Ispra, Italy* |
| 14:35-15:00 | **NAM 03** | **Inhalation exposure modeling for assessing health risks of toxic aerosols and vapors**<br>CORLEY R.A.<br>*Greek Creek Toxicokinetics Consulting (GCTC), LLC, Boise, ID 83714, U.S.A.* |
| *CET Time Zone* | | **PART 2** |
| 15:10-15:35 | **NAM 04** | **Assessing respiratory toxicity of chemicals in two human bronchial *in vitro* systems**<br>STUCKI A.O.<br>*PETA Science Consortium International e.V., Stuttgart, Germany* |
| 15:35-16:00 | **NAM 05** | ***In silico* toxicology as a New Approach Methodology in tobacco regulatory science**<br>VALERIO L.G.<br>*FDA/Center for Tobacco Products, Office of Science, U.S.A.* |
| 16:00-16:25 | **NAM 06** | **Application of mechanistic data in risk assessment: exposure alignment and evidence integration**<br>JARABEK A.M.<br>*U.S. Environmental Protection Agency's Office of Research and Development (ORD), U.S.A.* |
| 16:25-16:45 | **Discussion** | LEE K.M.(1); BELL S.(2)<br>*(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*<br>*(2) Integrated Laboratory Systems, 601 Keystone Park Drive, Suite 200, Morrisville, NC 27560, U.S.A.* |

## DAY 3

## WEDNESDAY 20 OCTOBER

### SESSION 1 - Heated tobacco products: modelling and numerical simulation

Chair: Jutta PANI

Co-Chair: Bin HU

*CET Time Zone*

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|---|---|---|
| 13:30-13:45 | ST 10 | **Modelling and simulation of electromagnetic heating smoking set with multi physical field**<br>BAO Jun(1); HAN Jingmei(2); LI Zhiqiang(2); ZHANG Jianming(3); LEI Ping(2); SHANG Shanzhai(2); YE Bo(1); MA Jun(1); LI Bin(3)<br>*(1) Faculty of Information Engineering and Automation, Kunming University of Science and Technology, Kunming 650500, China*<br>*(2) Technical Centre of Yunnan China Tobacco Industry Co., Ltd, Kunming 650202, China*<br>*(3) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* |
| 13:45-14:00 | ST 11 | **Simulation research on temperature field distribution of electric heating non-combustion cigarette heater**<br>DENG Weiquan(1); LI Zhiqiang(2); HAN Jingmei(2); WANG Le(3); LEI Ping(2); SHANG Shanzhai(2); MA Jun(1); ZHANG Ke(3)<br>*(1) Faculty of Information Engineering and Automation, Kunming University of Science and Technology, Kunming 650500, China*<br>*(2) Technical Centre of Yunnan China Tobacco Industry Co., Ltd, Kunming 650202, China*<br>*(3) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* |
| 14:00-14:15 | ST 12 | **The use of infra-red thermal imaging to evaluate the thermal performance of filtration segments in heated tobacco products**<br>AHMED N.<br>*Essentra Laboratories, Shaftesbury Avenue, Jarrow, Tyne & Wear NE32 3UP, U.K.* |
| 14:15-14:30 | ST 13 | **Numerical simulation of smoke flow field characteristics and temperature distribution of circumferential heated tobacco products**<br>SUN Zhiwei(1); DU Wen(1); WANG Zhiguo(1); WANG Wei(1); CHEN Jingbo(1); LUO Wei(1); WEN Jianhui(1); HUANG Ping(1); YIN Xinqiang(1); ZHANG Mingjian(2); LI Bin(2); HUANG Zhengwei(3); ZHANG Zhang(3); GUI Qinfeng(3); DAI Hongliang(3)<br>*(1) Technology Center, China Tobacco Hunan Industrial Co., Ltd, No. 386 Laodong Road, Changsha 410007, China*<br>*(2) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China*<br>*(3) College of Mechanical and Vehicle Engineering, Hunan University, No. 2 South Lushan Road, Changsha 410082, China* |
| 14:30-14:45 | ST 14 | **Numerical simulation of flow, heat and mass transfer in a heated tobacco stick**<br>JUNG Yongmi<br>*R&D Institute, KT&G Corp., 30, Gajeong-ro, Yuseong-gu, Daejeon, Republic of Korea* |

## DAY 3

## WEDNESDAY 20 OCTOBER

### SESSION 2 - Heated tobacco products: methods

Chair: Jutta PANI

Co-Chair: Bernhard EITZINGER

*CET Time Zone*

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| 15:15-15:30 | ST 15 | **Determination of tobacco specific nitrosamines (TSNAs) in heated tobacco product (HTP) blend by accelerated solvent extraction (ASE) and LC-MS/MS analysis**<br>RODRIGUEZ-LAFUENTE A.; WANG Jiaming; JOZA P.<br>*Labstat international Inc., 262 Manitou Dr., Kitchener, Ontario N2C 1L3, Canada* |
| 15:30-15:45 | ST 16 | **eHTP aerosol generation: TSNA method adjustments and mass balance methodology feasibility**<br>DUROT N.; ROUILLARD S.; RAVERDY-LAMBERT D.<br>*SWM INTL c/o LTR Industries, Usine Le Mans, 72702 Allonnes Cedex, France* |
| 15:45-16:00 | ST 17 | **LC-MS-MS method development for the analysis of tobacco-specific nitrosamines in heated tobacco products aerosols**<br>MULLIN L.; HAMMOND D.; McGUIGAN S.; GIBBONS P.; THOMAS J.<br>*Hall Analytical Laboratories Ltd, Waterside Court, 1 Crewe Road, Wythenshawe, Manchester M23 9BE, U.K.* |
| 16:00-16:15 | ST 18 | **Determination of aromatic amines in mainstream and sidestream cigarette tobacco smoke and heated tobacco products**<br>ZIERLINGER M.; JAINDL I.; PUMMER S.; KUBA M.; BAMMER S.; MAYER-HELM B.<br>*ÖKOLAB Gesellschaft für Umweltanalytik Ges.m.b.H., Japan Tobacco International, Hasnerstraße 127, 1160 Vienna, Austria* |
| 16:15-16:30 | ST 19 | **Substantial reductions in selected harmful or potentially harmful constituents in heated tobacco aerosol, compared to 1R6F reference cigarette smoke, correlated with substantially reduced *in vitro* toxicological outcomes**<br>CHAPMAN F.(1); TRELLES STICKEN E.(2); WIECZOREK R.(2); POUR S.J.(2); DETHLOFF O.(2); STEVENSON M.(1)<br>*(1) Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, U.K.*<br>*(2) Reemtsma Cigarettenfabriken GmbH, an Imperial Brands PLC Company, Albert-Einstein-Ring-7, D-22761 Hamburg, Germany* |

## DAY 4

# THURSDAY 21 OCTOBER

## SESSION 1 - E-vapour: analytical methods

Chair: Rob STEVENS

Co-Chair: Karl WAGNER

*CET Time Zone*

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|---|---|---|
| 13:30-13:45 | ST 20 | **Determination of organic acids by GC-FID: on cartridge derivatization by silylating reagent (MTBSTFA)**<br>PENNINGTON A.S.C.; PLUNKETT S.<br>*Enthalpy Analytical, LLC, 800 Capitola Dr., Durham, NC 27713, U.S.A.* |
| 13:45-14:00 | ST 21 | **Comparison of quartz filter collection and electrostatic precipitation for analysis of trace metals in ENDS aerosol**<br>JAMESON J.B.; YANG C.; COOK D.K.; JEONG L.N.; GILLMAN I.G.<br>*JUUL Labs Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.* |
| 14:00-14:15 | ST 22 | **Analysis of organic acids in e-vapor products by ion chromatography**<br>ZHU J.; SNEAD E.; HAGERTY G.<br>*ITG Brands, LLC, P.O. Box 21688, Greensboro, NC 27420, U.S.A.* |
| 14:15-14:30 | ST 24 | **Evidence for artefactual formation of glycidol during the analysis of e-liquids**<br>O'REGAN D.C.; OZVALD A.M.; COOK D.K.; JEONG L.N.; CHEN X.; GILLMAN I.G.<br>*JUUL Labs Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.* |

*[**ST 23** moved to Friday, 22 October, Session 1]*

## DAY 4

# THURSDAY 21 OCTOBER

## SESSION 2 - E-vapour: product analyses

Chair: Karl WAGNER

Co-Chair: Rob STEVENS

*CET Time Zone*

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| 15:15-15:30 | ST 25 | **Demonstration of capability of SIFT-MS to measure volatile carbonyl compounds in e-cigarettes**<br>BISHOP L.J.<br>*British American Tobacco, Regents Park Road, Southampton SO15 8TL, U.K.* |
| 15:30-15:45 | ST 26 | **Key parameters affecting the release of aldehydes in e-cigarette aerosols**<br>FAN Meijuan; CUI Huapeng; PAN Lining; CHEN Li; GUO Junwei; ZHAO Le; WANG Hongbo; HUA Chenfeng; LIU Shaofeng<br>*Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* |
| 15:45-16:00 | ST 27 | **Development of new chemicals for e-cigarette vaping based on machine learning**<br>HUANG Jiaruo; DU Wen; YIN Xinqiang; YI Jianhua; CAO Jun; CHEN Jingbo; WANG Zhiguo<br>*China Tobacco Hunan Industrial Co., Ltd, No. 386 Laodong Middle Road, Changsha 410007, Hunan, China* |
| 16:00-16:15 | ST 28 | **Compound identification process for GC-MS non-targeted analysis of JUUL aerosol using a custom mass spectral library**<br>JEONG L.N.(1); NOE M.R.(2); SHAH N.H.(2); CHAKRABORTY S.(2); MILLER IV J.H.(2); GILLMAN I.G.(1)<br>*(1) JUUL Labs, Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.*<br>*(2) Altria Client Services, LLC, 601 E Jackson St, Richmond, VA 23219, U.S.A. (a Service Provider to JUUL Labs, Inc.)* |

## DAY 5

# FRIDAY 22 OCTOBER

## SESSION 1 - Cigarettes and water pipes: analytical methods

Chair: Jutta PANI

Co-Chair: Karl WAGNER

*CET Time Zone*

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| 13:30-13:45 | ST 29 | **Development of 134 flavour compounds analysis in tobacco to comply with new Brazil ANVISA RDC 226/2018 regulatory demand – chemometric approach**<br>SILVA C.; QUINTERO J.A.A.; BELADINELI P.; MUSTO J.; RODRIGUES L.; GEYER J.; BHERING D.L.<br>*British American Tobacco Brazil, TSS-AmSSA, Av. Frederico A. Ritter 8000, Distrito Industrial, Cachoeirinha, RS, 94970-470, Brazil* |
| 13:45-14:00 | ST 30 | **Analysis of volatile and semi-volatile organic compounds in mainstream and intense cigarette smoke regimes by use of charcoal cartridge-trap system and GC/MS analysis**<br>PAPROCKI A.; ABAD F.; JANDREY A.; GEYER J.; FARIAS W.<br>*British American Tobacco Brazil, TSS-AmSSA, Av. Frederico A. Ritter 8000, Distrito Industrial, Cachoeirinha, RS, 94970-470, Brazil* |
| 14:00-14:15 | ST 31 | **Technical challenges in water pipe transfer studies**<br>MAYER-HELM B.; ZIERLINGER M.; JAINDL I.; PUMMER S.; KUBA M.; HOFBAUER L.; EILENBERGER G.; STEPAN H.<br>*ÖKOLAB Gesellschaft für Umweltanalytik Ges.m.b.H., Japan Tobacco International, Hasnerstraße 127, 1160 Vienna, Austria* |
| 14:15-14:30 | ST 32 | **Challenges in analysing contemporary flavoured shisha tobaccos**<br>LAUTERBACH J.H.<br>*Lauterbach & Associates, LLC, 211 Old Club Court, Macon, GA 31210-4708, U.S.A.* |
| 14:30-14:45 | ST 23 | **A quicker method for the analysis of ammonia in cigarette smoke by ion chromatography**<br>ZHU J.; SNEAD E.<br>*ITG Brands, LLC, P.O. Box 21688, Greensboro, NC 27420, U.S.A.* |

## DAY 5

# FRIDAY 22 OCTOBER

## SESSION 2 - Aromas and flavours: analytical methods

Chair: Bin HU

Co-Chair: Jutta PANI

*CET Time Zone*

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|---|---|---|
| 15:00-15:15 | ST 33 | **Studying the release of natural tobacco flavorants and nicotine in a variety of tobaccos at HnB and vaping temperatures (100-400 °C)**<br>MIHAYLOVA-KROUMOVA A.B.(1); WAGNER J.G.(2)<br>*(1) University of Kentucky, Kentucky Tobacco Research and Development Center, 1401 University Drive, Lexington, KY 40546, U.S.A.*<br>*(2) University of Kentucky, Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546, U.S.A.* |
| 15:15-15:30 | ST 34 | **Study of the interaction between aroma components and sweet taste characteristics of cigarettes by $\sigma$-$\tau$ model method**<br>LI Chao(1); LIU Jinyun(2); FAN Duoqing(1); CHEN Fangrui(1); HU Yan(1); WANG Meng(1); YE Ling(1); LIAO Tougen(1); LIU Xiuming(1); ZHANG Yipeng(1)<br>*(1) Cigarette Product Quality Test Center, Technology Center of China Tobacco Yunnan Industrial Co., Ltd, Kunming 650023, China*<br>*(2) Yunnan Industrial Tobacco HI-TECH Material Co., Ltd, Kunming 650106, China* |
| 15:30-15:45 | ST 35 | **Study of the saliva components of cigarette smokers by closed loop stripping analysis**<br>WU Bingyu; ZHOU Yan; FEI Ting; WANG Liang; WU Da; LIU Baizhan<br>*Shanghai Tobacco Group Co., Ltd, No. 3733 Xiupu Road, Shanghai 201315, China* |
| 15:45-16:00 | ST 36 | **Simultaneous detection of 35 organosulfur compounds in cigarette smoke by GC-MS/MS coupled with sulfur chemiluminescence detector**<br>QIN Yaqiong; LIU Ruihong; WANG Xiaoyu; XIE Fuwei; PAN Lining; CHEN Li; WANG Bing; YU Jingjing; CAI Junlan; LIU Shaofeng<br>*Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* |

## DAY 6

## MONDAY 25 OCTOBER

### SESSION 1 - Method for *in vitro* and *in vivo* toxicology testing: advancing methods in pre-clinical toxicology

Chair: Kei YOSHINO

Co-Chair: Paul HARP

*CET Time Zone*

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| 13:30-13:45 | ST 37 | **What is the best approach to assess the vapor-induced cytotoxicity of ENDS? A road map to the destination**<br>CARUSO M.(1,2); EMMA R.(1,2); RUST S.(2); DISTEFANO A.(1); CAROTA G.(1); PULVIRENTI R.(1); CARUSO T.(1); POLOSA R.(2,3); LI VOLTI G.(1,2)<br>*(1) University of Catania, Department of Biomedical and Biotechnological Sciences, Via S. Sofia 97, 95123 Catania, Italy*<br>*(2) University of Catania, Center of Excellence for the Acceleration of Harm Reduction (CoEHAR), Via S. Sofia 97, 95123 Catania, Italy*<br>*(3) University of Catania, Department of Clinical and Experimental Medicine, Via S. Sofia 97, 95123 Catania, Italy* |
| 13:45-14:00 | ST 38 | ***In vitro* to *in vivo* extrapolation (IVIVE) for evaluating exposure and health impacts of e-vapor products using ingredient and mixture *in vitro* data**<br>ZHANG J.(1); CHANG X.(2); HINES D.(2); BELL S.(2); LEE K.M.(1)<br>*(1) Altria Client Services LLC, Richmond, VA, U.S.A.*<br>*(2) ILS, RTP, NC, U.S.A.* |
| 14:00-14:15 | ST 39 | **Influence of antioxidant capacity on micronucleus induction by cigarette smoke in various mammalian cell lines**<br>HASHIZUME T.; YAMAMOTO H.; SHIBUYA K.; FUKUSHIMA T.<br>*Japan Tobacco Inc., R&D Group, Scientific Product Assessment Center, 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa, Japan* |
| 14:15-14:30 | ST 40 | **Development and characterization of an alternative rotary-like ENDS collection method for *in vitro* toxicology testing**<br>COFFA B.G.; KOSACHEVSKY P.; SMITH M.; McFADDEN L.; SOVICK C.; SMITH J.; KALLAM B.; PRESS E.; PICKELL T.; PATEL N.; KUMARI A.; SCIAN M.<br>*Enthalpy Analytical, LLC, 1470 E Parham Rd, Richmond, VA 23228, U.S.A.* |
| 14:30-14:45 | ST 41 | **Identification of a novel mode of action to induce micronuclei by 1-(4-methoxyphenyl)pent-1-en-3-one**<br>WATANABE T.; MUNAKATA S.; ISHII T.; SAITO J.; TOMOHIRO T.; HASHIZUME T.<br>*Japan Tobacco Inc., R&D Group, Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan* |
| 14:45-15:00 | ST 42 | **Method development of an *in vitro* waterpipe air-liquid interface exposure system**<br>GAFNER J.(1); MILLER-HOLT J.(1); FUKUSHIMA T.(2)<br>*(1) Scientific and Regulatory Affairs, JT International SA, Geneva, Switzerland*<br>*(2) Scientific Product Assessment Center, R&D Group, Japan Tobacco Inc., Kanagawa, Japan* |

## DAY 7

# TUESDAY 26 OCTOBER

## SESSION 1 - Biomarkers in clinical science: human data for assessing tobacco harm reduction

Chair: Paul HARP

Co-Chair: Kei YOSHINO

*CET Time Zone*

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| 13:30-13:45 | ST 43 | **Quantitative analysis of NNN in plasma: a suitable alternative to urinary determination to assess the exposure to this important toxicant**<br>PLUYM N.(1); SIBUL F.(1); JIN X.(2); EDMISTON J.(2); SARKAR M.(2); SCHERER G.(1); SCHERER M.(1)<br>*(1) ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany*<br>*(2) ALCS, Center for Research & Technology, 601 E. Jackson St., Richmond, VA 23219, U.S.A.* |
| 13:45-14:00 | ST 44 | **Analysis of 3-hydroxybenzo[a]pyrene in urine as a biomarker of exposure for BaP in smokers and users of potentially reduced risk products**<br>RÖGNER N.; SCHERER M.; SCHERER G.; PLUYM N.<br>*ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany* |
| 14:00-14:15 | ST 45 | **Meta-analysis study to establish population level estimates of NNAL in smokers and non-smokers**<br>AYALA-FIERRO F.(1); VERRON T.(2); LIZHNYAK P.(3); FREELAND R.(4); FROST-PINEDA K.(4); ELAMIN A.(5); PRASAD G.L.(4); SARKAR M.(3)<br>*(1) Juul Labs, Inc, U.S.A.*<br>*(2) Imperial Brands, U.K.*<br>*(3) Altria Client Services, U.S.A.*<br>*(4) RAI Services Company, U.S.A.*<br>*(5) Philips Morris International, Switzerland* |
| 14:15-14:30 | ST 46 | **Reductions in biomarkers of exposure to selected harmful and potentially harmful constituents following complete or partial transition to *my*blu™ ENDS (exclusive or dual use) compared to continued combustible cigarette smoking**<br>MORRIS P.(1); McDERMOTT S.(2); CHAPMAN F.(2); VERRON T.(2); CAHOURS X.(2); STEVENSON M.(2); THOMPSON J.(2); CHAUDHARY N.(2,3); O'CONNELL G.(2)<br>*(1) Nerudia Ltd - an Imperial Brands PLC Company, Wellington House, Physics Road, Speke, Liverpool L24 9HP, U.K.*<br>*(2) Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, U.K.*<br>*(3) Broughton Nicotine Services, Oak Tree House, West Craven Drive, Earby, Lancashire BB18 6JZ, U.K.* |
| 14:30-14:45 | ST 47 | **Biomarkers of potential harm in smoking abstinence and in the use of Vuse electronic nicotine delivery systems (ENDS)**<br>MAKENA P.; SCOTT E.; CHEN P.; LIU H-P.; JONES B.A.; PRASAD G.L.<br>*RAI Services Company, 401 N Main St, Winston Salem, NC 27101, U.S.A.* |

## DAY 7

## TUESDAY 26 OCTOBER

### SESSION 2 - Toxicological assessment: nonclinical toxicity assessment of nicotine products

Chair: Kei YOSHINO

Co-Chair: Paul HARP

*CET Time Zone*

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| 15:15-15:30 | ST 49 | **Assessment of alternative cell lines for adoption within the OECD TG 129 for cytotoxicity determination**<br>POUR S.J.(1); WIECZOREK R.(1); CZEKALA L.(2)<br>*(1) Reemtsma Cigarettenfabriken GmbH, an Imperial Brands PLC Company, Albert-Einstein-Ring 7, D-22761 Hamburg, Germany*<br>*(2) Imperial Tobacco Limited, 121 Winterstoke Road, Bristol BS3 2LL, U.K.* |
| 15:30-15:45 | ST 50 | ***In vivo* genotoxicity testing of aerosols generated from JUUL ENDS products**<br>LALONDE G.(1); ZHANG Jingjie(2); JALIGAMA S.(3); GUPTA A.(3); PSURNY E.(3); LEE K.M.(2); DOSHI U.(2); YAO J.(1)<br>*(1) JUUL Labs, Inc., San Francisco, CA, U.S.A.*<br>*(2) Altria Client Services, LLC, Richmond, VA, U.S.A. (a Service Provider to Juul Labs, Inc.)*<br>*(3) Battelle Memorial Institute, West Jefferson, OH, U.S.A.* |
| 15:45-16:00 | ST 51 | ***In vitro* evaluation of tobacco free nicotine pouches**<br>MILLER-HOLT J.(1); ABRAHAM I.(1); SAKIMURA M.(1); FUKUSHIMA T.(2); GAFNER J.(1)<br>*(1) Scientific and Regulatory Affairs, JT International SA, Geneva, Switzerland*<br>*(2) Scientific Product Assessment Center, R&D Group, Japan Tobacco Inc., Kanagawa, Japan* |

*[ST 48 cancelled]*

## DAY 8

# WEDNESDAY 27 OCTOBER

## SESSION 1 - Cigarettes: modelling and design

Chair: Bernhard EITZINGER

Co-Chair: Bin HU

*CET Time Zone*

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| 13:30-13:45 | ST 52 | **A prediction model for lip sticking force of cigarette tipping paper based on friction coefficient and grammage**<br>YANG Ji; ZHU Ruizhi; ZHANG Tao; GUO Lijuan; LIU Chunbo; XIANG Nengjun; LIU Zhihua; MIAO Mingming<br>*China Tobacco Yunnan Industrial Co., Ltd of CNTC, R&D Center, Kunming 650231, China* |
| 13:45-14:00 | ST 53 | **Analysis of volatile compounds in tipping glue by HS-SPME coupled with GC-MS using C3N4 coated fibers as SPME fibers**<br>WEI Min; LI Ran; PAN Xi; RONG Lin; DONG Aijun; SONG Xuyan; HE Yunlu; DONG Ping<br>*China Tobacco Hubei Industrial Co., Ltd, Yellow Crane Tower Science Park, No. 1355 Jinshan Avenue, Dongxihu District, Wuhan 430040, China* |
| 14:00-14:15 | ST 54 | **Effects of cigarette circumference on cigarette ventilation rate during smoking**<br>DENG Nan(1,2); ZHANG Qi(2); ZHAO Wenkang(2,3); WANG Hui(4); WANG Le(2); TAO Hong(5); YIN Xianzhong(6); DAI Lu(4); GU Junping(5); YU Hongxiao(7); WANG Bing(2); LI Bin(2)<br>*(1) Instrumental Analysis Center, Xi'an Jiaotong University, Xi'an, Shaanxi, China*<br>*(2) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan, China*<br>*(3) China Tobacco Guangxi Industrial Co., Ltd, Nanning, Guangxi, China*<br>*(4) China Tobacco Zhejiang Industrial Co., Ltd, Hangzhou, Zhejiang, China*<br>*(5) China Tobacco Guangdong Industrial Co., Ltd, Guangzhou, Guangdong, China*<br>*(6) China Tobacco Henan Industrial Co., Ltd, Zhengzhou, Henan, China*<br>*(7) China Tobacco Shandong Industrial Co., Ltd, Jinan, Shandong, China* |
| 14:15-14:30 | ST 55 | **Preparation of cigarette packet blanks with high barrier and moisture retention, moisture resistance and flavour keeping performance**<br>JI Xiaoying; HUANG Xin; LI Xiaopeng; SHI Fengcheng; GENG Zongze; LIU Minchang; ZHANG Rongya; LI Dongliang<br>*China Tobacco Sichuan Industrial Co., Ltd, Chenglong Road, Chengdu 610066, China* |
| 14:30-14:45 | ST 56 | **Influence of multi-layer packaging of cigarette packets on their moisture barrier**<br>LOU Jiaying; HUA Qing; WU Da; ZHANG Qian; FEI Ting; ZHANG Wei<br>*Shanghai Tobacco Group Co., Ltd, No. 3733, Xiupu Road, Shanghai 201315, China* |

## DAY 8

## WEDNESDAY 27 OCTOBER

### SESSION 2 - Nicotine pouches: nonclinical toxicity assessment

Chair: Bernhard EITZINGER

Co-Chair: Karl WAGNER

*CET Time Zone*

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| 15:15-15:30 | ST 57 | **Nonclinical toxicity assessment of oral tobacco-derived nicotine products: I. Framework**<br>ZHANG M.; SMITH C.; MORGAN R.; DOSHI U.; KUMAR A.; LEE K.M.<br>*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 15:30-15:45 | ST 58 | **Nonclinical toxicity assessment of oral tobacco-derived nicotine products: II. Ingredient evaluation**<br>MORGAN R.; AWOYEMI O.; ANDERSON C.; ZHANG M.; LEE K.M.<br>*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 15:45-16:00 | ST 59 | **Nonclinical toxicity assessment of oral tobacco-derived nicotine products: III. Extraction and test material characterization**<br>SMITH C.; ZHANG M.; HURST T.; DOSHI U.; KUMAR A.; MORGAN R.; LEE K.M.<br>*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 16:00-16:15 | ST 60 | **Nonclinical toxicity assessment of oral tobacco-derived nicotine products: IV. *In vitro* regulatory testing**<br>DOSHI U.; SMITH C.; KUMAR A.; MORGAN R.; ZHANG M.; LEE K.M.<br>*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 16:15-16:30 | ST 61 | **Nonclinical toxicity assessment of oral tobacco-derived nicotine products: V. *In vitro* mechanistic assays using human gingival fibroblasts**<br>KUMAR A.; DOSHI U.; SMITH C.; MORGAN R.; ZHANG M.; LEE K.M.<br>*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 16:30-16:45 | ST 62 | **Market survey of modern oral nicotine products: determination of select HPHCs and comparison to traditional smokeless tobacco products**<br>JABLONSKI J.J.; CHEETHAM A.; MARTIN A.M.<br>*Enthalpy Analytical, LLC, 1470 E Parham Road, Richmond, VA 23228, U.S.A.* |

## DAY 9

# THURSDAY 28 OCTOBER

## SESSION 1 - E-vapour: product chemistry

Chair: Rob STEVENS

Co-Chair: Bernhard EITZINGER

*CET Time Zone*

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| 13:30-13:45 | ST 63 | **An 18-month e-liquid stability study**<br>LAUTERBACH J.H.<br>*Lauterbach & Associates, LLC, 211 Old Club Court, Macon, GA 31210-4708, U.S.A.* |
| 13:45-14:00 | ST 64 | **Effect of pH and storage temperature on e-liquid metal concentrations**<br>COLLINS D.; HOCHSTETLER S.; CRABTREE R.; HEIGTER D.; NGO V.<br>*Enthalpy Analytical, LLC, 1470 E Parham Rd, Richmond, VA 23228, U.S.A.* |
| 14:00-14:15 | ST 65 | **A 12-month stability study on JUUL Virginia tobacco flavored aerosols using two non-targeted analytical methods**<br>CROSSWHITE M.R.; JEONG L.N.; JAMESON J.B.; LIOUBOMIROV A.; YANGA C.; OZVALD A.M.; GILLMAN I.G.<br>*JUUL Labs Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.* |
| 14:15-14:30 | ST 66 | **Comparison of collection strategies for the analysis of targeted compounds in e-cigarettes aerosol**<br>JAMESON J.B.; HIRAKI B.J.; OZVALD A.M.; JEONG L.N.; CHEN X.; GILLMAN I.G.<br>*JUUL Labs Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.* |
| 14:30-14:45 | ST 67 | **A comparison of the yield of select analytes from JUUL e-cigarette aerosols and eight Korean brand cigarettes**<br>YANG C.; CROSSWHITE M.R.; COOK D.K.; CHEN X.; HIRAKI B.J.; GILLMAN I.G.<br>*JUUL Labs Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.* |
| 14:45-15:00 | ST 68 | **Targeted characterization of the chemical composition of novel JUUL product aerosol to 3R4F cigarette smoke**<br>COOK D.K.; O'REGAN D.C.; OZVALD A.M.; YANG C.; GILLMAN I.G.<br>*JUUL Labs Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.* |

## DAY 9

# THURSDAY 28 OCTOBER

## SESSION 2 - Statistics and laboratory operations

Chair: Xavier CAHOURS

Co-Chair: Rob STEVENS

*CET Time Zone*

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|---|---|---|
| 15:30-15:45 | ST 69 | **A robust statistical approach for estimating method precision**<br>GUO M.; VERRON T.; CAHOURS X.<br>*SEITA, Imperial Brands, 200-216 Rue Raymond Losserand, 75014 Paris, France* |
| 15:45-16:00 | ST 70 | **Eliminate or accommodate outliers? A comparison between standard and robust approaches for the analysis of collaborative study data**<br>MORTON M.J.<br>*Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* |
| 16:00-16:15 | ST 71 | **Analysis of productivity in the laboratory and the role of outsourced asset management services**<br>FIELD B.; KERAS K.; FILOSA A.<br>*PerkinElmer, 940 Winter St, Waltham, MA 02451, U.S.A.* |
| 16:15-16:30 | ST 72 | **History of 10 years of the BAT TRM – tobacco reference material for total alkaloids and total sugar monitoring**<br>ALVES M.(1); BHERING D.L.(1); HERMES N.(2)<br>*(1) British American Tobacco Brazil, TSS-AmSSA, Av. Frederico A. Ritter 8000, Distrito Industrial, Cachoeirinha, RS, 94970-470, Brazil*<br>*(2) British American Tobacco, Research and Development, Regents Park Road, Southampton SO15 8TL, U.K.* |

# ABSTRACTS

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

## ST 01

### Development of consumer-reported outcome measure (CROM) standards for the tobacco industry with respect to psychometric CROM using a consortium-based approach: methodology and scope

McCAFFREY S.(1) on behalf of the CORESTA CROM Task Force (WG02): AFOLALU E.(2); BLACK R.(1); CHREA C.(2); CURTIN G.(3); GILES L.(4); NISHIHARA D.(4); PRASAD K.(5); SARKAR M.(6); SHETTY M.(5)

*(1) JUUL Labs, Inc., San Francisco, CA, U.S.A.*
*(2) Philip Morris Products S.A., Neuchâtel, Switzerland*
*(3) RAI Services Company, Winston-Salem, NC, U.S.A.*
*(4) JT International S.A., Geneva, Switzerland*
*(5) British American Tobacco, Southampton, U.K.*
*(6) Altria Client Services, Richmond, VA, U.S.A.*

In November 2018, CORESTA approved the formation of a new Task Force (TF) to establish best practices and guidelines for the integration of consumer-reported outcome measures (CROM) in tobacco regulatory research. The primary objective of the CROM TF is to provide guidance on how to identify, develop, and validate CROM and to provide access to CROM for evaluating tobacco and nicotine-containing products for pre-market and post-market purposes. Here, we describe the research completed by one of the TF working groups, WG02, with the purpose of developing CROM standards for tobacco regulatory research with respect to psychometric CROM (i.e. CROM intended to measure underlying psychological attributes – e.g. reinforcing effects).

WG02 comprises 11 researchers from the CROM TF, representing seven different tobacco companies and with experience in psychometrics, patient-reported outcomes (PRO), survey methodology, and product use behavior. WG02 members collaboratively drafted an operational definition of psychometric CROM and devised an approach for developing the standards.

Consistent with approaches taken by other outcomes research organizations, WG02 adopted a consensus-based approach for drafting the standards, which includes (1) review of literature and (2) an iterative peer-review process leveraging various sources, such as conference presentation and review by subject matter experts from tobacco, PRO, and related fields. Through literature review and WG02 member input, the scope was defined and initial content for the five components of the standards was drafted. These components include: (1) appropriate content, (2) development process and validation, (3) adaptation/modification of existing CROM, (4) application/implementation and interpretation of CROM, and (5) linguistic/cultural translation.

This research represents the development of draft standards for psychometric CROM for tobacco regulatory research, with the intention to complement the 2020 FDA draft guidance issued by the Food and Drug Administration Center for Tobacco Products. The initial draft content is being disseminated for peer review to gather feedback.

## ST 02

### Descriptive consumer reported outcome measures and definitions in tobacco and nicotine research: a consensus approach

WEI L.(1) on behalf of the CROM Task Force: CLERC E.(2); SHETTY M.(3); CHREA C.(2); PRASAD K.(3); SARKAR M.(1)

*(1) Altria Client Services LLC, Richmond, Virginia, U.S.A.*
*(2) Philip Morris International, Neuchâtel, Switzerland*
*(3) British American Tobacco, Southampton, U.K.*

The Consumer Reported Outcome Measures (CROM) Consortium Task Force (TF), consisting of academic researchers and members from various tobacco companies, was formed within the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) in 2018. The primary objective of the CROM TF is to provide guidance on how to develop, validate, select, access, and use CROM to evaluate tobacco and nicotine-containing products for pre-market and post-market purposes. Working Group 04 (WG04), as one of the CROM TF working groups, was established in 2020 and has the objective of developing recommendations and definitions related to Descriptive-CROM.

Descriptive-CROM are composed of factual items that are direct measures of individual characteristics and behaviors, such as demographics and tobacco product use patterns. Fifteen tobacco surveillance surveys that focus on adult populations were selected for review, including two international, eight European, one Japanese, and four US surveys. Question items from the most recent survey questionnaires were discussed and grouped into domains including key demographics, prevalence, use consumption, initiation, and cessation, etc.

Recommendations of survey items were made by the core team members and reviewed by advisory board members using a consensus-based approach. Differences between the international and country-specific surveys and gaps (e.g. consumption measures for new emerging tobacco categories) in the survey items were identified. Recommendations were also made to align definitions derived from Descriptive-CROM, such as tobacco product dual or poly use, initiation and cessation.

Based on identified domains and recommendations, WG04 will publish CORESTA Recommendations and Guidelines for Descriptive-CROM. Additionally, optimal survey questionnaire items will be summarized to create a publicly available knowledge repository, including conditions and context of use. The repository will facilitate the development and use of Descriptive-CROM to evaluate tobacco and nicotine-containing products for pre-market and post-market purposes.

## ST 03

### Psychometric validation of new scales expanding conceptual coverage of the ABOUT-Perceived Risk: perceived social risk and perceived practical risk

CLERC E.(1); SALZBERGER T.(2); CANO S.(3); AFOLALU E.(1); CHREA C.(1)

*(1) PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*
*(2) Institute for Statistics and Mathematics, University of Economics and Business, Welthandelsplatz 1, 1020 Vienna, Austria*
*(3) Modus Outcomes, Spirella Building, Letchworth Garden City SG6 4ET, U.K.*

The ABOUT™—Perceived Risk is a self-report instrument, developed by Philip Morris Products SA, designed to assess perceived risks associated with the use of a wide range of tobacco and/or nicotine containing products (TNP), such as cigarettes, e-cigarettes, smokeless tobacco, and heated tobacco products. Its original five-domain conceptual framework (perceived: health risk to self, addiction risk, harm to others, social risk, and practical risk) was developed on the basis of qualitative studies, literature review, and input from experts. Although draft versions of scales for representing each of the five domains were developed and cognitively debriefed, only the Perceived Health Risk and Addiction Risk scales were initially psychometrically validated in two quantitative studies, and "harm to others" was captured by three items to be treated as single item.

Here, we describe the subsequent psychometric evaluation of the Perceived Social and Practical Risk scales, using data collected in an independent cross-sectional study in the United States among adult TNP users.

A sample of exclusive TNP and poly-TNP users (n = 1250 in each sample) completed the Perceived Social Risk (13 items) and Perceived Practical Risk (9 items) scales in an online survey. Psychometric evaluation was based on Rasch Measurement Theory (RMT) and Classical Test Theory (CTT) analysis.

An item-reduced scale was derived for both Perceived Social (7 items) and Practical (6 items) Risk. The item-reduced Social and Practical Risk scales showed no indication of differential item functioning and exhibited high reliability (Rasch PSI 0.90 and 0.81; Cronbach's alpha 0.96 and 0.92, respectively) and good stability over time (test–retest reliability, 0.76 and 0.74, respectively).

The psychometric performance of the new Perceived Social Risk and Perceived Practical Risk scales supported the expansion of the ABOUT—Perceived Risk instrument to include better coverage of perceived risk measurement. The instrument will be made available through PROQOLID™.

## ST 04

### Risk perceptions and likelihood of use of (tobacco-free oral) nicotine pouches among current, former and never nicotine users

STONE C.; KNIGHT-WEST O.; O'CONNELL G.; TOPE A.

*Imperial Brands PLC., Research and Development, 121 Winterstoke Road, Bristol BS3 2LL, U.K.*

Nicotine pouches deliver nicotine to the blood via the oral mucosa; since they are not combusted and contain no tobacco leaf, they have the potential to be significantly less harmful than continued combustible cigarette smoking. There is limited data on how adults perceive and intend to use nicotine pouches, and none have been reported in the nascent UK market in the published literature. This cross-sectional online survey of 1,532 UK adults (aged 18-60) assessed intentions to try and purchase nicotine pouches as well as risk perceptions of nicotine pouches, combustible cigarettes and other nicotine products among current, former and never nicotine product users.

Most respondents (44 %-70 %) understood that nicotine pouches likely present a lower or much lower risk than continued combustible cigarette smoking but are not risk-free. A minority indicated nicotine pouches carry the same (12 %-24 %) or more (3 %-14 %) risk than combustible cigarettes; some were unsure of the risk (7 %-18 %). A good proportion of current adult smokers (11 %-22 %) expressed intentions to try or purchase Imperial Brands' high quality nicotine pouches, while such intentions were very low among former and never adult smokers (0 %-4 %). The main reasons for intent to purchase were that current adult smokers perceived nicotine pouches to be less harmful than combustible cigarettes (38 %-48 %) and to use them as an aid to stop smoking (37 %-50 %).

Accurate understanding of the relative risks of emerging nicotine products, as demonstrated in this study, are essential if they are to advance tobacco harm reduction. The fact that Imperial Brands' nicotine pouches overwhelmingly appealed to current adult smokers, and not former or never adult smokers suggests they have potential to serve as an "off-ramp" for adult smokers who would otherwise continue to smoke. Moreover, the data offers little evidence that these products would serve as a 'gateway' to regular nicotine use in nicotine naïve populations.

## ST 05

### Assessing the impact of next generation products on population health: a population modelling approach

PHILLIPS C.V.(1); KHAKWANI A.(2); O'CONNELL G.(2); CAHOURS X.(2); TOPE A.(2)

*(1) epiphi Consulting Group, New Hampshire, U.S.A.*
*(2) Imperial Brands PLC., 121 Winterstoke Road, Bristol BS3 2LL, U.K.*

Next Generation Products (NGPs) collectively represent novel nicotine-containing products that offer potentially reduced exposure to toxicants, toxicity and health risks to adult smokers compared to continued combustible cigarette smoking. To assess the potential impact of introducing a new NGP onto the market on the health of population as a whole, with respect to the reduction in smoking-related risk amongst users and people who do not currently use tobacco products, population health modelling is required. To this end, Imperial Brands PLC is developing a statistical simulation model to explore the possible effects on the prevalence of NGP and combustible cigarette use in a hypothetical population. In addition, the simulation model aims to predict the prevalence of NGP use at one, five and ten years post introduction in a market.

The simulation model is based on a combination of deterministic population effects and simulations modelling that takes into consideration the real-world trajectory of NGP uptake. The model will integrate information about relative risks between combustible cigarettes and NGPs from published literature and obtain base transition probabilities from nationally representative datasets where available. The model will allow for the calculation of 'what if' scenarios to assess health benefits of NGPs and the degree by which these are stable against external changes. In addition, a novel approach will be implemented by incorporating forecasting data in the model to allow assessment of multiple scenarios and estimate prevalence of the new NGPs amongst current NGP users and the extent to which introduction of the NGPs may displace combustible cigarette smoking.

Results from simulation modelling will be applied to the US market and shared at the CORESTA Conference 2021.

## ST 06

### A statistical methodology integrating resampling techniques to evaluate public health impact after introduction of reduced-risks products in Japan

LARROQUE S.; KUBOTA T.; CHARRIÈRE M.; SONNERAT D.; KIMURA Y.

*JT International SA, 8 Rue Kazem Radjavi, 1202 Geneva, Switzerland*
*Japan Tobacco, Tokyo, Japan*

Statistical modelling methodologies to predict the impact of reduced-risk products (with the potential to reduce the public health risks associated with smoking, "RRP"), namely e-cigarettes and heated tobacco products, require many input parameters; i) demographic projections (e.g. births, deaths, migrations), ii) status and evolution of tobacco consumers' prevalence (e.g. initiations, cessations, product transitions), and iii) estimates of the potential reduced-risk of new products (as compared to the risks associated with smoking conventional cigarettes).

Generally, due to the large volume of data and heavy computation processing time, a small range of input parameters is carefully selected (e.g. low, medium, high scenarios) and is evaluated for sensitivity purposes.

The objective of this work is to build a statistical platform to compute valid and reliable predictions integrating exhaustive variability by inputting not only point estimates but the full parameter distributions.

A standard SAS/IML® script using resampling techniques simulations has been created to mimic the density of the reduced-risk profile and smoking status transition rates probabilities coming either from assumptions defined by the users of the platform or from clinical biomarker and observational studies.

The script is producing automatic outputs showing the predictive demographics distribution over time including life-year gain in a population after introduction of an RRP. Sensitivity and robustness of the model can then be checked by looking at percentiles of all simulations (e.g. 5 % lowest percentile showing positive life-year gain after introduction).

This method has been applied using official Japanese demographic projections, vital statistics and smoking prevalence data to better describe what would be the impact of model parameters variability on public health predictions after introduction of a new RRP.

## ST 07

### Nicotine pharmacokinetics of electronic cigarettes: an updated analysis of pooled data from the literature

JACOBSON K.; MARTINEZ J.; LARROQUE S.; JONES I.; PASCHKE T.

*JT International SA, 8 Rue Kazem Radjavi, 1202 Geneva, Switzerland*

Several regulatory initiatives around the world restrict the content of nicotine in electronic cigarette (e-cigarette) liquids (e-liquids) in order to minimize the risks associated with nicotine. However, to which extent levels of nicotine in e-liquids influence e-cigarettes users' behaviour is complex and poorly defined. Extensive studies are conducted to assess the factors contributing to nicotine absorption in adults using e-cigarettes in a variety of laboratory conditions coupled with different e-cigarette devices. Nonetheless, it remains difficult to draw conclusions regarding nicotine absorption from e-cigarettes. The purpose of this study is to present a pooled analysis of publicly available studies that provide nicotine pharmacokinetic data for e-cigarettes, mainly $C_{max}$, to identify which factors influence nicotine blood absorption. The data gathered indicate that nicotine absorption is determined by a combination of many factors, including user experience, device type and e-liquid nicotine concentration. For e-cigarettes to be viable alternatives to smoking, they should provide to adult smokers who express their interest in switching to e-cigarettes an equally satisfying experience, including in terms of nicotine absorption into the body. Therefore, any regulation of e-cigarettes should take all these factors into account.

## ST 08

### Effects of various forms of nicotine delivery on the autonomic nervous system (ANS) and stress hormones

TROFIMOV A.V.(1); BERDNIKOVA N.G.(1,2); ZAGURSKAYA A.V.(3,4); MENSHOV V.A.(1); YABLONSKAYA O.I.(1)

*(1) Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow 119334, Russia*
*(2) I.M. Sechenov First Moscow State Medical University, Moscow 119991, Russia*
*(3) Medical Center AVC, Moscow 127018, Russia*
*(4) DNKom Laboratory, Moscow 127018, Russia*

The variety and continuing appearance of new nicotine delivery tools necessitate a comparative analysis of their influence on a human organism. The main objective of this work was a multivariate clinical study on a new generation of oral nicotine products. Oral nicotine products with low and moderate nicotine content (up to 6 mg per portion) were tested for six months on ten smokers, three ex-smokers and six users of alternative nicotine delivery systems (ANDS). Saliva and blood of volunteers were used as biomaterials. As a result, we have determined more than 500 different invasive and non-invasive indicators, including biochemical, cytological, electrophysiological, etc. The main emphasis of this work was placed on research on the hormonal and immune systems. It has been found that in smokers and ANDS users, the synthesis and metabolism of stress hormones from the number of catecholamines and cortisol derivatives is closely interrelated not only and not so much with the dose of nicotine, but with the way of delivering nicotine into the body, which, in turn, determines the rate of its entry into the blood and, in general, the pharmacokinetics of nicotine. Stress hormones, along with other factors, influenced the heart rate variability (HRV) measured with the help of electrocardiography. It has been demonstrated that smoking a cigarette causes the activation of the sympathetic division of the ANS, depending on the dose of nicotine. Interestingly, in experienced smokers, when testing oral nicotine products, a change in the balance of ANS activities does not correlate with a change in the concentration of nicotine and cotinine in blood, as well as in saliva. It was concluded that oral forms of nicotine, despite the many times higher content of nicotine in one portion, exert a milder (less stressful) effect on the body in comparison with a regular cigarette.

## ST 09

### Inhibitory effect of nicotine on monoamine oxidase B and its action against Parkinson's disease

CAO Yun(1); ZHOU Shun(1,2); WANG Chenghu(1); WANG Peng(1); WANG Xiaofeng(1); ZHANG Yaping(1,2); ZHANG Xiaoyu(1); LI Yanyan(1); GUAN Mingjing(1)

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Epidemiological investigation indicates that smoking is negatively correlated with the incidence of Parkinson's disease (PD), but its mechanism is still not fully elucidated. The aim of this study is to investigate the inhibitory effect of nicotine on monoamine oxidase B (MAO B, a PD biomarker) and its application in the therapy of Parkinson's disease. The interaction between nicotine and MAO-B was analyzed by using docking model simulation technology. Molecular biology and animal behaviour experiments verified that nicotine could improve the relevant biological indicators of Parkinson's disease in cell and drosophila PD models. The results showed that: 1) In the docking simulation, the shortest distance between nicotine and active sites of MAO-B protein was 3.8 Å, which was shorter than that of pargyline (5.1 Å). 2) In the *in vitro* protein activity inhibition assay, 0.01 µM nicotine induced 72 % inhibition rate for MAO-B. When the concentration of nicotine increased to 1 µM, the inhibition rate reached 80 %. 3) In the cellular experiments, nicotine reached mitochondria within 0.5 h and maintained at a level of 2.5 ng/mL for 6 h. Nicotine protected dopaminergic cell line, SH-SY5Y, from neurotoxicity induced by MPTP. The protective effect was more significant in the cells with MAO-B overexpression. The cell viability increased from 75 % to 90 %, and the proportion of early apoptosis decreased from 84.7 % to 77.5 %. 4) Nicotine significantly improved the motor function and dopamine level of drosophila PD model. In conclusion, nicotine is able to inhibit the activity of MAO-B and protects against PD *in vitro* and *in vivo*. This study provides a basis for clarifying the protecting mechanism of nicotine against PD via inhibiting MAO-B and is a new way of utilizing tobacco.

## NAM 00

### Advancing new alternative methods for tobacco harm reduction

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New approach methodologies (NAMs) represent *in vitro* and *in silico* or computational methodologies, an emerging set of chemical safety assessment tools without needing additional *in vivo* animal testing. This virtual symposium introduces the current status of NAMs and their potential to support the evaluation of potentially reduced-risk tobacco products in support of tobacco harm reduction. Significant progress has been made toward the reduction, refinement, and replacement (3R) of animal studies for pharmaceutical and environmental toxicity assessment through the adoption of NAMs. At the same time, NAMs acceptance beyond screening and prioritization, including regulatory decision making, remains challenging. At this symposium, external experts from regulatory agencies and research organizations will share insights on the current status, strengths, and opportunities in application of NAMs using case examples from safety assessments of chemicals and consumer products. Following the presentations, the panel will be available for questions from the symposium participants and discussion on opportunities and challenges in applying NAMs for toxicological assessment of tobacco and novel nicotine products.

The symposium intends to foster scientific engagement between CORESTA members (endorsed by the *In vitro* Toxicity Testing Sub-Group, Biomarker Sub-Group, and 21st Century Toxicology for Next Generation Tobacco and Nicotine Products Task Force) and the external NAMs community, together facilitating the utility of NAMs in support of evidence-based tobacco regulatory science. Highlights of the symposium will be submitted for a peer-review publication.

## NAM 01

### US federal efforts to develop and implement alternatives to animal testing

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The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is made up of representatives from US federal agencies that require or consider chemical safety testing data, and are interested in more rapid, human-relevant approaches to supplement or replace existing regulatory standard *in vivo* guideline tests. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) provides scientific and administrative support to ICCVAM through a variety of efforts including methods development and validation, construction of computational tools, communication and outreach, and stakeholder engagement, all driven by federal agency priorities and decision contexts. This talk will provide an overview of ICCVAM and NICEATM's progress in developing, evaluating, and implementing alternatives to animal testing. Emphasis will be on implementation of the ICCVAM strategic roadmap, ongoing efforts to replace the "six-pack" of acute toxicity tests, and development of computational resources such as *in vitro* to *in vivo* extrapolation workflows, with specific examples in the areas of inhalation toxicity testing.

## NAM 02

### Application of biokinetic modelling for *in vitro* to *in vivo* extrapolation (IVIVE) in chemical risk assessment

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*In vitro* chemical safety testing methods offer the potential for efficient and economical tools to provide relevant assessments of human health risk. To realize this potential, methods are needed to relate *in vitro* effects to *in vivo* responses, without relying on *in vivo* animal testing. To this end, *in vitro* to *in vivo* extrapolation (IVIVE) is key. IVIVE is essentially representing two main streams: 1) Upscale of *in vitro* measurements from molecular reaction to full organ to be used in biokinetic-dynamic modelling (biological scaling), and 2) The linking of *in vitro* concentration response (endpoints/effect) to external exposure doses by means of biokinetic modelling. In the current presentation we will highlight the main concepts of IVIVE, report the main biokinetic models and illustrate the IVIVE approach using case studies that were published as part of the recent OECD PBK model Guidance document (OECD, 2021).

## NAM 03

### Inhalation exposure modeling for assessing health risks of toxic aerosols and vapors

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Computational fluid dynamics (CFD)-based approaches, either alone or coupled with lower dimensional models such as physiologically-based pharmacokinetic (PBPK) and multiple-path particle dosimetry (MPPD) models (multiscale models) have been developed to facilitate the reduction in animal testing in human health risk assessments for inhaled materials. Case studies will be presented where these *in silico* approaches were used to establish human equivalent exposure concentrations for cytotoxic pesticide aerosols corresponding to exposure conditions used *in vitro* in toxicity studies with human air way cells grown at air-liquid interface as well as for reactive vapor constituents of tobacco smoke previously studied in animal models. CFD-based modeling of respiratory dosimetry under realistic human exposure vs. experimental conditions improves the relevance of new risk assessment approaches that utilize human cells *in vitro* as well as historically relevant *in vivo* studies thereby reducing the overall use of animals in tobacco harm reduction research.

## NAM 04

### Assessing respiratory toxicity of chemicals in two human bronchial *in vitro* systems

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Risk assessment and management relies on approaches that can accurately and efficiently predict the toxicity of chemicals in humans. Inhalation is a major route by which exposure to substances can occur, and is an area where resources have been dedicated to optimize human-relevant *in vitro* approaches. In this study a two-dimensional (2D) human bronchial epithelial cell line (BEAS-2B) and a three-dimensional (3D) human reconstructed tissue model (MucilAir™, Epithelix) were used to predict the ability of chemicals to cause portal-of-entry effects on the human respiratory tract. The human cell-based systems were exposed to different concentrations of silanes (triethoxysilane (TES) and trimethoxysilane (TMS)) surfactants (Triton X-100 and oleoyl sarcosine) at the air-liquid interface in a VITROCELL® 6/4 exposure module. Nitrogen dioxide ($NO_2$) was included as a positive control and sodium chloride and clean air (CA) or nitrogen gas (N) as negative controls. Endpoints assessed include cell viability (Prestoblue™ assay), cytotoxicity (lactate dehydrogenase assay; LDH), and expression of inflammatory markers (electrochemiluminescence immunoassay, Meso Scale Discovery) and, in addition for the 3D tissues, morphology (hematoxylin and eosin (H&E) staining), barrier integrity (transepithelial electrical resistance, TEER), and cilia beat frequency (SAVA system). Preliminary studies demonstrated a concentration-dependent decrease in cell viability and an increase in cytotoxicity after 1 hour exposure of BEAS-2B cells to TES (0.72 ppm, 25 ppm, and 85 ppm) as compared to CA. A significant increase in expression of inflammatory markers, including interleukin IL-6, IL-8, IL-2, and tumour necrosis factor-alpha (TNF-α), was observed at 25 ppm of TES. Studies are underway to assess the additional test chemicals and endpoints in both systems. The results of this project can be used to better understand the usefulness of different test systems and, therefore, help guide selection. They may also be used to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and inform regulatory decision-making.

## NAM 05

### *In silico* toxicology as a New Approach Methodology in tobacco regulatory science

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*In silico* (computational) toxicology is a new approach methodology (NAM) that is an integral part of the US federal collaboration Toxicology in the 21st Century, and FDA's Predictive Toxicology Roadmap. *In silico* methods are efficient, reliable, cost effective, and align with the '3Rs' principals of animal testing. Smoke from combusted tobacco products contains thousands of organics including harmful or potentially harmful chemicals. The aerosol from electronic nicotine delivery systems (ENDS) is also a complex mixture of constituents. In addition, flavored e-liquids used with ENDS have many chemical constituents. Collecting hazard identification information for every tobacco constituent using conventional *in vivo* toxicology studies would be impractical. *In silico* methods are versatile showing capabilities in toxicity prediction screening, data mining, structural alert identification, model building and many other techniques. This presentation will discuss how different *in silico* approaches, if properly used and in context, have the potential to overcome the challenges with traditional testing models to generate useful data in the frame of tobacco regulatory science. Limitations and other considerations inherent to use of *in silico* approaches will also be discussed. FDA/CTP's research on utility of NAMs to support toxicology evaluations is illustrated by *in silico* screening for genetic toxicity hazard identification of tobacco constituents using (quantitative) structure-activity relationship ((Q)SAR) computer models, results from predictive computational model validation testing to classify mutagenic potential, and research on integration of *in silico* and *in vitro* high throughput (HTP) screening technologies to identify the genotoxic mode of action of flavor compounds. With focus on flavor compounds relevant to tobacco products, the *in vitro* research using clastogen-sensitive (γH2AX and p53) and aneugen-sensitive (p-H3 and polyploidy) biomarkers of DNA damage and machine learning algorithms can classify the genotoxic mode of action (structural chromosome damage or aneuploidy). Such work underscores how leveraging different NAMs generates streams of evidence that can be productive to increase knowledge about toxicological hazards of compounds. As predictive models such as (Q)SARs and HTP multiplexed *in vitro* and machine learning methods are trained and garnered with empirical data, the quality of data used to make predictions as well as interpretations becomes paramount. Also, the integration of *in silico* and *in vitro* NAMs offers another approach to better understand toxicity profiles of constituents with the goal to increase the strength of evidence to make better decisions about potential toxicities that may lead to health risks.

## NAM 06

### Application of mechanistic data in risk assessment: exposure alignment and evidence integration

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Risk assessment relies on integration of evidence across several data streams by necessity. Conceptual constructs for source-to-outcome modeling based on aggregate exposure pathway (AEP) and adverse outcome pathway (AOP) frameworks can provide a mechanistic scaffold for evidence integration to now include application of new approach methods (NAMs). Based on the conceptual construct, this presentation will discuss the critical role of dosimetry models to provide exposure alignment across experimental platforms and illustrate impact by two recent integrated approaches to testing and assessment (IATA) under the Toxic Substances Control Act (TSCA) that formalized a role for NAMs. The IATA demonstrate how consideration of physicochemical properties and NAMs aimed at key events (KEs) of AOPs create context for evaluation of the need and strategy for higher-tiered testing based on mechanistic responses, dosimetry, and exposure information.

## ST 10

### Modelling and simulation of electromagnetic heating smoking set with multi physical field

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In heat-not-burn cigarettes, electromagnetic heating has the advantages of uniform heating and fast heating speed compared with traditional resistance plate heating. However, there are still some problems in domestic electromagnetic heating smoking sets, such as the lack of theoretical guidance for heating parameters and structure parameters, the defects in heating performance and endurance ability. In order to solve these problems, a commercial computational fluid dynamics software, Comsol Multiphysics®, was used to study the temperature field distribution law of micro electromagnetic coil heaters with different structure sizes and materials in the process of natural heat transfer under empty load. The finite element simulation model obtained by Comsol Multiphysics® was compared with the actual measurement results to verify the reliability of the simulation. With a variety of heater materials and continuous heater structure parameters, the simulation model can be applied to calculate the heating performance, which provides a reference for the optimal design of the heater. The experimental results show that the combination of permalloy and peek has a better heat insulation effect and lower cost. Considering the heater structure and heating time, the heating performance can meet the general product requirements when the coil turns number is 12. The maximum error between the simulation value and the experimental value is less than 10 %, which verifies the effectiveness of the method. This study can optimize the performance of existing products and provide a reference for the optimal design of the heater.

## ST 11

### Simulation research on temperature field distribution of electric heating non-combustion cigarette heater

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Compared with traditional cigarettes, heat-not-burn cigarettes only heat the tobacco materials or tobacco extracts instead of burning. The heating temperature of heat-not-burn cigarettes is generally about 300 °C, and the harmful substances released by the smoke of its tobacco products are greatly reduced. In order to achieve accurate temperature control and energy consumption optimization management, the temperature distribution of the whole cigarette set under different operating conditions was analyzed, and the change law of the temperature field of the electric heating non-combustion cigarette heater was obtained. Through theoretical simulation and actual experiment, the research results of heating comparison and analysis of the temperature field distribution law of the micro heaters of different structure sizes and materials during the no-load natural heat transfer process, can be optimized and designed for subsequent electric heating non-combustion cigarette heaters. Theoretical and experimental guidance and support are provided for in-depth analysis of heat transfer mechanisms, precise temperature control, and optimized management of energy consumption for subsequent products, and further promote the development and application of domestic heat-not-burn cigarette products.

## ST 12

### The use of infra-red thermal imaging to evaluate the thermal performance of filtration segments in heated tobacco products

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Heated tobacco products (HTPs) heat tobacco to create an aerosol that the consumer inhales. Tobacco in combustible cigarettes burn at temperatures over 700 °C, compared to tobacco in HTPs that is heated without combustion to temperatures up to 350 °C. Despite this lower temperature in HTPs, the aerosol the consumer inhales can be perceived to feel hotter than cigarette smoke. The objective of this study was to evaluate if thermal imaging can be used to assess the thermal performance of filtration segments in HTPs and assist in filter design. An infra-red camera was adopted for the study which is able to measure real time temperature with a high degree of accuracy. An in-house designed adapter was used to accurately position the product and camera. The temperature of the filter segments in a HTP varied across the smoking cycle. In a triple filter design study, the filter segment at the tobacco end increased in temperature over the smoke cycle increasing from 130 °C to 175 °C. The middle filter stayed relatively steady at around 70 °C. Lower temperatures were observed at the mouth end filter with aerosol temperatures decreasing with each puff from 65 °C to 40 °C. These observations are used to drive filter design, with the aim to reduce the temperature of the inhaled aerosol. We concluded that this method provides real time thermal imaging of emissions during HTP puffing. Thermal profiling allows the ability to assess the thermal performance of filter segments, rods and filtration materials in heated tobacco products.

## ST 13

### Numerical simulation of smoke flow field characteristics and temperature distribution of circumferential heated tobacco products

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Heated tobacco products have gradually become an important development direction and research hotspot of tobacco products because of their remarkable advantages in reducing the releases of harmful components. In order to carry out the research and development of heated tobacco products more comprehensively and systematically, an integrated development platform for heated tobacco products was constructed. On the basis of a computational fluid dynamics method, a three-dimensional numerical model for the smoke flow and heat transfer in circumferentially heated tobacco products was established. This was done by taking the heater of a circumferentially heating apparatus and dry base cigarette as the research objects. The simulation results of flow field distribution were verified by testing draw resistance and filter ventilation rate. The simulation results of temperature field distribution were verified by infrared thermometer and thermocouples. The model was used to predict the smoke flow and temperature distribution in circumferentially heated tobacco products during actual smoking. The results showed that: (1) The heat variation caused by the mass transfer of dry base cigarette in the process of heating-puffing at higher temperature was slight. (2) The temperature of different positions in the cigarette might rise or fall during puffing, depending on whether the smoke was fully heated enough at that position. (3) In the process of puffing, higher filter ventilation rate led to lower smoke flow rate passing through the tobacco section of the cigarette, which kept the internal temperature of the cigarette stable. (4) The radial temperature difference of circumferentially heated tobacco products was small. This method provides a technical support for the fast and accurate research and development of heated tobacco products.

## ST 14

### Numerical simulation of flow, heat and mass transfer in a heated tobacco stick

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An electrically heated tobacco stick is operated at a temperature below that of combustion and it is generally known that this results in the formation of an aerosol with reduced levels of harmful and potentially harmful constituents (HPHCs - Baker 1981, 1987, 2006; White 2001; Torikai 2005; McGrath 2007; Nordlund 2016, 2017). For this reason, heat-not-burn (HnB) tobacco products have recently been receiving much attention. For the stable and controllable release of aerosol, it is important to understand the flow pattern, the distribution of temperature, the concentration and the transport of smoke vapours inside a stick. This can be clearly aided by numerical simulation. In this work, the governing equations regarding mass, momentum, energy, and the species transport equations were solved numerically by commercial computational fluid dynamics (CFD) software (Comsol Multiphysics®). The software was used to demonstrate the operating conditions of the tobacco stick exposed to air flow (Health Canada Intense puffing regime) and the heater element during puffing. The 2D domains were employed with equations supplemented by simple Arrhenius expressions defining kinetic properties of generation (vaporization and/or pyrolysis (relatively low temperature)) of the smoke vapours (e.g. nicotine, glycerol, water) during the time-temperature history of a tobacco heater. Darcy's law was implemented in a tobacco substrate as a porous medium. The numerical results show the close correlation with the measured data and indicates that relevant physical and chemical physics inside a tobacco stick are reasonably accounted for in the numerical model. It was the aim of this study to develop such a physical and chemical model and to use it to numerically simulate a stick during multi-puffing, which will be useful to develop better electrically heated tobacco stick design technology.

## ST 15

### Determination of tobacco specific nitrosamines (TSNAs) in heated tobacco product (HTP) blend by accelerated solvent extraction (ASE) and LC-MS/MS analysis

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Some studies (Vuarnoz, 2015; Williams, 2015) have pointed to chemical binding between TSNA and the tobacco matrix that are not extracted using standard extraction procedures. This potential negative bias may cause inconsistencies in 'mass balance' determinations of HTP products. This study used a systematic approach to optimize an accelerated solvent extraction technique to differentiate the 'free' and 'bound' TSNA available in the tobacco product.

Accelerated solvent extraction was performed using a Dionex ASE 350 using the same extraction solutions and instrumental parameters as the standard procedure for TSNA determinations. However, the extraction process was sequentially optimized modifying the temperature, sample size, static time and the number of extraction cycles. To investigate the potential of artifact formation, l-ascorbic acid was added to the extraction solution.

The optimized conditions to extract both the 'bound' and 'free' TSNA were determined to be: 200 °C, for a 0.5 g sample size and a 15-minute static or cycle time, with a single cycle being adequate. The 'free' TSNA were extracted at 45 °C with all other parameters remaining the same, producing results consistent with those determined using the standard extraction procedures.

Detailed results of the optimization process will be presented. The impact of ASE on NNN, NAT and NAB was found to be negligible. However, the estimated 'free' NNK fraction was calculated to be anywhere from 16 % to 41.7 % of the total NNK content determined by ASE.

## ST 16

### eHTP aerosol generation: TSNA method adjustments and mass balance methodology feasibility

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Heated tobacco products (HTPs), whatever their design, deliver reduced harmful and potentially harmful constituents (HPHCs) aerosol emissions thanks to reduced temperature applied to tobacco. Last year we presented a mass balance method (2020_ST06) to figure out the water, glycerol and nicotine contents from the tobacco to the aerosol. We have complemented the method with the addition of tobacco specific nitrosamines (TSNAs) to the mass balance methodology.

The objectives of this study were to adjust the TSNA analytical method to the HTPs range and to assess the feasibility of a TSNA mass balance to understand the influence of the device and stick designs on aerosol TSNAs.

Modified Health Canada Intense puffing regime was used to generate aerosol on a linear smoking machine. A procedure for sample collection before and after heating was developed with some adjustments in order to have TSNA levels above quantification limits.

The following steps were added:

- total NNK (free and bound) quantification after sample autoclaving, measured in tobacco before heating and after heating,
- removal of binding agents in TSNA aliquot by precipitation before analysis to limit troubleshooting on the high pressure liquid chromatography system.

A control commercial HTP stick stored in a freezer was analyzed at the beginning and at the end of each puffing session to control and ensure the stability of the puffing and analytical system. The coefficient of variation of TSNAs is below 10 %.

This mass balance approach allows the actual TSNA transfer rate and the TSNA retention in cooling and filtering segments, which vary across different HTPs systems, to be defined.

## ST 17

### LC-MS-MS method development for the analysis of tobacco-specific nitrosamines in heated tobacco products aerosols

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Heated tobacco products (HTPs) are designed to deliver a tobacco/nicotine aerosol with a significant reduction in the toxicants associated with combustible cigarettes. Tobacco-specific nitrosamines (TSNAs) are N-nitroso-derivatives of pyridine-alkaloids present in tobacco smoke, although reported to be present at significantly reduced levels in HTP aerosols. N'-Nitrosonornicotine (NNN) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are included on the U.S. Food & Drug Administration (FDA) list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke. Therefore, manufacturers will be required to submit robust quantitation data to regulatory authorities and demonstrate a relative reduction in exposure to TSNA toxicants for HTP users.

The objective of this work was to develop and validate a sensitive and selective liquid chromatography with tandem mass spectrometry (LC-MS-MS) method, optimised for analysis of two TSNAs in HTPs. The work sought to adapt the existing CORESTA method (CRM 75) for determination of TSNAs in mainstream cigarette smoke to the aerosols of HTPs.

Generated eHTP aerosols were trapped in 12-puff collections, 2-s durations, 55-mL volumes, 30-s puff intervals, bell curve profiles. The collection was performed on a Cerulean SM450e vaping machine set up for HTP analysis with Cambridge filter pad collection. The chromatographic method showed limits of quantitation < 0.2 ng/mL (in solution) for NNN and NNK, with an appropriate linear range for eHTP TSNA analysis of 0.2-200 ng/mL based on reported literature data. The method has demonstrated a suitable degree of accuracy, precision and robust quantitation.

Initial experimental results obtained from a commercially available eHTP for NNN and NNK demonstrated a significant reduction in levels compared with the literature data for a 3R4F reference cigarette.

## ST 18

### Determination of aromatic amines in mainstream and sidestream cigarette tobacco smoke and heated tobacco products

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Aromatic amines are of public concern as they are known to be probable or possible carcinogens to humans. Furthermore, the US Food and Drug Administration (FDA) lists six aromatic amines as harmful or potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke.

Here, a state-of-the-art method is described for the determination of 1-aminonaphthaline, 2-aminonaphthaline, 3-aminobiphenyl, 4-aminobiphenyl, o-toluidine, 2,6-dimethylaniline and o-anisidine in mainstream, sidestream tobacco smoke and heated tobacco products.

Cigarettes or heated tobacco products were smoked on a routine analytical smoking machine and mainstream or sidestream smoke was collected by a filter pad according to ISO standards. Seven deuterated internal standards were added to the sample prior to extraction. After extraction the samples were purified by solid-supported liquid/liquid extraction. The extracts were derivatized and finally purified with a solid phase extraction column. Gas chromatography coupled to tandem mass spectrometry was used for the separation of the seven aromatic amines.

All ten possible isomers were chromatographically well separated from the target analytes. External standard calibration after correction with the internal standards was used for quantification.

The limits of quantification (LOQ) for seven aromatic amines ranged from 0.03 ng/cig to 1.54 ng/cig in mainstream tobacco smoke, from 0.40 ng/cig to 12.9 ng/cig in sidestream tobacco smoke and from 0.19 ng/cig to 1.54 ng/stick in heated tobacco products. The recoveries were within 80 to 120 %. The relative standard deviations of intraday and interday tests for 1R6F reference cigarette were less than 15 % and 25 %. Trueness is based on the comparison with data from proficiency testing (PT CIG-2018A, University of Kentucky, Center of Tobacco Reference Products) resulting in satisfactory z-scores.

In conclusion, a selective and accurate method for the simultaneous determination of seven aromatic amines in mainstream tobacco smoke, sidestream tobacco smoke and heated tobacco products has been developed and validated.

## ST 19

### Substantial reductions in selected harmful or potentially harmful constituents in heated tobacco aerosol, compared to 1R6F reference cigarette smoke, correlate with substantially reduced *in vitro* toxicological outcomes

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Heated tobacco products (HTPs) form a category of nicotine-containing next generation products (NGPs) which show potential for tobacco harm reduction (THR). This is attributed to the absence of combustion in producing a HTP aerosol, which in turn contains substantially reduced levels of the harmful and potentially harmful constituents (HPHCs) found in combustible cigarette smoke. This has also been correlated with decreased *in vitro* toxicological effects of HTP aerosols when compared to cigarette smoke. This study aimed to assess the aerosol chemistry of a prototype HTP (p-HTP), focusing on a select list of HPHCs, compared to 1R6F Reference Cigarette smoke and to measure the *in vitro* toxicological outcomes following exposure to these. A multiple endpoint testing approach was used (Ames test (TA98; TA100), micronucleus (MN) assay (V79), neutral red uptake (NRU; Beas-2B) and high content screening (HCS) for seven cell stress related endpoints). On a per puff basis, the p-HTP aerosol was found to contain substantially reduced (> 90-98 %) levels of selected HPHCs compared to 1R6F smoke. However, nicotine yields were comparable between two p-HTP puffs and one puff of 1R6F smoke. 1R6F smoke elicited strong *in vitro* toxicological responses upon exposure. In comparison, following exposure to p-HTP aerosols, no mutagenicity under the Ames test conditions was observed and decreased potency in the MN (8/13 (+S9/-S9)-fold) and NRU (39-fold) assays were recorded. Substantial decreases, at comparable nicotine concentrations, in effects of the p-HTP aerosol compared to 1R6F smoke were also observed in seven cellular stress related HCS endpoints (including DNA damage and mitochondrial stress) at both four and 24h. These findings contribute to the weight of evidence for HTPs' THR potential compared to combustible cigarette smoking and support other HTP studies which demonstrate that reduced HPHC levels in the aerosol translate directly to reduced *in vitro* toxicological outcomes.

## ST 20

### Determination of organic acids by GC-FID: on cartridge derivatization by silylating reagent (MTBSTFA)

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Organic acids are compounds that, when used in electronic nicotine delivery systems (ENDS) devices and e-liquids, can alter the perception and uptake of nicotine by the consumer. Traditional means of analysis by suppressed IC (Ion Chromatography) can lead to long runtimes in excess of 70 minutes and can contain coelutions or partial coelutions of some analytes, namely lactic acid and levulinic acid which coelute completely and benzoic acid, malic acid, and succinic acid which coelute partially. This may be overcome by the analysis of organic acids by gas chromatography-flame ionization detection (GC-FID). By using a weak anion sample preparatory exchange (SPE) cartridge, the organic acids may be trapped and then undergo silylation derivatization with N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and be eluted with acetonitrile. Standards prepared in the same fashion have shown to have quantitative recovery from the cartridge that has allowed for a production of a quadratic curve from 0.4 µg/mL to 1000 µg/mL with recoveries of 90 to 115 % for all compounds. By performing the derivatized analysis by GC-FID, coeluting compounds in IC are completely resolved, analytical runtime is decreased to 25 minutes, and comparable method detection limits to that of IC are maintained. Notably, the method detection limits for the 15 organic acids analyzed were between 0.02 µg/mL and 0.08 µg/mL. This process eliminated the need for multiple dilutions in order to analyze effectively the levels of organic acids in percent level nicotine salt e-liquids and vapor. Derivatization and GC-FID analysis has added the benefit of a reduced runtime as well as provided resolution to coelution of known compounds.

## ST 21

### Comparison of quartz filter collection and electrostatic precipitation for analysis of trace metals in ENDS aerosol

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The Premarket Tobacco Product Application (PMTA) process for electronic nicotine delivery systems (ENDS) recommends that a wide range of harmful and potentially harmful compounds (HPHCs) be determined to support the application. The U.S. Food and Drug Administration provides guidance specifying a number of constituents and chemicals that should be included for analysis. However, no guidance is provided on appropriate analytical methodologies or test procedures. The present study was designed to assess methodologies for collection of aerosol samples for trace metals analysis. In this study, collection of ENDS aerosols on quartz filter pads (QF) was compared to electrostatic precipitation (EP). Specifically, the puffed room air blank measurements collected across a number of historical studies were analyzed and the resulting method limit of detection (LOD) values were computed using CORESTA Guide No. 28. QF and EP blank levels were compared across eight long-term stability studies for chromium (Cr), nickel (Ni), cadmium (Cd), and lead (Pb). Under QF collection virtually all the blank measurements levels, with the exception of Cd, were above the instrument LOD while under EP collection, virtually all the blank measurements levels were equal to the instrument LOD. The computed method LODs for QF and EP collection, respectively, were 0.68 ng and 0.37 ng for Cd, 130 ng and 7.6 ng (94 % reduced) for Cr, 43 ng and 3.7 ng (91 % reduced) for Ni, and 11 ng and 3.6 ng (66 % reduced) for Pb. This study confirms that using EP based aerosol collection for analytical measurements of trace metals in ENDS products significantly improves method detection limits for Cr, Ni, and Pb.

## ST 22

### Analysis of organic acids in e-vapor products by ion chromatography

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Organic acids (formic acid, acetic acid, propionic acid and lactic acid) are of interest for e-vapor products testing, especially propionic acid which is included in guidance from US Food and Drug Administration Center for Tobacco Products (FDA-CTP). Herein we wish to present a method for the analysis of these organic acids in e-cigarette aerosols and e-liquids by ion chromatography. The trapping efficiency study indicated that one glass fiber filter pad (Cambridge Filter Pad) is sufficient for aerosol collection without the use of impingers. A Cerulean SM 450 smoking machine has been utilized to collect aerosol under the ISO regime, while a Cerulean CETI-8 e-cigarette testing instrument can be used for either ISO or intense regimes. The filter pad after the aerosol collection or an e-liquid aliquot is extracted with deionised (DI) water in a 50 mL polypropylene vial using a shaker for 30 min. The extracts are analyzed by ion chromatography. The run time is 19 minutes (including equilibrating time) with baseline separations between these analytes. The calibration range is 0.05 µg/mL to 5 µg/mL for all of these organic acids. The method limit of quantitation (LOQ) is defined as the lowest spiking level used during the validation, which is 0.05 µg/mL, 0.036 µg/puff for 50 puffs of aerosols, or 5 µg/g for 0.36 g of e-liquids. The new method has been validated on two Dionex ion chromatography systems (ICS 5000 and ICS 6000) with good recoveries on e-cigarette aerosols (80-109 %) and e-liquids (89-123 %).

## ST 23

### A quicker method for the analysis of ammonia in cigarette smoke by ion chromatography

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In 2019 at the TSRC, we presented a quicker and robust method using a Dionex CS18 column for the analysis of ammonia in e-cigarette aerosols and e-liquids by ion chromatography. The method has a short run time of 13 minutes (including equilibrating time). However, for cigarette smoke, the ammonia content is much higher than in e-cigarettes, presenting a challenge to achieve a baseline separation of the ammonium peak from the preceding sodium peak. By utilizing plastic impingers instead of glass impingers for smoke collection, the amount of sodium in sample solutions has been greatly reduced, and thus a baseline separation of ammonium and sodium peaks can be achieved. The trapping efficiency study indicated that a combination of one filter pad and one impinger is sufficient for smoke collection. When quartz filter pads (Whatman QM-A) are used, the sodium peak in sample solutions is even lower than when using Cambridge glass fiber filter pads. A Cerulean SM 450 smoking machine has been utilized to generate smoke from cigarettes, while a Dionex ICS 5000 ion chromatography system has been used for the analysis. The new method has a calibration range of 0.1 µg/mL to 10 µg/mL. The ammonia results generated for the 1R6F reference cigarettes using this new method are in good agreement with the certified values as documented on the Certificate of Analysis from the University of Kentucky.

## ST 24

### Evidence for artefactual formation of glycidol during the analysis of e-liquids

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The Premarket Tobacco Product Application (PMTA) process for electronic nicotine delivery systems (ENDS) recommends that a wide range of harmful and potentially harmful compounds (HPHCs) be determined to support the application. The U.S. Food and Drug Administration (FDA) provides guidance specifying the number of constituents and chemicals that should be included for analysis. However, no guidance is provided on appropriate analytical methodologies or test procedures for the determination of these HPHCs. One such chemical, glycidol, was recently added to the HPHC list of ENDS. Glycidol is a thermal degradant of glycerine and is potentially formed at temperatures achievable during gas chromatography (GC) sample analysis.

In this study, we investigated two different techniques for the determination of glycidol in e-liquid via gas chromatography-mass spectrometry (GC-MS): 1) Direct injection of the sample, 2) Derivatization of glycidol to form a stable compound. Differences between the two methods would indicate if artefactual glycidol was formed with direct injection method.

Glycidol was measured in two JUUL e-liquids, Royal Crème 3 % and Royal Crème 5 %, and was found to be 8.43 µg/g and 7.71 µg/g, respectively, by direct injection versus 0.14 µg/g and 0.13 µg/g, respectively, for the derivatization method. These results indicate that direct GC analysis can lead to the formation of relevant levels of glycidol. The derivatization method was found to be more selective and offered improved sensitivity versus the direct injection analysis method.

## ST 25

### Demonstration of capability of SIFT-MS to measure volatile carbonyl compounds in e-cigarettes

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Selected ion flow tube mass spectrometry (SIFT-MS) is a form of direct mass spectrometry that enables real time analysis of trace volatiles at ppb levels without the need for preconcentration or chemical derivatisation. As a new capability in our laboratory, we have investigated several applications for which the SIFT-MS could provide a solution. In this presentation we will focus on the use of SIFT-MS to directly measure volatile carbonyl compounds in the aerosol produced from an e-cigarette.

Analysis of carbonyl components in e-aerosol involves a derivatisation method and typically analysis of "puff blocks" of an aerosol, giving no detailed information of the puff-by-puff profile of carbonyl generation. When connected to a PM1 smoke engine, the SIFT-MS was shown to be able to analyse individual puffs of an e-cigarette for formaldehyde, acetaldehyde and most of the other volatile carbonyl compounds. Preliminary results are presented for a device that has been allowed to 'wick' to show the increase in carbonyl components as the device runs dry, thus demonstrating the capability of using the SIFT-MS to measure volatile carbonyl compounds on a puff-by-puff basis.

## ST 26

### Key parameters affecting the release of aldehydes in e-cigarette aerosols

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In recent years, the sales of electronic cigarettes (e-cigarettes) have grown exponentially, and the aldehydes in e-cigarette aerosols have raised concerns. This study aims to investigate the effects of key parameters, the ratio of propylene glycol (PG) to glycerin (GLY), the moisture content and the nicotine concentration of e-liquid, and the output power of the smoking device on the releases of aldehydes in aerosols. Laboratory-made e-liquids were filled in a commercial refillable e-cigarette, 50 puffs of aerosols were collected, and the releases of formaldehyde, acetaldehyde and acrolein in aerosols were analyzed by HPLC (high performance liquid chromatography) under the smoking parameters of puffing volume 55 mL, puffing interval 30 s and puffing duration 3 s. The results showed that PG and GLY were the main sources of aldehydes in e-cigarette aerosols, acetaldehyde was mainly from the thermal degradation of PG, formaldehyde was mainly from the thermal degradation of GLY, and acrolein was mainly from the dehydration of GLY at higher temperature. The releases of aldehydes decreased with the increase of the nicotine concentration in e-liquid, while they increased quickly as the output power increased. The effect of moisture content in the e-liquid on the releases of aldehydes was slight.

## ST 27

### Development of new chemicals for e-cigarette vaping based on machine learning

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In order to overcome the disadvantages of e-cigarettes with glycerol and propylene glycol as the carrier liquid during vaporing, a recurrent neural network (RNN) model based on glycerol was employed in this study to generate plenty of new type chemical molecules. Consequently, xylitol was selected as a new vaporing chemical according to the research results of toxicity analysis and artificial smoking. The results of aerosol collected matter (ACM), characterization of physicochemical properties and analysis of aerosol particle characteristics proved that the atomization properties of xylitol and other sugar alcohol molecules are similar with glycerol and propylene glycol in aqueous solution. Further toxicological studies indicated that xylitol had no significant inhalation toxicity, its no-observed-adverse-effect concentration (NOAEC) in rats was determined as 5 g/m$^3$. The detailed results elucidated the potential to develop a new type of electronic cigarette containing more water than previous solutions by using xylitol as a novel vaporing chemical. This study also indicates that the introduction of a machine learning method could promote chemical research efficiency and broaden the application prospect of chemical molecules.

## ST 28

### Compound identification process for GC-MS non-targeted analysis of JUUL aerosol using a custom mass spectral library

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The US Food and Drug Administration (FDA) Premarket Tobacco Product Application (PMTA) guidance for electronic nicotine delivery systems (ENDS) recommends that applicants evaluate unique constituents in the aerosol of ENDS products for potential health risks. There is a need for chemical characterization of the ENDS aerosol and emission level estimation for aerosol constituents. A semi-quantitative non-targeted analysis (NTA) screening method was developed to provide a comprehensive chemical characterization of the aerosol generated from ENDS products. This gas chromatography-mass spectrometry (GC-MS) based NTA method was validated for evaluation of volatile and semi-volatile compounds in ENDS. Many published NTA studies rely heavily on pre-established vendor libraries for the compound identification process. Chemical identifications of the JUUL aerosol constituents using only the National Institute of Standards and Technology mass spectral database (NIST17) revealed several limitations. For example, less than 50 % of the detected compounds had an acceptable tentative identification (i.e. match factor > 800). Also, many peaks eluting closely to primary constituents exhibited poor match factor due to matrix effects. Therefore, a custom library containing mass spectra from matrix matched reference standards, tentatively identified compounds, and unknown compounds previously observed in JUUL products, was utilized. Here we present a comparison of the results when performing a search including and excluding the custom library combined with the NIST17 database for JUUL aerosol. Only approximately 20 % of the identifications were found to be in common in this comparison. The use of a custom library greatly increased acceptable tentative identifications by providing consistent detection and peak tracking based on relative retention time (RRT) between studies. Taken together, the use of a custom mass spectral library is a valuable addition to the non-targeted assessment approach and evaluation of its use is recommended for comprehensive characterization of ENDS products.

## ST 29

### Development of 134 flavour compounds analysis in tobacco to comply with new Brazil ANVISA RDC 226/2018 regulatory demand – chemometric approach

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On April 30, 2018, the Brazilian national agency for sanitary surveillance (ANVISA) published Resolution RDC 226/2018, which introduced the obligation to report 134 new analytes in processed tobacco and cigarette filler for the brands registration and annual renewal processes. These compounds are flavourings or additives that comprise fourteen different chemical classes (esters, ketones, aldehydes, alcohols, carboxylic acid and lactones, predominantly). Although methods have been published for the measurement of some of these compounds in tobacco, there is no published reference method for the measurement of all the compounds of interest. A principal component analysis (PCA) was conducted using published data on physico-chemical properties of the target compounds to group them for measurement by the same methodology. PCA was performed in SIMCA software and chemical analytes properties evaluated were: molar mass, boiling point, partition coefficient (LogP), vapor pressure, polar surface area and polarizability. This study presented a set of five distinct hierarchical clusters that suggested they could be analyzed by the same number of methods. Glycyrrhizinic acid, ethyl acetate and isobutyraldehyde showed the strongest differentiation of the clusters. Chemometrics showed a strong correlation between boiling point, molar mass and polarizability in predictions with opposite behaviour to the vapor pressure. The partition coefficient and polar surface area were both useful to create a greater distinction in the PCA. To test the chemometric hypothesis on the bench, five methodologies were tested with mainly liquid and gas chromatography coupled with mass spectrometer detection. Chemometrics prediction was four groups for GC-MS (109 compounds) and one group for LC-MS/MS (25 compounds), and the experimental results enabled three GC-MS to cover 114 compounds and two LC-MS/MS for the other 18 compounds. Minor adjustments were done to include most volatiles analytes in the three GC MS methods and the higher polar analytes in two LC-MS/MS methods.

## ST 30

### Analysis of volatile and semi-volatile organic compounds in mainstream and intense cigarette smoke regimes by use of charcoal cartridge-trap system and GC/MS analysis

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The volatile and semi-volatile organic compounds (VOCs) benzene, 1,3-butadiene, toluene, isoprene, acrylonitrile, pyridine, quinoline and styrene are included in the group of substances known as Hoffmann analytes and they have been the focus of regulatory agencies as the object of cigarette smoke monitoring. There are several techniques for both collection and analysis available in literature for VOCs and semi-VOCs in mainstream cigarette smoke but, in terms of collection, the cooled-trap impingers have been the ones most applied and have even been adopted by ISO as a standard method - ISO 21330 (Mainstream smoke regime - MS) / ISO 23923 (Intense smoking regime - IR). Although this method has shown good reproducibility, it requires high glass handling, relatively complex system assembly and a laborious cryogenic bath step (using dry ice), which usually results in long sample-preparation times. The aim of this study is to present a newly developed method based on a charcoal cartridge-trap, which is simpler compared to the impinger cooled-trap and, consequently, allows for relatively high-throughput analysis (30 % expected time-saving effect) involving less glass handling and complex system assembly as well as considerably less solvent waste (~ 96 % less). Calibration, detection, and quantification of the eight VOC and semi-VOC analytes of interest were performed for 1R6F Kentucky reference cigarettes and commercial brands, and are presented in this study, as well as method validation results. All validation parameters showed a fit-for-purpose method, which included: linear quantification range, LODs (MS: 0.04-7.98 µg/cig; RI: 0.12-26.6 µg/cig) and LOQs (MS: 0.12-26.6 µg/cig; IR: 0.41-88.6 µg/cig), recovery (MS: 90.0-104 %; IR: 89.2-108 %) and variability range from 5 to 25 %, this last one being comparable with repeatability (*r*) values presented in the corresponding ISO standards (MS and RI).

## ST 31

### Technical challenges in water pipe transfer studies

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Water pipe smoking is a traditional form of tobacco use in India and Arabic regions. Due to a rise in popularity it is now used all over the world. Water pipe tobacco typically contains molasses and various flavourings resulting in a complex mixture of smoke constituents. The goal of a transfer study is to determine the amount of a certain ingredient which is transferred from water pipe tobacco to smoke and may be inhaled by the consumer.

Several technical challenges arise for an analytical chemist:

- The analytical methods are applied to two different matrices: water pipe tobacco and water pipe smoke.
- Although a water pipe tobacco smoking machine and its application are described by ISO standards, some practical details remain unspecified, e.g. the heating technique. While charcoal heating better reflects how consumers typically use waterpipes, electrical heating offers higher precision.
- Transfer rates can by determined only for single compounds. When the transfer of a mixture is investigated, characteristic targets need to be defined to give a representative transfer rate. E.g. *trans*-cinnamaldehyde was selected as the target for the plant extract cassia oil.
- The ingredients highly differ in their physicochemical properties and in their amount. Hence, the measurement technique needs to be carefully selected depending on the target compound and its concentration. E.g. HPLC-UV was selected to analyze the colorant Allura Red. GC-MS is the method of choice for many volatile flavour compounds.
- It is tricky to establish the mass balance, since there are several compartments along the smoke pathway where the target compound may be retained. Some compounds are partly degraded or burned, e.g. molasses. Other compounds partially remain in the tobacco (e.g. glycerol up to 58 %) or are trapped in the water bowl (e.g. linalool up to 74 %, eucalyptol up to 44 %) or in the connections between tobacco and consumer.

## ST 32

### Challenges in analysing contemporary flavoured shisha tobaccos

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Contemporary flavoured shisha (waterpipe) tobaccos present several challenges for the analytical scientists charged with characterizing them as the composition of such products differs considerably from other non-liquid tobacco products. Based on analytical data of 30 brand-styles from an ISO 17025 accredited laboratory, the ranges (% of sample weight) of the following analytes were found: glycerol, 27 to 70; propylene glycol (PG), BQL to 30, fructose, BQL to 19, glucose, 1 to 16, and sucrose, 0.3 to 11. Such shishas generally contain only 10-30 % tobacco (most are low-nicotine, high-sugar flue-cured tobaccos, some are dark air-cured or other tobaccos). Thus, the particles of tobacco are “wet” with a mixture of glycerol and/or PG and sugars. The pack sizes in that sample set ranged from 50 g to 1 kg in weight. Most popular sizes are the 200 g or 250 g sizes for individual use while the 1000 g sizes are for hookah bars. Most of the larger size packs of shisha will contain separate tobacco and liquid phases, with the liquid being glycerol and/or PG with dissolved sugars. The combination of analyte ranges and pack sizes and the likely need to determine shisha emissions using ISO 22486 as well as particle-size distribution require at least three types of representative samples: one for the chemical analyses, one for the particle-size distributions, and one for shisha emissions. This presentation will include data on sample homogeneity, particle-size distribution, a new high performance liquid chromatography (HPLC) technique for glycerol, PG, fructose, glucose, sucrose, and higher sugars, and examples of its use, estimation of blend composition using polyphenols, and profiling of flavours using HPLC scan techniques. These techniques are based on extraction of the shishas with 75/25 acetonitrile/water, use of commercial amine/diol columns as well as Type-C silica columns with amide, diol, phenyl hydride, and C18 stationary phases.

## ST 33

### Studying the release of natural tobacco flavorants and nicotine in a variety of tobaccos at HnB and vaping temperatures (100-400 °C)

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There are novel trends in the tobacco industry, motivated by the regulations aimed at reducing the risk of tobacco products. Many studies show significant reduction of harm-causing compounds released from heat-not-burn (HnB) or other non-combusted tobacco products. The question arises if or how the non-combusted products will change their natural flavor and the release of nicotine. Tobacco flavor is a criterion driving tobacco product sales. The objective of this study was to evaluate the release of volatile flavor compounds (and nicotine) from Burley, Virginia and Samsun tobacco varieties at heat-not-burn temperatures (100, 200, 300, and 400 °C). We applied a novel approach, to study known, and other flavor-related volatile emissions from fresh and cured leaves, and from extracts containing flavor-related leaf surface chemicals. We used an Agilent Thermal Separation Probe (TSP) to compare volatiles generated after heating the material for one minute or less at a specific inlet temperature. The method approximates HnB and vaping processes where consumers start puffing immediately upon heating the material in the smoking device. We have preliminary data for the volatile emissions of Virginia K326 which showed that important flavor compounds are released at specific temperatures. For example, at 400 °C the major observed fragrance compound of cured leaf was D-limonene; while the nicotine was released at highest abundance at 300 °C. The tobacco leaf surface extract volatiles were very different from those of cured and fresh leaves. The major extract compounds were cembratrien (CBT) and CBT-like-diols. 3-methyl valerate and isovaleric isomers were found only in the extracts. These results give an idea of which tobacco product types are appropriate for non-combusted cigarettes, which are worth exploring in e-liquids containing tobacco leaf extracts, and which heating temperature is likely most favorable to deliver particular desired flavor compounds and nicotine.

## ST 34

### Study of the interaction between aroma components and sweet taste characteristics of cigarettes by *σ-τ* model method

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To investigate the correlations between the aroma substances in mainstream smoke and the sweet taste of cigarettes via analyzing the interaction mechanism between 12 fragrance materials and glucose, the sweet taste values of different concentration glucose, fragrance materials and their mixtures were measured based on the combined evaluation methods of electronic tongue and sensory quality. The synergistic effect model for different aroma substances and smoke sweet taste was established by *σ-τ* method. The results showed that the fragrance materials in the order of the influences on sweet taste were eugenol > benzyl alcohol > phenethyl alcohol > vanillin > cinnamaldehyde > *γ*-decalactone > propionic acid > furanone. Eugenol, benzyl alcohol and phenethyl alcohol had synergistic effects on glucose, and the sweet taste value increased with the rise of their concentrations; vanillin, cinnamaldehyde and *γ*-decalactone had additive effects on glucose, and the sweet taste value also increased with the increase of their concentrations; while the sweet taste value decreased with the increase of propionic acid and furanone concentrations, indicating that these two materials had masking effects on glucose. However, the influences of limonene, 2-methylpyrazine, furfural and 2-acetylfuran on glucose were slight. When fragrance materials are mixed with glucose, the interactions between them are different, and the concentration ratio is the main factor affecting the interactions. The materials with spicy, sweet, fresh and milky scents have synergistic and additive effects on sweet taste. By analyzing the relationships between the aroma and sweet components in tobacco, the sensory characteristics of cigarettes can be determined from the aspects of aroma characteristics and taste characteristics, so as to improve the design and quality control technology of cigarette products.

## ST 35

### Study of the saliva components of cigarette smokers by closed loop stripping analysis

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It is of great significance to analyse the components in saliva of cigarette smokers in order to study the oral sensory materials of smokers. Therefore, a closed loop stripping analysis (CLSA) method was optimized and coupled with thermal desorption-gas chromatography-mass spectrometry to analyse the components in saliva. The saliva of smokers of cigarettes of different types (including Chinese-style Virginian type, modified Virginian type and blended type) was collected with cotton balls by chewing stimulation, and the components in the saliva were analysed under optimized pre-treatment conditions. The results showed that: 1) The volatile and semi volatile components in saliva of cigarette smokers were well enriched, and 254 components were detected. The established method featured good repeatability. 2) Compared with the chemical composition of mainstream cigarette smoke, the compositions and proportions of different kinds of compounds in saliva changed significantly, especially cyclopentanones, pyrazines, lactones and triacetin. 3) The major differential components in the saliva of smokers of different types of cigarettes were pyridines, pyrazines, cyclopentenones and furans, which were quite different from those in the mainstream smoke of different types of cigarettes. This study provides a support for further study of the relationships between smoke components and the sensory quality of cigarettes.

## ST 36

### Simultaneous detection of 35 organosulfur compounds in cigarette smoke by GC-MS/MS coupled with sulfur chemiluminescence detector

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Organosulfur compounds are of great concern in food science because they play an important role in the flavour and taste of food due to their extremely low odour threshold levels and high sensory impact. They also exist in cigarette smoke, mainly in the particulate phase matter. However, due to their low content and complex sample matrices, there is limited literature on the several identified and quantified organosulfur compounds. In this study, a method for analyzing 35 organosulfur compounds was developed, and 22 organosulfur compounds in mainstream smoke of cigarette samples were identified and determined. The particulate phase matter collected by Cambridge filter pads were extracted by dichloromethane, and the extract was purified with silica-based solid-phase extraction. The condensate was analyzed simultaneously by both tandem mass spectrometry and sulfur chemiluminescence detector, which were combined through Capillary Flow Technology Splitter modules to increase the accuracy of qualitative results. The developed method was systematically validated. The limits of quantification for 35 organosulfur compounds were 0.20-0.51 ng/cig. The inter-day precisions and the intra-day precisions for all the targets were controlled by less than 15 %. At low, medium, high spiked levels, the measured recovery rates for the targets were all between 70.3 % and 128 %. The releases of organosulfur compounds in mainstream smoke of six Virginia type and four blended type cigarettes were analyzed, and the 10 cigarette samples were clustered into two types through principal component analysis, which indicated that organosulfur compounds might contribute to the flavour type of cigarettes.

## ST 37

### What is the best approach to assess the vapor-induced cytotoxicity of ENDS? A road map to the destination

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Electronic nicotine delivery systems (ENDS) are rapidly growing in popularity. Nonetheless, comprehensive quality and safety requirements for regulatory purposes are still under development and currently the same approach is used that was recognized some time ago to study the cytotoxicity induced by smoke. Cytotoxicity studies are important initial steps in appraising potential ENDS toxicity. The objective of this study was to screen different *in vitro* cytotoxicity methods for the assessment of ENDS cytotoxicity. We used the classic neutral red uptake (NRU) assay, the MTT assay, the Annexin V apoptosis (AN-V) assay, the high-content screening (HCS) assays and the real-time cell analysis (RTCA), to compare cytotoxicity induced by vapor from two electronic cigarettes (e-cigs) and two heated tobacco products (HTPs) with the smoke of 1R6F reference tobacco cigarette (University of Kentucky). Human bronchial epithelial cells (H292) were exposed to tobacco smoke and ENDS vapor at air-liquid interface (ALI). All tests showed reduced cell viability following 1R6F smoke exposure and slight or no reduction with all the ENDS, at 24 hours. AN-V and RTCA exhibited a further significant reduction in cell viability following 1R6F exposure. AN-V allowed to discriminate viable cells from those in early/late apoptosis. RTCA and HCS, being time-resolved analyses, elucidate the kinetic dependency parameter for toxicity of smoke/vapor chemicals on cell viability. In conclusion, NRU assay may be considered a suitable test, especially when combined with a time-resolved analysis, for assessing the kinetic of cytotoxicity induced by these products. We can conclude that ENDS induce hugely reduced damage to cell viability on epithelial bronchial cells compared to tobacco cigarettes, but the correct test must be considered based on the final goal to be achieved.

## ST 38

### *In vitro* to *in vivo* extrapolation (IVIVE) for evaluating exposure and health impacts of e-vapor products using ingredient and mixture *in vitro* data

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*In Vitro* to *In Vivo* Extrapolation (IVIVE) uses reverse dosimetry to translate *in vitro* bioactivity data to clinically relevant exposure metrics. This study applies the IVIVE approach to e-vapor products using reported *in vitro* cytotoxicity data of the e-liquids and *in vitro* high-throughput screening (HTS) bioactivity data for individual ingredients. Open-source physiologically based pharmacokinetic models were used to calculate the plasma concentrations of ingredients, followed by reverse dosimetry to estimate the human equivalent administered doses (EADs) for the e-vapor products based on assumed usage patterns. Three approaches (single actor, additive effect, and data integration) were tested to calculate EADs of e-liquids considering bioactivity of all known ingredients including carriers (propylene glycol [PG] and glycerol [G]), flavors, benzoic acid, and nicotine. EADs were also estimated for hypothetical e-liquids with only flavors for comparison. The results suggest that 1) when considering bioactivity of the mixture, carriers (>80 % of the total e-liquid mass) minimally impacted the EAD outcomes due to their low bioavailability; 2) flavors in the tested e-liquids (less than 1.2 % of the total mass) were not the major *in vitro* cytotoxicity drivers when compared to the hypothetical e-liquids consisting of flavors only to the whole e-liquid; and 3) nicotine (5 % of the total mass) was the primary bioactive ingredient based on available HTS *in vitro* data, affecting EAD outcomes in the tested e-liquids. This study demonstrates that the IVIVE approach allows a quantitative way to use publicly available modeling tools and *in vitro* database to support human risk assessment. This work also identifies gaps for future improvement, demonstrating how model assumptions, biological endpoints, assay selection, and data integration approach for mixtures can influence IVIVE results and interpretations.

## ST 39

### Influence of antioxidant capacity on micronucleus induction by cigarette smoke in various mammalian cell lines

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Several cell lines can be used in the *in vitro* micronucleus (MN) assay to evaluate the genotoxic potential of various chemicals. However, less information is available on cell types suitable for genotoxic assessment of cigarette smoke. Therefore, we compared the potential of MN induction by cigarette smoke in L5178Y, TK6, and CHL/IU cell lines. The total particulate matter of reference cigarette (3R4F) smoke was suspended in DMSO as a test sample. After 3 h of treatment followed by 24 h of recovery, a dose-dependent MN increase was observed in all cell lines. However, the lowest concentrations with significant genotoxicity and cytotoxicity differed among the cell lines. 3R4F smoke generates reactive oxygen species (ROS). Therefore, we explored the relationship between the different sensitivities to 3R4F smoke in each cell line and their antioxidant capacity. Total antioxidant capacity and cellular glutathione (GSH) were higher in CHL/IU cells than in L5178Y cells. Furthermore, pre-treatment of CHL/IU cells with N-acetylcysteine (NAC) or buthionine sulfoximine (BSO), a precursor and inhibitor of GSH biosynthesis, respectively, resulted in higher or lower 3R4F concentrations for significant genotoxicity and cytotoxicity. The same effect exerted by NAC or BSO was also seen after treatment with allyl isothiocyanate, a ROS-generating chemical, whereas treatment with mitomycin C, a genotoxin without ROS generation, was not affected by NAC or BSO pre-treatment. Pre-treatment with NAC increased the cellular thiol level, which may have prevented genotoxicity and cytotoxicity caused by 3R4F or allyl isothiocyanate. These results indicate that the degrees of genotoxicity and cytotoxicity induced by cigarette smoke differ among these cell lines and such differences might be related to the antioxidant capacity of the employed cell line.

## ST 40

### Development and characterization of an alternative rotary-like ENDS collection method for *in vitro* toxicology testing

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Testing of ENDS aerosol collected condensate in the *in vitro* toxicology assays is limited by OECD guidelines for the maximum allowable solvent levels that can be tested, as well as the final concentration of the generated aerosol condensate. The concentration of condensates collected using organic solvents such as ethanol, are limited in the neutral red uptake (NRU) assay to 0.5 % of vehicle in the dosing medium. Using our standard linear smoking machine collection procedure for ENDS aerosol in ethanol yields an average final condensate concentration of 60 mg/ml, with a resultant maximum NRU testing dose of 300 ug/mL. Testing of higher doses requires exceeding OECD guideline solvent limits or the generation of more concentrated condensates. Here we describe the development and characterization of a proprietary ENDS aerosol collection device designed to smoke multiple devices in sequential order to generate a continuous aerosol stream with similarities to a rotary smoke machine. Puffing is accomplished using a dual puffing engine and the aerosol generated is captured onto a 92 mm pad. This setup allows for collection of larger amounts of aerosol with final concentrations of ~200 mg/ml, thereby tripling the standard collection concentration. An additional advantage with this novel aerosol collection method is that it allows for collection times that are 10 times faster when compared to the standard linear smoking machine collection. Condensates collected from the standard linear smoking method and this novel alternative rotary-like device were characterized for nicotine and carbonyl levels, and response in the NRU assay. The nicotine and carbonyl levels measured were comparable between the two aerosol collection methods. Condensates collected from both collection methods, under non-intense and intense regimes, were compared in the NRU assay using 3T3 cells and A549 cells.

## ST 41

### Identification of a novel mode of action to induce micronuclei by 1-(4-methoxyphenyl)pent-1-en-3-one

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Compounds that test positive in the *in vitro* micronucleus test (MNT) need to be followed-up with *in vivo* MNT. However, it is not uncommon for some compounds to test negative in *in vivo* MNT, which are "misleading positives" in *in vitro* MNT. The well-known mode of actions (MoAs) of these results are induction of non-DNA reactive micronuclei (MN) by reactive oxygen species (ROS) or aneuploidy. We previously developed a platform consisting of ToxTracker and High Content Analysis (HCA) to determine the MoAs of *in vitro* MN positive compounds, as one of the indicators for clarifying "misleading positives". ToxTracker is a reporter gene assay that uses mouse embryonic stem cells to measure six endpoints related to DNA damage, oxidative stress, protein damage and cellular stress. With HCA, γH2AX, centromere, ROS and glutathione (GSH) are visualized directly in our platform. In this study, based on well-known compounds that induce MN through primary DNA damage, secondary ROS generation or aneugenicity, our objective was to investigate the MoA of 1-(4-methoxyphenyl)pent-1-en-3-one (MPP), a flavouring compound that has been discussed to give a misleading positive via aneuploidy by the European Food Safety Authority. The results of ToxTracker showed that treatment with MPP increased the expression of reporter genes for oxidative stress, which were suppressed by simultaneous treatment of cells with an antioxidant. In HCA analysis, MPP promoted the increases of γH2AX and ROS and a reduction in GSH. Aneuploidy was not detected by intracellular localization analysis of centromeres. To validate our findings, we performed *in vitro* MNT after pre-treatment with antioxidants, which resulted in suppression of MN induction. In this study, the reported MoA, aneuploidy, was not determined by our platform, but we determined the unknown MoA of MPP that causes a misleading positive was an indirect MN induction via ROS.

## ST 42

### Method development of an *in vitro* waterpipe air-liquid interface exposure system

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Waterpipe tobacco smoke (WTS) bubbles through water before being inhaled and is a complex mixture of particulate gases formed by the heating of tobacco, flavourings and humectants, usually using an external charcoal piece. To date, there are no validated methods to test the toxicity in an *in vitro* setting. So far, only a limited number of publications have investigated the effects of WTS on cell cultures, and even less have used 3D human airway epithelia combined with a standardized waterpipe smoking machine. There is a clear need to develop methods to ensure that data is obtained in a repeatable and reproducible manner. Following the puffing regime set in ISO Standard 22486, this study aimed at investigating the optimal system settings of an air-liquid interface (ALI) exposure system (Vitrocell Shisha testing device combined with VC1) to achieve dose delivery with minimal run-to-run variation. This was achieved by using a trapping DMSO solution in the exposure chambers which was analyzed by fluorescence. Subsequently, nicotine was quantified in the same trapping solution.

The method was first validated using reference 3R4F cigarettes smoked according to the Health Canada Intense regime. By simply changing the smoke dilution, a clear dose-response in fluorescence was observed. Waterpipe smoke was then analyzed but did not produce significant delivery with the dilution parameters used for 3R4F. Therefore, puff exhaust time, number of smoking runs, and set-up of the dilution bar were modified to try to achieve a similar deposition as for 3R4F.

This preliminary work is a prerequisite for the interpretation and comparison of future experiments using this system. The standardization of this set-up will identify the parameters required to reach biological significance and extrapolate *in vitro* results on 3D cell cultures to *in vivo* models by measuring endpoints such as cell viability, trans-epithelial electrical resistance (TEER) and cilia beating frequency (CBF).

## ST 43

### Quantitative analysis of NNN in plasma: a suitable alternative to urinary determination to assess the exposure to this important toxicant

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*N*-Nitrosonornicotine (NNN) is classified as a Group 1 carcinogen by the International Agency for Research on Cancer. Urinary NNN has often been used as a biomarker of exposure. However, urinary NNN excretion is highly variable, possibly due to formation of NNN by nitrosation of nornicotine in urine samples in acidic conditions. Hence, we developed an LC-MS/MS method for analyzing NNN in human plasma samples as an alternative to urinary NNN determination.

1 mL of plasma is purified through a two-step liquid-liquid extraction with methyl-tert-butylether followed by LC-MS/MS analysis utilizing positive mode electrospray ionization (ESI) and multiple reaction monitoring (MRM) detection.

The method was validated according to FDA guidelines on bioanalytical method validation. Inter-day accuracy and precision was measured on three different days (LLOQ, low, medium and high concentrations), with an average accuracy of 98.7 % and precision of 7.5 % CV. The method proved to be selective and highly sensitive with an LLOQ of 0.5 pg/mL and a broad linear range of 0.5 to 1000 pg/mL.

The assay was applied to plasma samples collected from ten US adult traditional smokeless tobacco users after a single, 40-minute, 2 g product use. Blood was sampled at 15 time points over a 6 h time course. NNN was quantifiable in 97 % of the study samples. The maximum NNN concentration ranged from 3.5 to 10 pg/mL (mean $C_{max}$: 7.1 pg/mL) with the time to maximal concentration ranging from 25 to 45 minutes (mean $t_{max}$: 31.5 min). A strong correlation in the pharmacokinetics (PK) of NNN and nicotine was observed in this study suggesting that the obtained NNN concentrations in plasma reflect the product use specific exposure.

Given the high sensitivity, specificity, and throughput, this newly developed analytical method is well suited for both PK and exposure assessment of NNN during use of traditional and emerging new tobacco products.

## ST 44

### Analysis of 3-hydroxybenzo[a]pyrene in urine as a biomarker of exposure for BaP in smokers and users of potentially reduced risk products

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Benzo[a]pyrene (BaP), which is classified as a Group 1 carcinogen by the IARC, is formed during the incomplete combustion of organic matter, like tobacco smoke. Urinary 3-hydroxybenzo[a]pyrene (3-OH-BaP) is commonly assessed as a biomarker of BaP exposure. The determination of 3-OH-BaP in non-occupationally exposed populations is challenging due to the very low concentrations of this biomarker. Therefore, we developed and validated a sensitive method for the quantification of 3-OH-BaP to investigate the contribution of smoking and use of potentially reduced risk products to the exposure to BaP in non-occupationally exposed subjects.

After enzymatic hydrolysis, the urine samples were purified using solid phase extraction, and finally the hydroxyl group was derivatized with 2-fluoro-methylpyridinium-p-toluenesulfonate. The extracts were analyzed by LC-MS/MS using an Acquity UPLC BEH C18 column (50 × 2.1 mm i.d., 1.7 µm; Waters) and MRM mode in ESI positive ionization (API 5000; Sciex).

The method was validated according to US FDA guidelines. The calculated accuracy rates for the low, the medium and the high concentration levels were 101.8 %, 92.6 %, and 110.5 %, respectively. Intra- and inter-day precision ranged from 4.3 % to 14.3 % CV. A precision of 8.4 % CV and an accuracy of 103.7 % was determined at the LLOQ of 50 pg/L.

The method was applied to 24 h-urine samples collected in a controlled clinical trial to distinguish between five different nicotine user groups including users of e-cigarettes, heated tobacco products, nicotine replacement therapy, oral tobacco, as well as smokers, and non-users (controls). A mean concentration of 149.0 pg/24 h was observed for smokers with a high correlation to urinary cotinine levels. For the other four nicotine user groups and non-users, values were significantly lower, mostly below LLOQ (99 % < LLOQ in these groups).

In conclusion, 3-OH-BaP is a suitable biomarker to assess the exposure to the carcinogen BaP by cigarette smoking.

## ST 45

### Meta-analysis study to establish population level estimates of NNAL in smokers and non-smokers

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The purpose of this research (a project of the CORESTA Biomarkers Sub-Group) was to establish a population level estimate for the biomarker of cigarette smoke exposure, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), to serve as a baseline against changes in exposure. We conducted a meta-analysis based on published literature between 2008-2020. A protocol for literature assessment was developed, followed by an evidence-based table to identify and select studies. The data template identified elements in four major categories: Study identification, Study Design, Results, and Demographics.

A total of 76 scientific articles were identified and reviewed for potential inclusion resulting in 42 studies that met the pre-set criteria (reported clinical studies and/or observational studies with reportable original values). The dataset was normalized to pmol/mg creatinine, a unit most commonly used in research studies. This was accomplished with the use of a "conversion template" based on parameters and assumptions (e.g. healthy individuals with average creatinine excretion of 2.208 gm/day (0.601-2.936 gm/day)). Total NNAL reported as "geometric" mean (excluding "median") were considered that resulted in 19 studies for the statistical evaluation. The database was organized by categories, filtered, and data was weighted according to the size of the groups. Most of the data was derived from smokers (n=12,218) followed by non-smokers (n=1,160).

Smokers had the highest level of NNAL (1.112 pmol/mg creatinine; 95 % CI 0.161-2.047) compared to non-smokers (0.008 pmol/mg creatinine; 95 % CI 0.001-0.017). Statistical analysis indicated that the two groups are significantly different ($p < 0.0002$). This research establishes population level estimate for NNAL levels that can be used to determine changes in exposure for smokers switching to potentially reduced-risk tobacco products (RRPs).

## ST 46

### Reductions in biomarkers of exposure to selected harmful and potentially harmful constituents following complete or partial transition to *my*blu™ ENDS (exclusive or dual use) compared to continued combustible cigarette smoking

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E-cigarettes offer an alternative, potentially reduced harm, method of nicotine delivery to adult combustible cigarette (CC) smokers, with harm reduction attributed to reduced levels of harmful or potentially harmful constituents (HPHCs) in their aerosols compared to cigarette smoke. The objective of these two randomised, open label studies was to measure reductions achieved, through exclusive or partial switching from CC to *my*blu™ products, in 15 biomarkers of exposure (BoE) to selected HPHCs associated with tobacco smoking in healthy US adult smoker subjects (n=72). After nine days of exclusive switching to a range of allocated *my*blu™ products, substantial reductions in 14 non-nicotine BoE ranging from 46-97 % were demonstrated, relative to baseline values representing CC use. Reductions were maintained in subjects who then continued to use *my*blu™ exclusively (n=25) for a further five days, and returned to near baseline levels in subjects who exclusively returned to CC use (n=21). Dual users of no more than 50 % self-reported CC use supplemented with *my*blu™ products (n=24) demonstrated reductions in BoE to a lesser extent than those who continued to switch exclusively. As expected, levels of nicotine equivalents did not significantly change throughout the study. These data suggest that exclusive use of high quality e-cigarettes provides substantially reduced exposure to HPHCs in adult smokers compared to continued CC smoking whilst achieving satisfying levels of nicotine delivery. Dual use involving some substitution of CC use may also provide some benefits in this regard, but to a lesser extent than complete substitution. Overall, these data contribute to the weight of evidence that e-cigarettes are an important tool in tobacco harm reduction strategies for adult smokers unwilling to or uninterested in quitting smoking.

## ST 47

### Biomarkers of potential harm in smoking abstinence and in the use of Vuse electronic nicotine delivery systems (ENDS)

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Qualified biomarkers of potential harm (BoPH) are useful in evaluating the beneficial effects of abstinence from cigarette smoking or switching to potentially reduced risk tobacco products. We benchmarked BoPH changes in a 14-day smoking abstinence study in two age groups and used those BoPH to assess the effects of Vuse ENDS products. This smoking abstinence study was conducted under confinement conditions, and enrolled 70 subjects into younger (24-34 years, n=33) and older (35-60 years, n=37) groups. Several biomarkers of exposure and BoPH were evaluated.

Significant declines in Leukotriene E4 (LTE4), 2,3-dinor-thromboxane B2 (2,3-d-TXB2), neutrophils, white blood cells (WBC) and select arterial blood cell parameters were observed in both age groups at Day 7 and 14 compared to baseline, while other BoPH, (e.g. arterial blood gases, fractional exhaled nitric oxide) showed age-related effects. In a separate confinement study where smokers abstained from smoking or switched to three Vuse ENDS products for seven days, complete blood counts were analyzed. The results showed that WBC, neutrophil and red blood cell (RBC) counts, along with hematocrit and hemoglobin levels, decreased in smokers who were switched to Vuse ENDS to the same extent observed in seven days of smoking abstinence. The BoPH assessed in these studies are indicators of platelet activation (2,3-d-TXB2) and inflammation (LTE4, WBC and neutrophils), and provide useful clinical risk markers for assessing candidate modified risk tobacco products in short-term studies. In each study, rapid and reproducible reductions in LTE4, 2,3-d-TXB2, WBC and neutrophil counts were consistently detected following smoking abstinence or switching to Vuse ENDS, indicating the value of these markers as BoPH.

## ST 49

### Assessment of alternative cell lines for adoption within the OECD TG 129 for cytotoxicity determination

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Neutral red uptake assay (NRU), part of the *in vitro* CORESTA test "battery" is one of the most commonly used cytotoxicity assays for the evaluation tobacco and next generation products (NGPs). OECD TG 129 guideline for the NRU assay was originally adapted for mouse fibroblasts (BALB/c 3T3) and normal human epidermal keratinocytes (NHK), and was validated in the NICEATM-ECVAM multi laboratory *In vitro* Basal Cytotoxicity Study with 72 reference substances (ICCVAM 2006).

The aim of the present study was to validate Imperial Brands' approach for cytotoxicity response determinations, which follows the OECD TG 129 methodology with more human-relevant cellular systems for target organ (BEAS-2B human lung cells) and systemic toxicity predictions (HepG2 human liver cells).

Cytotoxicity responses to selected substances from the ICCVAM list, expressed as $EC_{50}$ (effective concentration triggering 50 % reduction in viability) were evaluated in the proposed cell lines, and compared to the OECD TG 129 recommended cell line (BALB/c 3T3) and to historical data.

The results show a high correlation in ranking between cell lines. The data indicates that BEAS-2B and Hep-G2 cells deliver comparable results in the NRU assay to that of the BALB/c 3T3 cells as recommended by OECD. Additionally, a significantly increased responsiveness of human cell lines to glycerine, ethylene glycol and particularly to nicotine was found.

The results of this internal validation study confirm the applicability of human-relevant cell lines for the cytotoxicity determination following the OECD method. Moreover, the superior sensitivity of BEAS-2B and HepG2 cell lines over BALB/c 3T3 to nicotine- and NGP-relevant substances, such as glycerine, is particularly advantageous for human-relevant NGP assessments.

## ST 50

### *In vivo* genotoxicity testing of aerosols generated from JUUL ENDS products

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We evaluated the *in vivo* genotoxicity potential of aerosols generated from two commercial nicotine electronic delivery systems (ENDS) products, P1 and P2, with a combined micronucleus (MN) and comet study design based on OECD TG 474 and TG 489. P1 and P2 were found to give positive and equivocal responses in *in vitro* micronucleus (MN) assays. P1 and P2 were puffed using an intense regimen (110 ml puff volume, 6-second puff duration, and a 30-second interval) to generate aerosols at 3000 µg/L or 4000 µg/L aerosol mass with a mass median aerodynamic diameter of 0.9-1.0 µm and a geometric standard deviation of 1.6. Male Sprague Dawley rats were exposed to filtered air (negative control) or to ENDS aerosols via nose-only inhalation for up to 6 hours/day for four consecutive days. Blood samples were collected immediately after the Day 3 exposure and analyzed for biomarkers of exposure (nicotine, cotinine, and propylene glycol). At necropsy, bone marrow samples were collected for MN evaluation and liver, lung, and nasal tissue samples were collected for the comet assay (DNA breakage). For both P1 and P2, the plasma nicotine, cotinine, and PG levels increased with increasing aerosol exposure duration in the test article-treated groups, with the plasma nicotine concentrations reaching 1240 ± 158 ng/mL and 1260 ± 265 ng/mL, respectively. There were no significant increases in the bone marrow % MN or in the liver, lung, and nasal tissue % Tail DNA (DNA breakage) compared to the negative control group. Therefore, under the test conditions, P1 and P2 aerosols were concluded negative *in vivo* for genotoxicity risk.

## ST 51

### *In vitro* evaluation of tobacco free nicotine pouches

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Non-clinical *in vitro* studies were conducted to investigate the characteristics of tobacco free nicotine pouches alongside a reference snus product and marketed snus product. The investigation included the evaluation of nicotine transfer to artificial saliva, Ames tests in five *Salmonella typhimurium* strains, micronucleus assay, and neutral red uptake (NRU) cytotoxicity assay. Results from *in vitro* nicotine transfer tests suggested that most of the nicotine was extracted after approximately 20 minutes. Additionally, results from the Ames, micronucleus and NRU cytotoxicity assays indicated that the tobacco free nicotine pouch products were non-mutagenic, non-genotoxic and non-cytotoxic. Tests to investigate the cytotoxicity in human gingival epithelial cell cultures were also conducted, the results similarly showed a non-cytotoxic response.

## ST 52

### A prediction model for lip sticking force of cigarette tipping paper based on friction coefficient and grammage

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To quickly and accurately determine the adhesive force of cigarette tipping paper to lips, a prediction method was proposed based on friction coefficient and grammage. A peel strength tester was used to measure the adhesive force between tipping paper of different brands and artificial mouth skins bonded together by artificial saliva. Through comparison and optimization, the experimental conditions were determined as follows: use of medical silica gel as artificial mouth skin, prepared artificial saliva containing proteins, enzymes and other biologically active and inorganic salts, bonding length of tipping paper and artificial lips 10 mm, bonding area 150 $mm^2$, tensile speed 50 mm/min, artificial saliva volume 6 µL, and standing time 1 min. The lip sticking force of tipping paper strongly correlated to its friction coefficient and grammage, therefore a prediction model for lip sticking force was developed by using the dynamic friction coefficient and grammage. The predicted values basically agreed with the measured values with the coefficient of determination ($R^2$) between predicted and observed values of 0.98 and the normalized root mean square error (nRMSE) of 6.4 %, which indicated that the model was accurate and reliable. This method could quickly, conveniently and quantitatively determine the lip sticking force of tipping paper dispensing with the influence of subjective factors and differential representations of panel sensory evaluation. This study provides a theoretical basis for material testing, quality control and product improvement in the tobacco industry.

## ST 53

### Analysis of volatile compounds in tipping glue by HS-SPME coupled with GC-MS using C3N4 coated fibers as SPME fibers

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Volatile organic compounds (VOCs) in tipping glues influence the sensory quality of cigarettes and it is important to monitor the VOCs in tipping glues. However, due to the complex matrices and the low content, sample pre-treatment prior to analysis is necessary. Headspace solid-phase microextraction (HS-SPME) is suitable for the VOCs in trace amounts from complex samples, but fibre coating is crucial. The purpose of this study was to prepare a new coated fibre used as HS-SPME material for the analysis of VOCs in tipping glues. A graphic carbon nitride (C3N4) coated fibre was prepared via the pyrolysis of melamine on a quartz fibre. The extraction performance of the C3N4 coated fibre for the VOCs from tipping glues was compared with that of commercial fibres, such as polyacrylate (PA), through HS-SPME coupled with gas chromatography-mass spectrometry (GC-MS). The C3N4 coated SPME fibre extracted more compounds than PA due to its unique physiochemical properties. A method for determining the VOCs in cigarette adhesives by SPME-GC-MS with the C3N4 coated fibre was established. More than 20 VOCs in tipping glues were initially screened with more than 85 % of similarity through the alignment with NIST library after C3N4 SPME. Among these compounds, three phthalate compounds were qualitatively and quantitatively analyzed. The linear range of the method was from 10 ng/g to 1000 ng/g. The limits of detection (LODs) were 2.3-7.9 ng/g. The recoveries ranged from 77.9 % to 95.4 %. The intra- and inter-day relative standard deviations (RSDs) of the three phthalate compounds were 3.3-11.4 % with the fibre-to-fibre reproducibility (n=3) of 18.9 %. The established C3N4 HS-SPME-GCMS method was used to analyze the VOCs in tipping glues from different manufacturers. The results indicate that the developed C3N4 coated fibre might be a promising alternative for the SPME of volatile compounds in cigarette products.

## ST 54

### Effects of cigarette circumference on cigarette ventilation rate during smoking

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Cigarette ventilation is one of the key parameters during cigarette design, that strongly affects cigarette combustion behaviour and the smoke components released. When combustion occurs, smoke is produced and transferred from the cut tobacco to the filter. Air viscosity, moisture and other parameters along the cigarette axial direction are altered compared with the unburned cigarette. There are few studies characterizing the instantaneous ventilation of a burning cigarette due to the rapid change during combustion. The purpose of this study was to develop a simple and accurate method to determine the puff-by-puff ventilation along the axial direction during cigarette burning, and the ventilation for the unburned and burning cigarette. Three cigarettes with different circumference (super-slim 17 mm, 20 mm and king-size 24 mm) and with the same blend were compared.

The combustion coal and the unburned part of the cigarette were separated by a movable sealing tube. A soap film flow tube with low resistance was used to measure the ventilation air flow of the unburned part in real time and the total real time ventilation of the burning cigarette was obtained. The results were as follows: 1) Along with the burning, the total real time ventilation of the cigarette decreased gradually. 2) Compared with the first puff, the total real time ventilation of super-slim cigarettes in the last puff decreased 37.5 %. While for king-size cigarettes, the corresponding decrease is only 29.5 %. 3) Because of the resistance of combustion coal, same residual length of cut-tobacco segments showed great increases of real time ventilation in each puff for burning cigarettes compared to unburned cigarettes. Furthermore, the puff-by-puff average increase of burning super-slim cigarettes (74.5 %) is greater than that of king-size cigarettes (55.7 %) and 20 mm cigarettes (60.5 %).

## ST 55

### Preparation of cigarette packet blanks with high barrier and moisture retention, moisture resistance and flavour keeping performance

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In order to improve the moisture retention, moisture resistance and flavour keeping performance of cigarette products in different environments, a series of coatings with high barrier properties were prepared by using an environmentally friendly polymer material as matrix modified by a natural lipid. Cigarette packet blanks were coated with the prepared coating solutions, the water vapor barrier of the blanks was tested before and after coating. In addition, the effects of coating on the physical moisture retention, moisture resistance and flavour keeping performance of cigarettes were studied. The results showed that compared with the uncoated blanks, the water vapor transmission rates of coated blanks significantly decreased with the largest drop of 83.23 %; under the same conditions, the total moisture loss in a dry environment and the total moisture absorption in a humid environment of cigarettes packed in a packet with a coated blank decreased by 16.81 % and 18.03 %, respectively. In conclusion, the water vapor barrier of packet blanks was increased by coating, and the physical moisture retention and moisture resistance of the cigarettes were significantly improved. Moreover, the flavour keeping performance and sensory quality of the cigarettes were also improved.

## ST 56

### Influence of multi-layer packaging of cigarette packets on their moisture barrier

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The barrier performance of cigarette packaging plays a key role in the quality consistency of cigarette products. In order to study the influence of multi-layer packaging on the moisture barrier in a dry environment, an automatic dynamic moisture analysis climate chamber was developed to investigate the moisture diffusion process through different packaging materials under specific temperature and humidity conditions. A moisture barrier index was used to characterize the moisture barrier performance quantitatively. The results showed that: the evaluation method for moisture barrier performance of multi-layer packaging of cigarette packets could accurately quantify and characterize the moisture barrier performance of the packaging, and the model calculation values were consistent with experimental values. Under low humidity conditions, the amount of moisture permeating through the clearance between layers and the materials of different layers of the cigarette pack was different. For BOPP films, moisture mainly diffused through the film. For different labels and inner liners, the distribution ratios of moisture diffusion through the material and the clearances were different. The moisture barrier of BOPP films was the key factor influencing the moisture consistency of cigarettes. The moisture barrier performance of hard packets was better than that of soft packets. The moisture diffusion processes of cigarettes in different packets were predicted accurately. In this paper, a moisture barrier test and evaluation method for multi-layer cigarette packaging was established. The moisture barrier performance of each layer and different parts of each layer of cigarette packets was quantitatively characterized and the key factors affecting the moisture consistency of cigarettes in packets were identified. Moreover, the prediction of moisture content in cigarettes within different packets in a known environment was realized.

## ST 57

### Nonclinical toxicity assessment of oral tobacco-derived nicotine products: I. Framework

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There is an increasing acknowledgement of a broad "continuum of risk" among tobacco products, with combustible cigarettes at the highest end and non-combustible products toward the lower end of health risk. The oral tobacco-derived nicotine (OTDN) product category is one of the fast-growing tobacco product segments in the United States, offering an alternative form of nicotine products that do not contain tobacco. A rigorous nonclinical toxicological assessment is an integral part of a scientific evaluation of the potential health risks of OTDN products compared to other tobacco products, including combustible cigarettes. We present an overview of our nonclinical toxicity assessment framework that includes: 1) ingredient evaluation, 2) test article extraction and characterization, 3) *in vitro* OECD regulatory testing, and 4) *in vitro* oral health mechanistic testing. Four companion presentations will follow to provide additional details on each of these areas. Briefly, individual ingredients are evaluated through *in silico* and expert judgement risk assessment in addition to review of published literature. A range of different categories of tobacco products encompassing cigarettes, moist smokeless tobacco, snus, and OTDN products are selected and extracted with ethanol (cigarette smoke condensate) or artificial saliva (oral products) and characterized for nicotine prior to *in vitro* testing. For *in vitro* testing, standard OECD regulatory assays (cytotoxicity, mutagenicity, and genotoxicity) and mechanistic inflammatory endpoints in primary human gingival fibroblast cells were used. Results from these tests show that oral tobacco products have substantially lower biological activities compared to cigarette smoke, supporting the reduced-risk potential upon complete switching from smoking cigarettes.

## ST 58

### Nonclinical toxicity assessment of oral tobacco-derived nicotine products: II. Ingredient evaluation

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The oral tobacco-derived nicotine (OTDN) product category is one of the fastest growing tobacco product segments in the United States offering alternative nicotine products that do not contain tobacco. A rigorous nonclinical toxicological assessment is an integral part of a scientific evaluation of the potential health risks of OTDN products compared to other tobacco products. The toxicological risk assessment strategies for evaluating these products and their non-tobacco ingredients are evolving; in this study *in silico* or computational toxicology is incorporated as a part of the hazard identification step. Using a tiered approach, we first perform a thorough literature review on ingredient-specific toxicological information to determine if the ingredient is appropriate for inclusion in an OTDN. We then examine each ingredient using a statistical-based (CASE Ultra) and a knowledge-based (Lhasa Derek) *in silico* hazard prediction software for systemic cancer and non-cancer risk (i.e. carcinogenicity, mutagenicity, irritation). For each ingredient, we also conduct a quantitative risk assessment (QRA) and compare the reference toxicological threshold (e.g. acceptable daily intake [ADI]) identified from the literature review, with relevant uncertainty factors and calculate a margin of exposure (MOE) under usage scenarios based on consumer actual use data (e.g. eight or higher OTDN pouches/day, 100 % intake assumption). For the ingredients evaluated, the estimated daily exposure from typical product use does not exceed the reference safety limit (MOE = 1). The risk assessment outcomes (predicted low toxicity of OTDN) are supported by experimental *in vitro* testing results. The *in silico* assessment could be an important tool to characterize the toxicological profile of novel tobacco products which can inform the overall health risks.

## ST 59

### Nonclinical toxicity assessment of oral tobacco-derived nicotine products: III. Extraction and test material characterization

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The oral tobacco-derived nicotine (OTDN) product category is one of the fastest growing tobacco product segments in the United States, offering alternative nicotine products that do not contain tobacco. A rigorous nonclinical toxicological assessment is an integral part of a scientific evaluation of the potential health risks of OTDN products compared to other tobacco products. In this study, we established the methodologies for preparing test materials from different tobacco product categories using various solvents and characterized them prior to *in vitro* testing. For combustible cigarettes, smoke from 3R4F reference cigarettes was collected in ethanol (condensate), capturing both particulate matter (TPM) and gas vapor phase (GVP), and was analyzed for nicotine and select harmful and potentially harmful chemicals (HPHCs). For smokeless tobacco products, CORESTA reference products (CRP 2.1 and CRP 1.1) and commercial OTDN products were extracted with ethanol and artificial saliva and analyzed for nicotine and select HPHCs. The results showed that ethanol was efficient at the extraction of nicotine and tobacco-specific nitrosamines (TSNAs; > 80 % of CORESTA reference values) for cigarettes and smokeless tobacco (CRP) products. Similarly, within the smokeless CORESTA reference products, artificial saliva demonstrated equivalent extraction efficiency with respect to nicotine and TSNAs (> 80 %) when compared to ethanol. The nicotine extraction of OTDN was efficient (> 80 % of product label) in both solvents, with little to no detectable levels of additional HPHCs in either solvent. The nicotine levels in all extracts, irrespective of solvent, were stable for a minimum of eight weeks (stored at -70 °C) to accommodate the time needed to complete *in vitro* testing. The results support the use of both ethanol and artificial saliva in preparing test materials from various tobacco product categories with an overlapping range of nicotine to allow subsequent *in vitro* result comparison.

## ST 60

### Nonclinical toxicity assessment of oral tobacco-derived nicotine products: IV. *In vitro* regulatory testing

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The oral tobacco-derived nicotine (OTDN) product category is one of fastest growing tobacco product segments in the United States offering alternative nicotine products that do not contain tobacco. A rigorous, nonclinical, toxicological assessment, including *in vitro* assays, is an integral part of a scientific evaluation of the potential health risks of OTDN products compared to other tobacco products. The toxicity assessment was performed using the standard regulatory *in vitro* assay battery: neutral red uptake (NRU) for cytotoxicity, Ames assay for mutagenicity, and micronuclei (MN) assay for genotoxicity. Ethanol extracts from a commercial OTDN product were first evaluated in comparison to condensate from reference cigarette (3R4F), extracts from reference moist smokeless tobacco (CRP2.1), and reference snus (CRP1.1). The 3R4F condensate was cytotoxic ($IC_{50}$: 3.67 µg/mL nicotine), mutagenic (strain TA98+S9 and TA1537+S9), and genotoxic (4h+S9) when tested up to 13.5 µg/mL nicotine. Extract from CRP 2.1 was not cytotoxic, weakly mutagenic (strain TA1537+S9), and non-genotoxic, whereas CRP 1.1 and OTDN extracts did not demonstrate cytotoxicity, mutagenicity or genotoxicity at the concentrations tested (up to 7-14 µg/mL nicotine). Two commercial OTDN products, CRP 1.1 and CRP 2.1, were also extracted using artificial saliva and subjected to *in vitro* testing at concentrations up to 162.5-367.5 µg/mL nicotine. The oral product extracts were found to be noncytotoxic, non-mutagenic, and non-genotoxic at the concentrations tested, except for CRP 2.1, which was positive in the MN assay (27h-S9). The results demonstrated that OTDN products had substantially lower toxicity than cigarettes, thus supporting their reduced-risk potential upon complete switching from smoking cigarettes.

## ST 61

### Nonclinical toxicity assessment of oral tobacco-derived nicotine products: V. *In vitro* mechanistic assays using human gingival fibroblasts

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The oral tobacco-derived nicotine (OTDN) product category is one of the fastest growing tobacco product segments in the United States, offering alternative nicotine products that do not contain tobacco. A rigorous nonclinical toxicity assessment is an integral part of a scientific evaluation of the potential health risks of OTDN products compared to other tobacco products. In this *in vitro* study, we used primary human gingival fibroblasts (HGF) as the *in vitro* test system to evaluate mechanistic toxicity responses of ethanol extracts of a commercial OTDN product, reference cigarette (3R4F) smoke condensate, reference moist smokeless tobacco (CRP 2.1) and reference snus (CRP 1.1). HGF cells were exposed to eight concentrations (up to 14 µg/mL nicotine) of each extract for up to 48 hours. The 3R4F smoke condensate extracts elicited concentration dependent cytotoxicity, increased oxidative stress (increased lipid peroxidation and decreased GSH/GSSG ratio) and inflammation (increased IL-8, MMP-1, and PGE-2). In contrast, OTDN extracts showed minimal cytotoxicity and no changes in oxidative stress (lipid peroxidation, GSH/GSSG ratio) or inflammation endpoints (e.g. IL-8) tested at similar nicotine concentrations. Other oral tobacco products (CRP 1.1 and CRP 2.1) showed responses that mostly overlapped with the OTDN product, except for CRP 1.1 being slightly more cytotoxic and CRP 2.1 eliciting increased oxidative stress (decreased GSG/GSSG ratio) compared to the OTDN product. The results demonstrate that the HGF cells are suitable as the test system to evaluate oral health-related mechanistic endpoints across different tobacco product categories and that oral tobacco products had substantially lower toxicity than cigarettes, thus supporting the reduced-risk potential upon complete switching from smoking cigarettes.

## ST 62

### Market survey of modern oral nicotine products: determination of select HPHCs and comparison to traditional smokeless tobacco products

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In an effort to combat the risks associated with traditional tobacco products, tobacco science and product innovation has been redirected towards providing the consumer with products that may limit their potential exposure to harmful or potentially harmful constituents (HPHCs). Among these product innovations are modern oral nicotine products (MONPs), or tobacco-free nicotine products (TFNPs). This product class aims to deliver nicotine while reducing the consumer's potential for toxicant exposure. This body of work sought to investigate the potential for select HPHC exposure (tobacco-specific nitrosamines, carbonyls, benzo[a]pyrene, nitrite, metals) from MONPs and to compare it to that from traditional tobacco products. Given the recent push towards reducing the consumer's potential risk, this work expands on previously published studies both in terms of diversity of products assessed and analytes tested. In total, twenty-one unique MONPs were assessed and compared to four traditional tobacco products.

We found that there was a difference in the potential exposure based on the MONP filler—plant material vs granulate/powder. Typically, the HPHC levels observed in plant-based MONPs were higher than those observed for granulate/powder products with this trend most significant within the metals analysis. Here, the observed levels of select metals were generally higher in plant-based MONPs, the levels of which were, in some instances, also greater than those seen in traditional smokeless tobacco products. Generally, the overall HPHC levels observed in MONPs were at or below those levels observed in traditional tobacco products.

## ST 63

### An 18-month e-liquid stability study

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There has been a trend by vendors of e-liquids to offer larger containers of e-liquids than in the past. Thus, it is possible that larger containers may be kept at ambient temperatures for several months or more. Therefore, we began an ageing study in October 2019 with four highly flavoured, high-glycerol e-liquids (all 6 mg/mL nicotine) in 120-mL bottles. The flavour descriptors on the labels and sample numbers were: 1) crunchy cookie and coconut; 2) blue raspberry and candy; 3) choco chip cookie and vanilla ice cream; and 4) cherry pie and custard. Samples were taken weekly through February 2020 with samples put in frozen storage each week. At the end of six months, samples of each e-liquid and their frozen controls were sent to an ISO 17025 accredited laboratory for cytotoxicity assays by the neutral red uptake (NRU) method. No differences were reported. Shortly thereafter, the study was suspended due to Covid-19 restrictions. The frozen samples that had been taken weekly since the first sampling were kept frozen, and the remaining samples in the 120-mL bottles were kept at ambient temperature. Samples of both frozen and ambient aged e-liquids were dissolved in methanol at 1 % (w/v) and 10 % (w/v) concentrations. Initial analyses were done on a Cogent Bidentate C18 column (250 × 4.6 mm) with a mobile phase of 25 % acetonitrile and 75 % water at a flow rate of 1 mL/min with UV detection at 280 nm. Based on the chromatograms obtained under those analytical conditions, Samples 3 and 4 were the most stable. Sample 2 was the least stable, showing apparent chemical reactions between the ethyl maltol and other components of the e-liquid. Sample 1 showed loss of early eluting peaks as well as what may have been reactions of the propylene glycol acetals of aromatic aldehydes with glycerol.

## ST 64

### Effect of pH and storage temperature on e-liquid metal concentrations

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E-liquids are available in a variety of nicotine formats and concentrations. Nicotine formats can be either nicotine freebase or nicotine salt. Nicotine salt is formed by combining nicotine with an organic acid. The pH of these nicotine salts ranges from 4-6 depending on the molar ratio, while the freebase is 7-9. The pH of the e-liquid has been shown to have an effect on the corrosion rate of the heater coils. To illustrate this effect quantitatively, different nicotine formulations at varying pH levels (pH 4-9) were formulated and tested. The nicotine was added to a 50:50 PG/VG base and the pH was then buffered to a range between 4-6 with lactic acid or benzoic acid to form nicotine lactate and nicotine benzoate respectively. Pods that contained a Nichrom wire were filled with these nicotine formulations and were stored at an elevated temperature (40 °C) for 7 days. In this study, a direct correlation was observed between decreases in pH and the concentration of metals measured in the e-liquid, specifically nickel and chromium. In addition, increases in the storage temperature also led to increases in metal levels observed. Further investigation is needed to evaluate the relationship of temperature on the chemical reactions of the e-liquid and metal wire.

## ST 65

### A 12-month stability study on JUUL Virginia tobacco flavored aerosols using two non-targeted analytical methods

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Combustible cigarette (CC) smoking is the leading cause of preventable human death, owing to the toxicants produced during combustion and the chemical complexity of the CC smoke. The United States Food and Drug Administration (FDA) Family Smoking Prevention and Tobacco Control Act, protects public health by regulating the manufacturing, distribution, and marketing of tobacco products. Beyond the analysis of target compounds, the FDA Premarket Tobacco Product Application guidance recommends that applicants evaluate chemical changes in their product over its shelf-life. Samples were aged using International Conference on Harmonization's climate zones III and IV long term conditions. Aerosols were collected from three replicates, each from three production batches of JUUL Virginia Tobacco 5.0 % nicotine by weight (VT5) using intense and non-intense puffing conditions. The objectives of this study were to 1) thoroughly chemically characterize the aerosols over a 12-month stability study and monitor the changes in aerosol chemistry, and 2) compare the chemical complexity of aerosols to combustible cigarette smoke (CCS). Therefore, two non-targeted analytical methods were applied to initial aerosol (T=0); remaining samples were stored in their original packaging and aerosolized and analyzed at six months (T=6) and again at 12 months (T=12). Trends from T=0 through T=12 show an increase in the total number of compounds detected, from 91 rising to 114 and spanning approximately 0.2 %-0.4 % of the total aerosol mass. There were 24 compounds formed comparing combined T=0 and T=6 results to T=12 results – which amounted to only 0.04 % by mass. After thoroughly evaluating VT5 for unique constituents it was observed that during the 12-month stability study there were only minimal chemical changes to the aerosols, and the chemical complexity increased with storage time, but remained approximately 35-fold less chemically complex than CC smoking.

## ST 66

### Comparison of collection strategies for the analysis of targeted compounds in e-cigarettes aerosol

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The Premarket Tobacco Product Application (PMTA) process for ENDS recommends that a wide range of harmful and potentially harmful compounds (HPHCs) be determined to support the application. The U.S. Food and Drug Administration provides guidance specifying a number of constituents and chemicals that should be included for analysis. However, no guidance is provided on appropriate analytical methodologies or test procedures. The majority of published literature reports targeted compound yields using the collection of a single puff block, often 50 puffs, or collection of multiple puff blocks over a single sample. In this study, first 50-puff collection was compared to whole pod measurement (WPM), using 1-end of pod life collections. Data was collected under both ISO 20768 (55/3/30) and intense (110/6/30) puffing regimes, using eight commercially marketed products from a number of manufacturers. Yields were normalized to total device mass loss. Analytes included primary constituents, carbonyls, metals, and glycidol. Two limitations of the first 50-puff approach were identified: (1) some targeted analytes, i.e. carbonyls, were found to be non-linear with aerosol yield over the entire pod life, thus whole pod yield could not be determined from 50-puff measurements, and (2) WPM improved the method limit of detection (LOD) and limit of quantitation (LOQ) by five-fold or more, compared to 50-puff, due to increasing collected aerosol mass. As such, some analytes that were below LOD/LOQ for 50-puffs, were found to be reportable (>LOQ) with WPM. Formaldehyde, acetaldehyde, acrolein, and diacetyl showed significant differences between collection approaches, with up to 500 % difference observed in analytical results. This study confirms that WPM improves the accuracy of carbonyl and other analyte yields for all ENDS products tested.

## ST 67

### A comparison of the yield of select analytes from JUUL e-cigarette aerosols and eight Korean brand cigarettes

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Combustible cigarette (CC) smoke contains known toxicants which are produced during pyrolysis and combustion of tobacco-plant materials, paper, and non-tobacco ingredients – 93 of which are listed as harmful or potentially harmful constituents (HPHC) by the Food and Drug Administration (FDA). In this study, HPHC levels were measured in aerosol generated from a JUUL ENDS device with two e-liquid formulations (Virginia Tobacco, Glacier Mint, 0.77 %). HPHC levels measured in these aerosols were compared with reported HPHC levels measured in smoke from eight Korean in-market "low yield" "light" cigarettes.

An assessment of aerosol constituent levels was determined from 10 aerosol replicates each using validated methods. Puffing topography was 70 mL volume, 3-second puff duration, and 30-second inter-puff interval. Selected aerosol constituents include: 1-aminonapthalene, 1,3-butadiene, 2-aminonapthalene, 4-aminobiphenyl, acetaldehyde, acetoin, acetyl propionyl, acrolein, acrylonitrile, ammonia, anabasine, benzene, benzo(a)pyrene, cadmium, chromium, copper, crotonaldehyde, diacetyl, diethylene glycol, ethylene glycol, formaldehyde and glycerol.

An evaluation of average level for all analytes (excluding glycerol and nicotine), when normalized to yield per puff, showed approximately 186-fold higher emissions in "low yield" cigarettes, which corresponded to 99.5 % reduction in JUUL aerosol. Metals – cadmium and lead were determined to be approximately 290-fold and 60-fold higher in CC smoke, respectively. Tobacco specific nitrosamine – NNN and NNK – were respectively 667-fold and 2054-fold higher in CC smoke. Carbonyls – formaldehyde, acetaldehyde, acrolein and crotonaldehyde – were on average found to be approximately 2763-fold higher. In general, 20 of 22 analytes in JUUL aerosol were below the level of quantification. Given the significantly lower amounts of HPHC and chemicals in JUUL aerosols, our results indicate that the chemical composition is substantially different from CC smoke.

## ST 68

### Targeted characterization of the chemical composition of novel JUUL product aerosol to 3R4F cigarette smoke

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Electronic nicotine delivery systems (ENDS) generally emit reduced levels of harmful and potentially harmful constituents (HPHCs) compared to combustible cigarettes; the degree of constituent reduction can vary across ENDS product designs. In this study, HPHC levels were measured in aerosol generated from a novel JUUL ENDS device with four novel e-liquid formulations. HPHC levels measured in these aerosols were compared with reported HPHC levels measured in smoke from the 3R4F reference cigarette.

An assessment of aerosol constituent levels was determined as a function of number of puffs based upon device mass loss (whole pod yield). Based on U.S Food and Drug Administration, Premarket Tobacco Application and European Tobacco Products Directive guidance, selected aerosol constituents include: nicotine, menthol, propylene glycol, ethylene glycol, diethylene glycol, glycerin, diacetyl, 2,3-pentanedione, formaldehyde, acetaldehyde, acrolein, crotonaldehyde, butyraldehyde, furfural, benzyl acetate, ethyl acetate, ethyl acetoacetate, isoamyl acetate, isobutyl acetate, methyl acetate, n-butanol, anabasine, anatabine, ß-nicotyrine, cotinine, mysomine, nornicotine, nicotine-N-oxide, chromium, iron, nickel, copper, zinc, arsenic, cadmium, tin, silver, lead, beryllium, cobalt, selenium, gold, 1,3-butadiene, isoprene, acrylonitrile, benzene, toluene, propylene oxide, propionic acid, benzoic acid, NNK, NNN, water, and glycidol. Aerosol pH were also measured. Seven replicates were performed for each constituent measured.

Reference cigarette 3R4F HPHC values measured in smoke generated under non-intense, [ISO 3308] and intense [ISO 20778] puffing regimes were obtained from the literature. These were compared to matched compounds measured in the aerosols from the novel JUUL devices. There were substantial reductions in the number of detectable HPHCs in JUUL aerosols relative to 3R4F reference cigarette smoke. Overall, the HPHC levels, excluding nicotine, menthol, propylene glycol and glycerin, measured in aerosols from the novel JUUL device/e-liquids generated under intense and non-intense puffing regimens were, on average, ≥96 % lower than those reported in 3R4F reference cigarette smoke.

## ST 69

### A robust statistical approach for estimating method precision

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Collaborative studies are widely used for evaluating the performance of analytical methods. The ISO standards suggest first checking for the removal of any atypical observations (outliers) before estimating the repeatability and reproducibility of methods. However, a usual rejection of such data might reduce the reliability of evaluations, especially for smaller samples. Robust statistics provide an alternative procedure capable of estimating the method performance without exclusion of data. However, this alternative procedure does not provide information about the alignment of lab results with the estimated global mean, repeatability and reproducibility as requested for their accreditation. To obtain this information, two *a posteriori* statistical tests were implemented: one for checking the distance between labs' means and estimated global mean, and the other for comparing labs' variabilities to the robust intra-laboratory variability.

In this presentation, the application of ISO robust statistical methods to assess data precision (repeatability and reproducibility) and these two *a posteriori* tests will be explained, with a worked example using a cigar collaborative study. This example shows that the robust method allows reliable estimation of the method precision when less than eight laboratories are involved in a collaborative study.

## ST 70

### Eliminate or accommodate outliers? A comparison between standard and robust approaches for the analysis of collaborative study data

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As part of the definition of an analytical method, collaborative studies are carried out to characterize the variability of the method and are generally analyzed using the recommendations of ISO 5725-2. An important part of the analysis using those recommendations is the identification and elimination of outlying laboratories and the details of how this is carried out can have an unsettlingly large effect on the estimated variability of the analytical method. If outliers are not eliminated, the variability can be estimated to be too large and if too many outliers are identified the variability can be substantially underestimated. Though, the approach is nominally standardized in ISO 5725-2, much latitude is given to the analyst and the choices made can greatly impact the results. A different approach is taken in ISO 5725-5 wherein, instead of eliminating outliers, the calculation procedure reduces the effect of the outliers. Under perfect conditions (normality of data, not excessive outliers, no borderline cases of outliers, etc.) the robust method sacrifices some efficiency. However, in practice, the conditions are rarely “perfect”, and the robust procedure is strongly worth considering.

In this presentation, the two approaches will be explained and compared on real, artificial, and simulated data sets. For small data sets, the standard approach offers statistical efficiency advantages, however the robust approach is less subjective and may often be preferred, particularly for larger data sets.

## ST 71

### Analysis of productivity in the laboratory and the role of outsourced asset management services

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With the ever-increasing regulatory and social pressure on quality and safety of goods, companies from multiple sectors of the economy are investing more time and money into R&D and analytical testing groups. This growing trend introduces the added complexity of pro-actively managing technical equipment to discover heretofore unexplored efficiencies. The pharmaceutical industry in particular, has benefited from outsourced asset management services for a number of years, but there are other industries that have been slower in shifting their instrument services to a more consolidated and hands-off model. Here we evaluated the potential effectiveness of asset management in the tobacco sector by analyzing an asset management program for a current tobacco client. Our testing methods included asset utilization analytics to measure instrument downtimes and usage. Additionally, a thorough analysis of purchasing contracts and replacement parts expenditures was performed to assess cost savings/avoidances. All this data was compared to multiple manufacturer's historical response and repair times to the new service model. The results showed that beyond giving time back to the scientists for higher value tasks, service response time was reduced to between five and eight hours per event, and downtime to as little as 16 to 19 hours—compared to days or even weeks under the previous service model. In addition to approximately $9,000 in parts savings each month, having strategic replacement parts on-site positively impacted the downtime improvements. A larger sample size is needed to fully understand the value service placement in the tobacco industry as a whole, but our initial findings do suggest an effective model that could potentially be replicated within the industry as a way to allow research teams to focus on core activities to ensure delivery of safe and high quality products to the marketplace.

## ST 72

### History of 10 years of the BAT TRM – tobacco reference material for total alkaloids and total sugar monitoring

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*(2) British American Tobacco, Research and Development, Regents Park Road, Southampton SO15 8TL, U.K.*

In 2010, British American Tobacco implemented a program to prepare, certify and distribute a tobacco monitoring sample to be used as a reference material for specific analysis in BAT and non-BAT (partners) Green Leaf Threshing (GLT) units and Analytical Services Laboratories (ASL).

The objective was to verify the results of total alkaloids (as nicotine) and total sugar (as glucose) within the BAT network using the data generated from analysis of the BAT Tobacco Reference Material (TRM). The program monitored the analytical process of each participant continuously during the crop season starting a week before the beginning of the season. The first program occurred in 2010 with 28 participants and expanded over ten years to include 69 participants in all parts of the world. The initial program produced and sent 36 twenty-gram packages to GLT laboratories and 52 packages to ASL, this increased to 4500 packages by 2020.

To test the homogeneity of each TRM batch, samples were collected every 100 packages, analyzed and statistical evaluation performed using TSS-AmSSA (Cochran, h test, Z-Score and ANOVA). TRM production includes the following steps: 1) tobacco\blend selection, 2) grinding and packing, 3) statistical evaluation of homogeneity, 4) sample shipment including certification specifying acceptable TRM result range, 5) weekly analysis with monthly report, 6) quarterly partial results report.

The TRM programs greatest learnings were harmonization of analytical methodologies which led to improvements in total alkaloids and total sugar results over the years. Data was used to verify analytical tendencies and to support action plans. Examples of implemented actions were the introduction of a settlement step in the nicotine salt purity procedure, and the addition of cooling to the storage of humectants which expanded shelf life of the samples to one year.


# AUTHOR INDEX

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# PROGRAMME SUMMARY

| DAY 1 | | DAY 2 | | DAY 3 | | DAY 4 | | DAY 5 | |
|---|---|---|---|---|---|---|---|---|---|
| | **Monday 18 Oct** | | **Tuesday 19 Oct** | | **Wednesday 20 Oct** | | **Thursday 21 Oct** | | **Friday 22 Oct** |
| | **Session 1** | | **Part 1** | | **Session 1** | | **Session 1** | | **Session 1** |
| | **Chair: CAHOURS Co-Chair: HARP** | | **Chair: LEE Co-Chair: BELL** | | **Chair: PANI Co-Chair: HU** | | **Chair: STEVENS Co-Chair: WAGNER** | | **Chair: PANI Co-Chair: WAGNER** |
| CET | **PERCEPTION & BEHAVIOUR** | CET | **SYMPOSIUM: NAMs** | CET | **HTP: MODELING** | CET | **E-VAPOUR: ANALYTICAL METHODS** | CET | **CIGARETTES & WATER PIPES** |
| 13:30-13:45 | **ST01** McCAFFREY | 13:30-13:35 | **Welcome** COLARD | 13:30-13:45 | **ST10** YE Bo | 13:30-13:45 | **ST20** PENNINGTON | 13:30-13:45 | **ST29** BHERING |
| 13:45-14:00 | **ST02** WEI | 13:35-13:45 | **Intro** LEE KM | 13:45-14:00 | **ST11** MA Jun | 13:45-14:00 | **ST21** JAMESON | 13:45-14:00 | **ST30** PAPROCKI |
| 14:00-14:15 | **ST03** CLERC | 13:45-14:10 | **NAM01** KLEINSTREUER | 14:00-14:15 | **ST12** AHMED | 14:00-14:15 | **ST22** ZHU | 14:00-14:15 | **ST31** MAYER-HELM |
| 14:15-14:30 | **ST04** STONE | 14:10-14:35 | **NAM02** PAINI | 14:15-14:30 | **ST13** SUN Zhiwei | 14:15-14:30 | **ST24** GILLMAN | 14:15-14:30 | **ST32** LAUTERBACH |
| 14:30-14:45 | **ST05** PHILLIPS | 14:35-15:00 | **NAM03** CORLEY | 14:30-14:45 | **ST14** JUNG | | | 14:30-14:45 | **ST23** ZHU |
| 14:45-15:00 | **ST06** LARROQUE | | **BREAK** | | | | | | |
| | **Session 2** | | **Part 2** | | **Session 2** | | **Session 2** | | **Session 2** |
| | **Chair: YOSHINO / CAHOURS** | | **Chair: LEE / BELL** | | **Chair: PANI Co-Chair: EITZINGER** | | **Chair: WAGNER Co-Chair: STEVENS** | | **Chair: HU Co-Chair: PANI** |
| CET | **NICOTINE SCIENCE** | CET | **SYMPOSIUM: NAMs** | CET | **HTP: METHODS** | CET | **E-VAPOUR: PRODUCT ANALYSES** | CET | **AROMAS & FLAVOURS** |
| 15:30-15:45 | **ST07** JACOBSON | 15:10-15:35 | **NAM04** STUCKI | 15:15-15:30 | **ST15** RODRIGUEZ-LAFUENTE | 15:15-15:30 | **ST25** BISHOP | 15:00-15:15 | **ST33** MIHAYLOVA-KROUMOVA |
| 15:45-16:00 | **ST08** TROFIMOV | 15:35-16:00 | **NAM05** VALERIO | 15:30-15:45 | **ST16** DUROT | 15:30-15:45 | **ST26** FAN Meijuan | 15:15-15:30 | **ST34** LI Chao |
| 16:00-16:15 | **ST09** CAO Yun | 16:00-16:25 | **NAM06** JARABEK | 15:45-16:00 | **ST17** GIBBONS | 15:45-16:00 | **ST27** HUANG Jiaruo | 15:30-15:45 | **ST35** WU Bingyu |
| | | 16:25-16:45 | **Discussion** LEE / BELL | 16:00-16:15 | **ST18** ZIERLINGER | 16:00-16:15 | **ST28** JEONG | 15:45-16:00 | **ST36** QIN Yaqiong |
| | | | | 16:15-16:30 | **ST19** CHAPMAN | | | | |

## Full Session Titles

**Day 1: Session 1 -** PERCEPTION AND BEHAVIOUR: Understanding how nicotine products are perceived and used

**Session 2 -** NICOTINE SCIENCE: Brain and body effects

**Day 2: SYMPOSIUM -** Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction

**Day 3: Session 1 -** HEATED TOBACCO PRODUCTS: Modelling and Numerical Simulation

**Session 2 -** HEATED TOBACCO PRODUCTS: Methods

**Day 4: Session 1 -** E-VAPOUR: Analytical Methods

**Session 2 -** E-VAPOUR: Product Analyses

**Day 5: Session 1 -** CIGARETTES & WATER PIPES: Analytical methods

**Session 2 -** AROMAS & FLAVOURS: Analytical Methods

## DAY 6

| CET | Monday 25 Oct |
|---|---|
| | **Session 1** |
| | **Chair: YOSHINO**<br>**Co-Chair: HARP** |
| | ***IN VITRO* & *IN VIVO* TOX** |
| 13:30-13:45 | **ST37** CARUSO |
| 13:45-14:00 | **ST38** ZHANG |
| 14:00-14:15 | **ST39** HASHIZUME |
| 14:15-14:30 | **ST40** COFFA |
| 14:30-14:45 | **ST41** WATANABE |
| 14:45-15:00 | **ST42** GAFNER |

## DAY 7

| CET | Tuesday 26 Oct | CET | Session 2 |
|---|---|---|---|
| | **Session 1** | | **Chair: YOSHINO**<br>**Co-Chair: HARP** |
| | **Chair: HARP**<br>**Co-Chair: YOSHINO** | | **TOX ASSESSMENT** |
| | **BIOMARKERS** | 15:15-15:30 | **ST49** POUR |
| 13:30-13:45 | **ST43** PLUYM | 15:30-15:45 | **ST50** LALONDE |
| 13:45-14:00 | **ST44** SCHERER | 15:45-16:00 | **ST51** MILLER-HOLT |
| 14:00-14:15 | **ST45** AYALA-FIERRO | | |
| 14:15-14:30 | **ST46** MORRIS | | |
| 14:30-14:45 | **ST47** MAKENA | | |

## DAY 8

| CET | Wednesday 27 Oct | CET | Session 2 |
|---|---|---|---|
| | **Session 1** | | **Chair: EITZINGER**<br>**Co-Chair: WAGNER** |
| | **Chair: EITZINGER**<br>**Co-Chair: HU** | | **NICOTINE POUCHES** |
| | **CIGARETTES: MODELING & DESIGN** | 15:15-15:30 | **ST57** ZHANG |
| 13:30-13:45 | **ST52** YANG Ji | 15:30-15:45 | **ST58** MORGAN |
| 13:45-14:00 | **ST53** WEI Min | 15:45-16:00 | **ST59** SMITH |
| 14:00-14:15 | **ST54** ZHANG Qi | 16:00-16:15 | **ST60** DOSHI |
| 14:15-14:30 | **ST55** JI Xiaoying | 16:15-16:30 | **ST61** KUMAR |
| 14:30-14:45 | **ST56** LOU Jiaying | 16:30-16:45 | **ST62** JABLONSKI |

## DAY 9

| CET | Thursday 28 Oct | CET | Session 2 |
|---|---|---|---|
| | **Session 1** | | **Chair: CAHOURS**<br>**Co-Chair: STEVENS** |
| | **Chair: STEVENS**<br>**Co-Chair: EITZINGER** | | **STATISTICS & LAB OPERATIONS** |
| | **E-VAPOUR: PRODUCT CHEMISTRY** | 15:30-15:45 | **ST69** GUO |
| 13:30-13:45 | **ST63** LAUTERBACH | 15:45-16:00 | **ST70** MORTON |
| 13:45-14:00 | **ST64** COLLINS | 16:00-16:15 | **ST71** FIELD |
| 14:00-14:15 | **ST65** CROSSWHITE | 16:15-16:30 | **ST72** ALVES |
| 14:15-14:30 | **ST66** JAMESON | | |
| 14:30-14:45 | **ST67** YANG | | |
| 14:45-15:00 | **ST68** COOK | | |

### Full Session Titles

**Day 6:** Method for IN VITRO & IN VIVO TOXicology testing: Advancing methods in pre-clinical toxicology

**Day 7: Session 1 -** BIOMARKERS in clinical science: Human data for assessing Tobacco Harm Reduction

**Session 2 -** TOXICOLOGICAL ASSESSMENT: Nonclinical Toxicity Assessment of nicotine products

**Day 8: Session 1 -** CIGARETTES: MODELING & DESIGN

**Session 2 -** NICOTINE POUCHES: nonclinical toxicity assessment

**Day 9: Session 1 -** E-VAPOUR: Product Chemistry

**Session 2 -** STATISTICS & LABoratory OPERATIONS

# Appendix: TIME ZONES

Time zone equivalents to CET 13:30 Conference start time

| Time Zones | City | Time | Hour difference with Paris |
|---|---|---|---|
| **CET** | **Paris** | **13:30** | **0** |
| **PDT** | San Francisco | 04:30 | **-9** |
| **CST** | Managua | 05:30 | **-8** |
| **EDT** | New York | 07:30 | **-6** |
| **BRT** | São Paulo | 08:30 | **-5** |
| **BST** | London | 12:30 | **-1** |
| **CAT** | Harare | 13:30 | **0** |
| **EET** | Bucharest | 14:30 | **+1** |
| **GST** | Dubai | 15:30 | **+2** |
| **IST** | New Delhi | 17:00 | **+3:30** |
| **CST** | Beijing | 19:30 | **+6** |
| **JST** | Tokyo | 20:30 | **+7** |
| **AEST** | Sydney | 21:30 | **+8** |

CET Central European Time
PDT Pacific Daylight Time
CST Central Standard Time
EDT Eastern Daylight Time
BRT Brasilia Time
BST British Summer Time
CAT Central Africa Time
EET Eastern European Time
GST Gulf Standard Time
IST Indian Standard Time
CST China Standard Time
JST Japan Standard Time
AEST Australian Eastern Standard Time