PROGRAM BOOKLET AND ABSTRACTS

Volume 73

73rd Tobacco Science Research Conference

September 15-18, 2019
Leesburg, Virginia USA

Host:
Altria Client Services

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GENERAL PROGRAM

Sunday, September 15, 2019

2:00 pm – 6:00 pm	Registration	Conference Center Foyer
2:00 pm – 6:00 pm	Speaker Ready Room	Bacon
3:00 pm – 5:00 pm	CTRP Workshop	Amphitheater
6:30 pm – 9:30 pm	Welcome Reception *Hosted by – Altria Client Services*	Buses depart beginning at 6:10 pm from the hotel lobby

Monday, September 16, 2019

7:30 am – 8:30 am	Session Chairs Breakfast	Dogwood C
7:30 am – 8:30 am	U.S. TAG: ISO/TC 126 Breakfast	Kettering A
7:30 am – 8:30 am	Tobacco Science Council Meeting	Dogwood A
7:30 am – 5:00 pm	Registration	Conference Center Foyer
7:30 am – 5:00 pm	Speaker Ready Room	Bacon
8:00 am – 8:45 am	Morning Coffee	Ballroom Foyer
8:45 am – 12:05 pm	Symposium "*Tobacco Harm Reduction: Addressing Complexities Across the Risk Continuum*" Chair: Summer Hanna, British American Tobacco	Lansdowne Ballroom
10:05 am – 10:35 am	Coffee Break	Ballroom Foyer
12:05 pm – 1:00 pm	Lunch	Riverside Hearth
1:00 pm – 2:20 pm	Poster Session	Ballroom Foyer
2:20 pm – 5:30 pm	Session A: *E-Cigarettes*	Ballroom B
2:20 pm – 5:30 pm	Session B: *Human Smoking & Toxicology and in vitro Studies*	Ballroom C
3:40 pm – 4:10 pm	Coffee Break	Ballroom Foyer

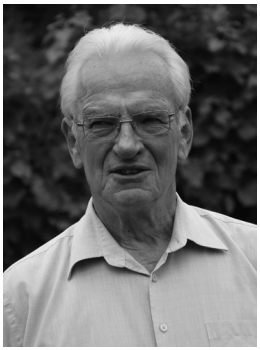
Tuesday, September 17, 2019

8:30 am – 5:00 pm	Registration	Conference Center Foyer
8:30 am – 5:00 pm	Speaker Ready Room	Bacon
8:30 am – 9:00 am	Morning Coffee	Ballroom Foyer
9:00 am – 11:30 pm	Session A: *Agronomy and Method Development*	Ballroom B
9:00 am – 12:10 pm	Session B: *Method Development*	Ballroom C
10:20 am – 10:50 am	Coffee Break	Ballroom Foyer
12:00 pm – 1:00 pm	Lunch	Riverside Hearth
12:00 pm – 1:30 pm	Policy Committee Lunch	Sarnoff
1:00 pm – 2:30 pm	Poster Session	Ballroom Foyer
2:30 pm – 4:40 pm	Session A: *Smokeless Tobacco Products*	Ballroom B
2:30 pm – 5:00 pm	Session B: *E-Cigarettes*	Ballroom C
3:30 pm – 4:00 pm	Coffee Break	Ballroom Foyer
5:15 pm – 6:00 pm	TSRC Business Meeting	Amphitheater
6:30 pm – 7:15 pm	Social Hour	Ballroom Foyer
7:30 pm – 10:00 pm	Award Banquet	Ballroom

Wednesday, September 18, 2019

8:30 am – 9:00 am	Morning Coffee	Ballroom Foyer
8:30 am – 11:30 am	Speaker Ready Room	Bacon
9:00 am – 10:20 am	Combined Session: *Materials, Manufacturing and Finished Product*	Ballroom C
10:20 am	Adjourn	

LIFETIME ACHIEVEMENT AWARD
Hubert Klus



Dr. Hubert Klus was born in Vienna, Austria, in 1941. He studied chemistry and physics at the University of Vienna and with funding from Austria Tabak, obtained his doctorate. Dr. Klus wrote his thesis, "Investigation on the Advancement of the Biological Activity of the Smoke of Cigarettes Made from Reconstituted Tobacco," under the supervision of Professor Mathias Pailer, head of the department for Food Chemistry and Natural Products.

While working on his thesis, Dr. Klus also investigated volatile N-nitrosamines in tobacco smoke and developed methodology for the determination of these compounds in tobacco smoke. After joining the R&D group at Austria Tabak in 1972, and under the guidance of the then department head Dr. Hans Kuhn, Dr. Klus expanded his analytical research on tobacco and tobacco smoke. The scope of his research focused on tobacco specific N-nitrosamines, nitro-phenols in cigarette smoke condensate, optical activity of nicotine in cigarette smoke. Building on work conducted by the Hoffmann Group at the American Health Foundation, he found that aside from nitroso-nornicotine two more tobacco-specific nitrosamines must be formed from nicotine. These were NNK [4-methylnitrosamino-1-(3-pyridyl)-1-butanone] and NNA [4-(methylnitrosamino)-4-(3-pyridyl)butanal]. Additionally, he studied the occurrence of nitrogen containing polycyclic aromatic hydrocarbons in cigarette mainstream smoke, and more specifically on the question of whether the S-(-) nicotine present in tobacco could be thermally racemised by smoking, and the impact of filter ventilation on the pH of mainstream smoke. He also evaluated methods for the cigarette sidestream smoke collection.

Dr. Klus expanded his work into product development and in 1981 was named R&D Department Head, a position he held until his retirement. As part of his increasing responsibilities Dr. Klus was also named General Manager of ÖKOLAB, a subsidiary of Austria Tabak which focused on the development of analytical methods, among them for the so-called Hoffmann analytes, the determination of pesticide and herbicide residues on tobacco and the determination of artificial genetical modifications in foodstuffs and tobacco.

During his professional career in tobacco science research, Dr. Klus authored or co-authored over 70 technical papers and reports, including contributing to 3 books on various aspects of nicotine, pharmacology and nitrosamines. In addition, he was on the editorial board of Beiträge zur Tabakforschung, participated in numerous CORESTA Task Forces, and served as a member of CORESTA's Scientific Commission and of the CORESTA Board. Dr. Klus is also a recipient of the CORESTA Gold Medal.

Dr. Klus and his wife Traudl have celebrated 55 years of marriage and have two daughters, two sons, eleven grandchildren and two great-grandchildren.

73rd TOBACCO SCIENCE RESEARCH CONFERENCE

Monday Morning, September 16, 2019
Combined Session

8:45 **WELCOME & OPENING REMARKS:** Karl Wagner, Altria Client Services, 73rd TSRC Chair and Maria Gogova, Altria Client Services

9:00 **SYMPOSIUM: "Tobacco Harm Reduction: Addressing Complexities Across the Risk Continuum"** Chair: Summer Hanna, British American Tobacco, Southampton UK

9:05 **1. IS CIVIL DIALOGUE AND ENGAGEMENT BETWEEN DIVERSE STAKEHOLDERS WITH RESPECT TO TOBACCO HARM REDUCTION FEASIBLE? A REVIEW OF THE PAST. PRESENT AND FUTURE.** Scott D. BALLIN; Health Policy Consultant, Washington, DC USA

9:35 **2. FDA REGULATING TOBACCO PRODUCTS ALONG A CONTINUUM OF RISK.** Deirdre Lawrence KITTNER; FDA, Center for Tobacco Products, Calverton, MD USA

10:05 ***Break***

10:35 **3. TOBACCO HARM REDUCTION: WEIGHING THE EVIDENCE.** Brian E. ERKKILA; Foundation for a Smoke-Free World, Washington, DC USA

11:05 **4. TOBACCO HARM REDUCTION: ADDRESSING COMPLEXITIES ACROSS THE RISK CONTINUUM.** Willie J. MCKINNEY; JUUL Labs, San Francisco, CA USA

11:35 **Panel discussion with all symposium speakers**

12:05 ***Lunch***

1:00 **Poster Session**

5. INCORPORATING ANALYTICAL VARIANCE INTO A COMPARATIVE QUANTITATIVE RISK ASSESSMENT (QRA) APPROACH FOR TOBACCO PRODUCTS. Chastain A. ANDERSON, Vanessa Haase, Kimberly D. Ehman, Paige N. Wiecinski and Donna C. Smith; Altria Client Services, Richmond, VA USA

6. ***IN VITRO* TESTING OF AN ETHANOL COLLECTION METHOD COMBINING PARTICULATE AND GAS-VAPOR PHASE COMPONENTS: BACTERIAL REVERSION MUTATION (AMES) ASSAY.** Sanjay Kumar BHARTI, Bhagyalaxmi Sukka Ganesh, Mariano J. Scian and I. Gene Gillman; Enthalpy Analytical, Henrico, VA USA

7. **CONVERSION OF A PHARMACEUTICAL SALT-SCREENING ROBOT PLATFORM TO AN AUTOMATED STATION FOR UNATTENDED WEIGHING E-CIGARETTES AND SMOKING MACHINE FILTERS.** J. Anthony COX and Justin Lu; Sirius Automation Group, Buffalo Grove, IL USA

8. **WORKSHOP SERIES TO IDENTIFY, DISCUSS AND DEVELOP RECOMMENDATIONS FOR THE OPTIMAL GENERATION AND USE OF *IN VITRO* GENOTOXICITY ASSAY DATA FOR TOBACCO AND NICOTINE PRODUCTS.** Martha M. MOORE[1] and Rodger Curren[2]; [1]Ramboll US Corporation, Little Rock, AR USA and [2]Institute for *In Vitro* Sciences, Gaithersburg, MD USA

9. **VOLATILE ORGANIC COMPOUNDS IN ENDS AEROSOL: COLLECTION ON ACTIVATED CARBON.** Kathy HUMPHRIES, Mitch Zimmerman, Trevor Lott and Gene Gillman; Enthalpy Analytical, Durham, NC USA

10. **QUALIFICATION OF CELL LINE A549 FOR THE NEUTRAL RED UPTAKE (NRU) ASSAY.** Shannon BRUCE, Khushbu Garala, Deepa Srinivasan, Sandra Springer, Douglass Dey, Kayla Campasino and Rohan Kulkarni; MilliporeSigma (BioReliance® Toxicology Testing Services), Rockville, MD USA

11. **COMPARISON OF VEHICLE CONTROLS FOR CELL LINE A549 IN THE NEUTRAL RED UPTAKE (NRU) ASSAY.** Shannon BRUCE, Khushbu Garala, Deepa Srinivasan, Sandra Springer, Douglass Dey, Kayla Campasino and Rohan Kulkarni; MilliporeSigma (BioReliance® Toxicology Testing Services), Rockville, MD USA

12. **REAL-TIME MONITORING OF VOLATILE ORGANIC COMPOUNDS AND AEROSOL IN EXHALED BREATH FROM INHALATION OF VAPOR PRODUCTS AND COMBUSTIBLE CIGARETTES.** Devon C. O'REGAN[1], Adam M. Ozvald[1], Nadja Heine[1], Rene Gutmann[2] and Bea Rosenkranz[2]; [1]JUUL Labs, San Francisco, CA USA and [2]Ionicon, Innsbruck, Austria

13. **SIMULTANEOUS DETERMINATION ANALYSIS OF GLYCEROL, PROPLYLENE GLYCOL, NICOTINE, MENTHOL AND WATER BY DISCHARGE IONIZATION DETECTOR ON GAS CHROMATOGRAPHY.** Akihito SHIMAZU and Fumihiro Omori; Japan Tobacco, Tokyo Japan

14. **DETERMINATION OF DICARBONYLS AND CROTONALDEHYDE IN E-VAPOR PRODUCTS.** David ZICH; ITG Brands, Greensboro, NC USA

15. **LEVELS OF ENDOTHELIAL PROGENITOR CELLS (EPCS) IN SMOKERS AND MOIST SNUFF CONSUMERS.** Subhashini ARIMILLI[1], Peter Chen[2] and G. L. Prasad[2]; [1]Eurofins Professional Scientific Services, Winston-Salem, NC USA and [2]RAI Services Company, Winston-Salem, NC USA

16. **BELIEFS, PERCEPTIONS, AND BEHAVIORAL INTENTIONS RELATED TO NICOTINE, LOW NICOTINE CIGARETTES, AND REDUCED RISK PRODUCTS AMONG U.S. ADULTS.** Tiffany PARMS and Kimberly Frost-Pineda; RAI Services Company, Winston-Salem, NC USA

17. **BELIEFS AND PERCEPTIONS ABOUT NICOTINE AND "MINIMALLY ADDICTIVE" CIGARETTES AMONG ADULT CURRENT, FORMER, AND NEVER SMOKERS.** Tiffany PARMS, Kimberly Frost-Pineda and Geoffrey Curtin; RAI Services Company, Winston-Salem, NC USA

18. **TOBACCO FLASH PYROLYSIS. EFFECT OF THE ADDITION OF MESOPOROUS CATALYSTS.** Emilio CALABUIG, Antonio Marcilla and Maria Isabel Beltrán; University Institute of Chemical Process Engineering, San Vicente del Raspeig, Spain

19. **SIMULATED LEACHABLES ASSESSMENT FOR CRITICAL COMPONENTS USED IN CARTRIDGES OF ELECTRONIC NICOTINE DELIVERY SYSTEMS.** Courtney CULBERT[1] and Randy Weidman[2]; [1]RAI Services Company, Winston-Salem, NC USA and [2]R.J. Reynolds Tobacco Company, Winston-Salem, NC USA

20. **NEUTRAL RED UPTAKE (NRU) CYTOTOXICITY ANALYSIS OF AEROSOL GENERATED FROM A TEMPERATURE-REGULATED NICOTINE SALT BASED PRODUCT UTILIZING COTTON WICKING MATERIAL.** Bryant HIRAKI, David Cook and Manoj Misra; JUUL Labs, San Francisco, CA USA

21. **INFLUENCE OF THE TIME OF AND STIRRING RATE DURING THE FIRST STEP OF SYNTHESIS OF SBA-15 ON ITS CATALYTIC EFFECT FOR REDUCING TOXICANTS CONCENTRATION IN TOBACCO SMOKE.** Nerea JUÁREZ-SERRANO, Javier Asensio-Morant, Isabel Martínez-Catellanos, Desiré Berenguer, Inmaculada Blasco, María Isabel Beltrán and Antonio Marcilla; University of Alicante, Alicante, Spain

22. **A SEVEN-MONTH SYSTEMS TOXICOLOGY INHALATION STUDY IN C57BL/6 MICE DEMONSTRATES REDUCED PULMONARY INFLAMMATION AND EMPHYSEMA FOLLOWING SMOKING CESSATION OR SWITCHING TO E-VAPOR AEROSOL EXPOSURES.** Ashutosh KUMAR[1], Ulrike Kogel[2], Marja Talikka[2], Julia Hoeng[2], Manuel Peitsch[2], Anthony Skowronek[3] and K. Monica Lee[1]; [1]Altria Client Services, Richmond, VA USA, [2]Philip Morris International, Neuchâtel, Switzerland and [3]Battelle, West Jefferson, OH USA

23. **EFFECT OF SBA-15 MORPHOLOGY IN THE COMPOSITION OF THE MAINSTREAM TOBACCO SMOKE.** Isabel MARTÍNEZ-CASTELLANOS, Antonio Marcilla, María Isabel Beltrán, Desiré Berenguer, Inmaculada Blasco, Nerea Juarez, Javier Asensio, Emilio Calabuig; University of Alicante, Alicante, Spain

24. **DETERMINATION OF AEROSOL PARTICLE SIZE DISTRIBUTION FOR SEVERAL ELECTRONIC NICOTINE DELIVERY SYSTEM PRODUCTS.** Chen SONG; R.J. Reynolds Tobacco Company, Winston-Salem, NC USA

25. ***IN VITRO* TESTING OF AN ETHANOL COLLECTION METHOD COMBINING PARTICULATE AND GAS-VAPOR PHASE COMPONENTS: *IN VITRO* MICRONUCLEUS ASSAY.** Bhagyalaxmi SUKKA GANESH, Sanjay K. Bharti, Mariano J. Scian and I. Gene Gillman; Enthalpy Analytical, Henrico, VA USA

26. **NICOTINE DISSOLUTION IN SMOKELESS TOBACCO PRODUCTS.** Nancy QIAN, Tracy Derry, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

27. **TOXICITY REFERENCE VALUES: RELEVANCE FOR THE EVALUATION OF TOXICITY AND RISK.** Felix AYALA-FIERRO and Ashley Turner; ITG Brands, Greensboro, NC USA

28. **GLYCIDOL IN HEATED TOBACCO PRODUCTS.** Norman E FRALEY, Elizabeth Anderson, Chris Almond, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

29. **COMPREHENSIVE INSIGHTS INTO TOBACCO SMOKE USING FLOW-MODULATED GCXGC-TOF MS.** Mark LEMONS[1], Laura McGregor[2], Bob Green[2], Anthony Buchanan[2] and Matthew Edwards[3]; [1]Markes International, Sacramento, CA USA, [2]SepSolve Analytical, Peterborough UK and [3]SepSolve Analytical, ON Canada

30. **IDENTIFICATION OF PREDICTIVE CLINICAL BIOMARKERS FOR DEVELOPING CHRONIC OBSTRUCTIVE PULMONARY DISEASE USING REAL WORLD EVIDENCE DATA.** Gang Michael LIU, Patrudu Makena, Kyung Soo Hong, Eric Scott and G. L. Prasad; RAI Services Company, Winston-Salem, NC USA

31. ***IN VITRO* TESTING OF AN ETHANOL COLLECTION METHOD COMBINING PARTICULATE AND GAS-VAPOR PHASE COMPONENTS: NEUTRAL RED ASSAY.** Mariano J. SCIAN, Bhagyalaxmi Sukka-Ganesh, Sanjay K. Bharti and I. Gene Gillman; Enthalpy Analytical, Henrico, VA USA

32. **A SIMPLIFIED METHOD FOR THE ANALYSIS OF MONO-CARBONYL COMPOUNDS IN E-CIGARETTE AEROSOLS BY LC-MS.** Jeff ZHU and Aaron Heredia; ITG Brands, Greensboro, NC USA

33. **A QUICKER METHOD FOR THE ANALYSIS OF AMMONIA IN E-CIGARETTE AEROSOLS AND E-LIQUIDS BY ION CHROMATOGRAPHY.** Jeff ZHU, Brittany Moore and Aaron Heredia; ITG Brands, Greensboro, NC USA

34. **COMPARISON OF A FLAME IONIZATION DETECTOR (GC/FID) TO A NITROGEN-PHOSPHORUS DETECTOR (GC/NPD) FOR GAS CHROMATOGRAPHIC DETERMINATION OF NICOTINE IN CONVENTIONAL AND ULTRA-LOW NICOTINE TOBACCO BLENDS.** Darren STEELMAN, Andy Stinson and T. Jeffrey Clark; Liggett Group, Mebane, NC USA

Monday Afternoon, September 16, 2019

Session A
E-Cigarettes
Session Chair: Rob Stevens

2:20 PM

35. FINGERPRINTING OF E-LIQUIDS BY THE DETERMINATION OF UNTARGETED COMPOUNDS USING TWO MODERN ANALYTICAL APPROACHES. Paulina BIERNACKA, Kenneth Chalcraft, Peter Joza and David Li; Labstat International, Kitchener, ON Canada

2:40 PM

36. REAL-TIME CHEMICAL PUFF PROFILING OF VAPOR PRODUCT AEROSOL WITH PROTON TRANSFER REACTION - MASS SPECTROMETRY. Luca CAPPELLIN[1] and Nadja Heine[2]; [1]University of Padua and Tofwerk AG, Padova Italy and [2]JUUL Labs, San Francisco, CA USA

3:00 PM

37. IMPACT OF DEVICE VARIABILITY ON THE DETERMINATION OF ALDEHYDE COMPOUNDS IN E-CIGARETTE EMISSIONS. I. Gene GILLMAN, Alexander S.C. Pennington and Kathy E. Humphries; Enthalpy Analytical, Durham, NC USA

Session B
Human Smoking &
Toxicology and *in vitro* Studies
Session Chair: Rana Tayarrah

2:20 PM

43. AN OPEN-LABEL, RANDOMIZED, PARALLEL-GROUP, CONTROLLED STUDY TO EVALUATE CHANGES IN BIOMARKERS OF CIGARETTE SMOKE EXPOSURE AND BIOMARKERS OF POTENTIAL HARM IN ADULT SMOKERS WHO COMPLETELY SWITCH TO USING E-VAPOR PRODUCTS FOR 12 WEEKS. Jeff EDMISTON, Douglas Oliveri, Qiwei Liang and Mohamadi Sarkar; Altria Client Services, Richmond, VA USA

2:40 PM

44. ABUSE LIABILITY OF VERY LOW NICOTINE CONTENT CIGARETTES WITH CHARACTERIZATION OF NICOTINE EXPOSURE PROFILES IN ADULT SMOKERS. Naama LEVY-COOPERMAN[1], Megan J. Shram[1], Debra Kelsh[2], Bradley Vince[2], and Ed Carmines[3]; [1]Altreos Research Partners, Toronto, ON Canada, [2]Altasciences/Vince and Associates, Overland Park, KS USA and [3]Carmines Consulting, Scottsdale, AZ USA.

3:00 PM

45. ADVANCING THE COMMERCIAL AND PUBLIC HEALTH GOALS OF POTENTIALLY REDUCED-RISK PRODUCTS THROUGH THE ASSESSMENT OF CONSUMER SATISFACTION. Neil SHERWOOD; Neil Sherwood Consulting, Nyon, Switzerland

Monday Afternoon, September 16, 2019

3:20 PM

38. OPTIMIZATION AND COMPARISON OF 2,4-DINITROPHENYLHYDRAZINE (DNPH) DERIVATIZATION CONDITIONS FOR THE DETERMINATION OF CARBONYL COMPOUNDS. Lena JEONG[1], John H. Miller IV[2] and Niti Shah[2]; [1]Eurofins Lancaster Laboratories, Richmond, VA USA and [2]Altria Client Services, Richmond, VA USA

3:20 PM

46. CHARACTERIZATION OF ADULT CIGARETTE SMOKERS' BEHAVIOR DURING SHORT AND LONGER-TERM USE OF REDUCED NICOTINE CONTENT CIGARETTES. Andrea VANSICKEL, Mingda Zhang and Jan Angel; Altria Client Services, Richmond, VA USA

BREAK

4:10 PM

39. GLYCIDOL BEHAVIOR IN GC SYSTEMS. Norman E. FRALEY; Eurofins Professional Scientific Services, Winston-Salem, NC USA

4:10 PM

47. ELECTRONIC NICOTINE DELIVERY SYSTEM PUFFING TOPOGRAPHY CHARACTERISTICS FROM HUMAN STUDIES. Ian M. FEARON[1] and Elaine K. Round[2]; [1]whatIF? Consulting Ltd, Harwell, UK and [2]RAI Services Company, Winston-Salem, NC USA

4:30 PM

40. I STEEP MY TEA, SO DO I NEED TO STEEP MY E-LIQUIDS? John H. LAUTERBACH; Lauterbach & Associates, Macon, GA USA

4:30 PM

48. A BLOOD-BASED SMOKING-RELATED GENE EXPRESSION SIGNATURE USING A MACHINE LEARNING APPROACH. Gang Michael LIU and G. L. Prasad; RAI Services Company, Winston-Salem, NC USA

Monday Afternoon, September 16, 2019

4:50 PM

41. ANALYSIS OF E-LIQUIDS OF ELECTRONIC CIGARETTES CONTAINING NICOTINE SALTS. HAN Shulei, Fu Yaning, Liu Tong, Wang Hongjuan, Chen Huan and Hou Hongwei; China National Tobacco Quality Supervision and Test Center, Henan China

4:50 PM

49. HIGH THROUGHPUT AIR LIQUID INTERFACE EXPOSURE MODULES: CHARACTERIZATION OF SMOKE/ AEROSOL DOSIMETRY AND *IN VITRO* MUTAGENICITY AND CYTOTOXICITY OF TWO TOBACCO PRODUCT TYPES. Robert LEVERETTE[1], Brian Keyser[1], Michael Hollings[2] and Adam Seymour[2]; [1]RAI Services Company, Winston-Salem, NC USA and [2]Covance Laboratories, North Yorkshire UK

5:10 PM

42. DETERMINATION OF AMOUNTS OF HARMFUL SUBSTANCES MIGRATING FROM PLASTIC ASSEMBLIES IN ELECTRONIC CIGARETTES. Fan Meijuan[1], Pan Lining[1], Cui Huapeng[1], Duan Yuanxing[2], Liu Yibo[3], Niu Jiajia[1], Mao Youan[4], Chen Li[1], Guo Junwei[1], Liu Shaofeng[1], Wang Hongbo[1], Liu Huimin[1] and ZHAO Le[1]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China, [2]Technology Center of China Tobacco Yunnan Industrial, Kunming, China, [3]Technology Center of China Tobacco Guangdong Industrial, Guangzhou, China and [4]Technology Center of China Tobacco Hunan Industrial, Changsha, China

5:10 PM

50. CYTOTOXICITY AND ACUTE TOXICITY ASSESSMENT OF SEVERAL TYPICAL NICOTINE SALTS. WANG Hongjuan, Chen Huan, Han Shulei, Fu Yaning, Liu Tong, Hou Hongwei and Hu Qingyuan; China National Tobacco Quality Supervision & Test Center, Henan, China

ADJOURN

Tuesday Morning, September 17, 2019

Session A
Agronomy and Method Development
Session Chair: Jason Flora

9:00 AM

51. THE EFFECT ON TSNAS OF STICK SPACING IN THE BARN. Anne Jack FISHER, Colin Fisher and Huihua Ji; University of Kentucky, Lexington, KY USA

9:20 AM

52. RELATIONSHIP OF ALKALOIDS, PON, TSNAS, NITRATE AND NITRITE: A TWO-YEAR FIELD ANALYSIS. Ying WU, Huihua Ji, Anne Fisher, Franklin Fannin, Nabanita Chattopadhyay and Lowell Bush; University of Kentucky, Lexington, KY USA

9:40 AM

53. OPTIMIZED METHOD FOR DETERMINATION OF SELECTIVE PHENOLIC COMPOUNDS IN CIGARETTE AND CIGAR SMOKE BY UHPLC-FLD. Xiaohong Cathy JIN, Thomas J. Hurst and Karl A. Wagner; Altria Client Services, Richmond, VA USA

10:00 AM

54. VARIATION OF SUGAR LEVELS IN TOBACCO UPON HEATING. Serban C. MOLDOVEANU and Karen Kilby; R.J. Reynolds Tobacco, Winston-Salem, NC USA

Session B
Method Development
Session Chair: Candice Cunningham

9:00 AM

57. CTP SUBMISSIONS – TIPS AND INSIGHT FOR PREPARING AN ELECTRONIC SUBMISSION. Jeffrey K. SMITH[1], Deborah Sholtes[1], Seth Glatstein[1] and Glenn Angermeier[2]; [1]US FDA, Silver Spring, MD USA and [2]WiseDesign, McLean, VA USA

9:20 AM

58. USP ELEMENTAL IMPURITIES: LIMIT TEST FOR METALS IN NICOTINE BY ICP-MS. Hamid LOTFI and Margaret Arroyo; Global Laboratory Services, Wilson, NC USA

9:40 AM

59. ANALYSIS OF COUMARIN IN ELECTRONIC CIGARETTE LIQUIDS AND SMOKELESS TOBACCO PRODUCTS BY UHPLC-MS/MS. Jingcun WU[1], Xia Geng[2] and Feng Qin[1]; [1]PerkinElmer, Woodbridge, ON Canada and [2]PerkinElmer, Shanghai, China

Not Presented

10:00 AM

60. METHOD OPTIMIZATION ON ANALYSIS OF TSNA IN ELECTRONIC CIGARETTE LIQUIDS AND NSAR IN SMOKELESS TOBACCO PRODUCTS BY UHPLC-MS/MS. Jingcun WU and Feng Qin; PerkinElmer, Woodbridge, ON Canada

Not Presented

BREAK

Tuesday Morning, September 17, 2019

10:50 AM

55. VARIATION OF TSNAS LEVELS IN TOBACCO UPON HEATING. Serban C. MOLDOVEANU and Marlene Adams; R.J. Reynolds Tobacco, Winston-Salem, NC USA

11:10 AM

56. PREDICTION OF TOTAL AMOUNT OF TOBACCO-SPECIFIC NITROSAMINES IN TOBACCO LEAVES BY FLUORESCENCE EXCITATION-EMISSION MATRIX. Hirotaka NAITO and Takumi Koike; Japan Tobacco, Yokohama, Kanagawa Japan

10:50 AM

61. HPHC MARKET MAP STUDY FOR US MACHINE-MADE CIGARS – PART 1 PHYSICAL PROPERTIES, FILLER AND SMOKE HPHC VARIABILITY. Karl A. WAGNER, Michael J. Morton, Raquel M. Olegario, Lara L. Baker and Jennifer H. Smith; Altria Client Services, Richmond, VA USA

11:10 AM

62. HPHC MARKET MAP STUDY FOR US MACHINE-MADE CIGARS – PART 2 PREDICTIVE MODELS. Michael J. MORTON, Karl A. Wagner, Raquel M. Olegario, Lara L. Baker and Jennifer H. Smith; Altria Client Services, Richmond, VA USA

11:30 AM

63. TOBACCO HEATING PRODUCTS (THP) : EXPERIMENTAL CONSIDERATIONS FOR ACHIEVEING REPRESENTATIVE YIELDS. Ian TINDALL[1], Linda Crumpler[2] and James Okpeh[1]; [1]Cerulean, Milton Keynes UK and [2]Cerulean, Richmond, VA USA

11:50 AM

64. COMPARISON OF TWO DIFFERENT HIGH-RESOLUTION MASS SPECTROMETERS FOR UNTARGETED LC/MS ANALYSIS OF CIGARETTE SMOKE EXTRACTS. Yuichiro TAKANAMI, Nobumasa Kitamura, Norimichi Orikata and Tetsuya Tobita; Japan Tobacco, Yokohama, Kanagawa Japan

LUNCH

Tuesday Afternoon, September 17, 2019

1:00 PM **Poster Session**

65. EFFECT OF SBA-15 MORPHOLOGY ON THE THERMAL DECOMPOSITION OF NICOTINE. Javier ASENSIO, Antonio Marcilla, Maria Isabel Beltrán and Nera Juárez; Alicante University, Alicante Spain

66. ANALYSIS OF BENZO[A]PYRENE IN TOBACCO AND RELATED PRODUCTS BY GC-MS/MS. Jingjing WU[1], Xia Geng[2], Lizhong Yang[1] and Feng Qin[1]; [1]PerkinElmer Inc, ON Canada and [2]PerkinElmer Management (Shanghai) Co, Shanghai China

67. CHARACTERIZATION OF SMOKELESS TOBACCO PRODUCTS EXTRACTED WITH DIFFERENT SOLVENTS FOR *IN VITRO* TESTING. Jingjie ZHANG[1], Doshi Utkarsh[1], Jyoti Thaikoottathil[1], Monica K. Lee[1] and Russell L. Wolz[2]; [1]Altria Client Services, Richmond, VA USA and [2]Enthalpy Analytical, Richmond, VA USA

68. TRACE METALS ANALYSIS OF TOBACCO HEATED PRODUCT BY ICP-MS. Danielle BENNER, Donald Stogner, Jamil Gray, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

69. APPLICATION OF ISOTHERMAL MICROCALORIMETRY TO MST PRODUCTS -- A NEW METHOD TO MONITOR AGING. Robert B. RAGLAND and Mark J. Rusyniak; Altria Client Services, Richmond, VA USA

70. COMPARATIVE LEVELS OF CARBONYL DELIVERY BETWEEN MASS-MARKET CIGARS AND CIGARETTES. Joseph J. JABLONSKI, J. Hunter Maines, Andrew G. Cheetham, Alexandra M. Martin and I. Gene Gillman; Enthalpy Analytical, Richmond, VA USA

71. NICOTINE VARIABILITY OF LOW NICOTINE CULTIVARS VERSUS NORMAL NICOTINE CULTIVARS. Kenny LION, Andrew Adams, Whit Morris, Emily Brown, Brittany Irving, Marcos Lusso and Dongmei Xu; Altria Client Services, Richmond, VA USA

72. A COMPARISON OF QUARTZ FILTER COLLECTION VERSUS ELECTROSTATIC PRECIPITATION COLLECTION IN E-CIGARETTE AEROSOL SAMPLES. I. Gene GILLMAN, Alexandra Martin, Samuel Hochstetler, Justin Lata, Nicholas Race, Darybelle Collins and Patrick Kelly; Enthalpy Analytical, Richmond, VA USA

73. COLLECTION AND CHARACTERIZATION OF MAINSTREAM CIGARETTE SMOKE CONDENSATES USING A GLASS FIBER FILTER AND ETHANOL CONTAINING IMPINGER. I. Gene GILLMAN, Jacob P. Hilldrup, Sarah R. Packett and Jacqueline M. Collins; Enthalpy Analytical, Henrico, VA USA

74. DETERMINATION OF PRIMARY AROMATIC AMINES IN SMOKELESS TOBACCO PRODUCTS. Andrew G. CHEETHAM and Alexandra M. Martin; Enthalpy Analytical, Richmond, VA USA

75. ANALYSIS OF NICOTINE, MENTHOL, WATER, PROPYLENE GLYCOL, AND GLYCERIN IN TOBACCO HEATED PRODUCT. Tiffany LANDINGHAM, Michael Siernos, Corey Posten, Chris Almond, David D. Mickey, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

76. NICOTINE *IN VIVO* EXTRACTION AND PHARMACOKINETICS OF NON-TOBACCO-BASED NICOTINE POUCHES (ZYN®) COMPARED WITH TOBACCO-BASED SWEDISH SNUS AND AMERICAN MOIST SNUFF. Mikael STAAF, Tryggve Ljung and Robert Pendrill; Swedish Match, Stockholm, Sweden

77. COMPARISON OF METHODS FOR MEASURING THE PARTICLE SIZE DISTRIBUTION OF SMOKELESS TOBACCO PRODUCTS. Sean P. PLATT, Charnise Jackson, Kathryn Dill and Mark Rusyniak; Altria Client Services, Richmond, VA USA

78. PROBABILISTIC RISK ASSESSMENT TO COMPARE HEALTH RISKS OF ORAL TOBACCO PRODUCTS. Annette B. SANTAMARIA[1], Marshall E. Krotenberg[2], Scott M. Drouin[1], Kimberly D. Ehman[3], Chastain A. Anderson[3], Vanessa Haase[3] and Donna C. Smith[3]; [1]Rimkus Consulting Group, Houston, TX USA, [2]Rimkus Consulting Group, Phoenix, AZ USA and [3]Altria Client Services, Richmond, VA USA

79. COMPARISON OF THE ACETALDEHYDE, FORMALDEHYDE, AND ACROLEIN YIELDS IN CIGARS UNDER DIFFERENT SMOKING REGIMENS USING A LINEAR CIGARETTE SMOKING MACHINE. Mimy YOUNG, Todd L. Cecil, Tricia Johnson and Shixia Feng; Food and Drug Administration, Calverton, MD USA

80. USE OF THE BENCHMARK DOSE APPROACH IN CARCINOGENIC RISK ASSESSMENT FOR NNK. Ashley TURNER and Felix Ayala-Fierro; ITG Brands, Greensboro, NC USA

81. REVIEW OF RELEVANT TOXICOLOGICAL DATA USING BMDS TO ESTIMATE INHALATION UNIT RISK FOR RISK ASSESSMENT PRACTICES. Ashley TURNER and Felix Ayala-Fierro; ITG Brands, Greensboro, NC USA

82. HPHC ANALYSIS OF SEVEN FLAVORS OF A TEMPERATURE-REGULATED NICOTINE SALT-BASED POD SYSTEM. David COOK, Bryant Hiraki and Manoj Misra; JUUL Labs, San Francisco, CA USA

83. TOBACCO EMISSIONS FOR CANADIAN CIGARETTES: A LOOK BACK ON 10 YEARS. Dilara JAKUPOVIC, Huda Masoud, Nemanja Mladjenovic and Trevor Mischki; Health Canada, Ottawa, ON Canada

84. ALTERED LUNG BARRIER FUNCTION IS A PHYSIOLOGICALLY-RELEVANT BIOMARKER OF POTENTIAL HARM. Patrudu MAKENA, Sarah Baxter-Wright, Peter Chen, and G. L. Prasad; RAI Services Company, Winston-Salem, NC USA

85. ESTABLISHMENT OF THE METHOD FOR THE ANALYSIS OF GASEOUS COMPOUNDS IN E-CIGARETTE EMISSION USING A SORBENT CARTRIDGE. Seok Heo, Hoi Kang, Hyoung Joon Park, Jin Hee Lee, Dong Gu Lee and Seongsoo Park; Ministry of Food and Drug Safety, Chungju-Si, Chungcheongbuk-do, Republic of Korea

86. DETERMINATION OF TOBACCO ALKALOIDS IN CONSUMER PRODUCTS BY UPLC-MS/MS USING A MODIFIED QUECHERS METHOD. John R. SHIFFLETT, James B. Wittenberg and Dawit Z. Bezabeh; Alcohol and Tobacco Tax and Trade Bureau, Beltsville, MD USA

87. ASSESSMENT OF *IN VITRO* TOXICITIES DEMONSTRATED BY TOTAL PARTICULATE MATTER (TPM) AND GAS VAPOR PHASE (GVP) SAMPLES GENERATED FROM TOBACCO HEATING PRODUCTS (THPS) COMPARED WITH A COMBUSTIBLE CIGARETTE. Thomas J. SHUTSKY, Casandra K. West, Kristen G. Jordan and Christopher S. Junker; RAI Services Company, Winston-Salem, NC USA

88. HOW DID WE MOVE FROM SMOKING TOPOGRAPHY TO VAPING TOPOGRAPHY? Elise POULIN-DELORME, Kevin Menager and Florian Lozano; SODIM, Saint Jean de Braye, France

89. DETERMINATION OF NICOTINE, TOBACCO-SPECIFIC NITROSAMINES, AND POLYCYCLIC AROMATIC HYDROCARBONS IN CANDIDATE REFERENCE MATERIAL 8112 TOBACCO SMOKE CONDENSATE SOLUTION. Walter B. WILSON and Lane C. Sander; National Institute of Standards and Technology, Gaithersburg, MD USA

90. DIFFERENTIAL REGULATION OF ION CHANNEL FUNCTION FROM EXPOSURES TO CIGARETTE SMOKE AND ENDS PREPARATIONS. Rachael E. RAYNER[1], Patrudu Makena[2], G. L. Prasad[2] and Estelle Cormet-Boyaka[1]; [1]The Ohio State University, Columbus, OH USA and [2]RAI Services Company, Winston-Salem, NC USA

91. TOBACCO-SPECIFIC NITROSAMINES IN THE MAINSTREAM SMOKE OF COMMERCIAL LITTLE CIGARS. Selvin H. EDWARDS[1], Matthew Hassink[1], Kenneth M. Taylor[1], Cliff Watson[2], Peter Kuklenyik[2], Ruth Wang[2], Patrick Chen[2], Liza Valentin-Blasini[2] and Brett Kimbrell[2]; [1]Food and Drug Administration, Silver Spring, MD USA and [2]Centers for Disease Control and Prevention, Atlanta, GA USA

92. WITHIN BRAND AND ACROSS BRAND CONTENT AND VARIABILITY OF NICOTINE AND TOBACCO-SPECIFIC NITROSAMINES IN 8 COMMERCIAL US CIGAR BRANDS. Jacob P. HILLDRUP, I. Gene Gillman and Katilyn N. L. Brooks; Enthalpy Analytical, Henrico, VA USA

93. CIGAR USAGE PATTERNS AMONG ADULT TOBACCO USERS: RESULTS OF A LARGE, NATIONALLY REPRESENTATIVE SURVEY. Susan MORRIS; Altria Client Services, Richmond, VA USA

94. A FIFTY NEW METHOD OF TOBACCO FOR SUSTAINABLE TOBACCO CROPPING IRAN (MAZANDARAN PROVINCE) AND TOBACCO GROWERS DISPUTE. Mohsenzadeh REZA; Iranian Tobacco Company, Behshahr, Iran

95. EFFECT OF PLANT GROWTH REGULATORS ON MATURATION, AND QUALITY OF ORIENTAL TOBACCO. Mohsenzadeh REZA; Iranian Tobacco Company, Behshahr, Iran

96. DESIGN AND MANUFACTURE OF MODERN BARN AND RACK FOR CURING TOBACCO. Mohsenzadeh REZA, Ahmadi Mojtaba and Moravvej Majid; Iranian Tobacco Company, Behshahr, Iran

Tuesday Afternoon, September 17, 2019

Session A
Smokeless Tobacco Products
Session Chair: Felix Ayala-Fierro

2:30 PM

97. METHODOLOGY TO DETERMINE THE PARTICLE SIZE DISTRIBUTION, MEAN, AND STANDARD DEVIATION FROM SIEVE DATA. F. Kelley ST. CHARLES[1] and Walter T. Morgan[2]; [1]St. Charles Consultancy, Lewisville, NC USA and [2]RAI Services Company, Winston-Salem, NC USA

2:50 PM

98. *IN VITRO* DISSOLUTION TESTING OF NICOTINE RELEASE FROM SMOKELESS TOBACCO PRODUCTS. Fadi ALDEEK, John H. Miller IV, Tim L. Danielson, Yezdi B. Pithawalla, Celeste T. Wilkinson, Anthony P. Brown and Karl A. Wagner; Altria Client Services, Richmond, VA USA

3:10 PM

99. EXTRACTION OF BAP IN WATER FROM MOIST SNUFF. Serban C. MOLDOVEANU and Andrew Harrison, R.J. Reynolds Tobacco, Winston-Salem, NC USA

Session B
E-Cigarettes
Session Chair: Fraser Williamson

2:30 PM

102. NOVEL APPLICATION OF DIFFERENTIAL ION MOBILITY SPECTROMETRY-TANDEM MASS SPECTROMETRY FOR IMPROVED ASSAY SELECTIVITY AND SENSITIVITY IN THE QUANTITATIVE DETERMINATION OF TOTAL NNN, TOTAL NNAL AND 2- / 3-HPMA IN HUMAN URINE. Jeff PLOMLEY; Altasciences, Laval, Canada

2:50 PM

103. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 1: SELECTION OF REPRESENTATIVE FLAVOR MIXTURES FOR TOXICOLOGICAL EVALUATIONS USING A STRUCTURAL GROUPING APPROACH. Kimberly D. EHMAN[1], Timothy B. Langston[1], Ashutosh Kumar[1], Monica Lee[1], Davide Sciuscio[2,] Patrick Vanscheeuwijck[2] and Julia Hoeng[2]; [1]Altria Client Services, Richmond, VA USA and [2]PMI R&D, Neuchâtel, Switzerland

3:10 PM

104. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 2: PREPARATION AND STABILITY CHARACTERIZATION OF REPRESENTATIVE FLAVOR MIXTURES. Cameron R. SMITH[1], John H. Miller IV[1], Niti Shah[1], Ashutosh Kumar[1], Monica Lee[1], Felix Frauendorfer[2], Philippe Guy[2], Pierrick Diana[2] and Anneke Glabasnia[2]; [1]Altria Client Services, Richmond, VA USA and [2]PMI R&D, Neuchâtel, Switzerland

BREAK

Tuesday Afternoon, September 17, 2019

4:00 PM

100. EVALUATION OF CONSTITUENTS RELEASED FROM SMOKELESS TOBACCO PRODUCTS TO HUMAN SALIVA. Siqi GUAN, Huihua Ji and Lowell Bush; University of Kentucky, Lexington, KY USA

4:20 PM

101. CHARACTERIZATION OF THE BACTERIAL COMMUNITY ASSOCIATED WITH SMOKELESS TOBACCO REFERENCE PRODUCTS UNDER DIFFERENT STORAGE CONDITIONS. Shuang LIU, Isaac Greenhut and Luke Moe; University of Kentucky, Lexington, KY USA

4:00 PM

105. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 3: *IN VITRO* CYTOTOXICITY AND GENOTOXICITY OF REPRESENTATIVE FLAVOR MIXTURES. Utkarsh B. DOSHI, Jingjie Zhang, Ashutosh Kumar and K. Monica Lee; Altria Client Services, Richmond, VA USA

4:20 PM

106. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 4: FLAVOR TRANSFER FROM THE LIQUID TO THE AEROSOL FOR INHALATION EXPOSURE. Jingjie ZHANG, Cameron Smith, Chase Anderson, Nicholas McCutcheon, John Miller and K. Monica Lee; Altria Client Services, Richmond, VA USA

4:40 PM

107. PREDICTED IMPACTS OF E-CIGARETTES ON US MORTALITY AND HEALTH CARE COSTS. Bill POLAND[1] and Sylvain Larroque[2]; [1]Certara USA, Menlo Park, CA USA and [2]JT International, Geneva, Switzerland

ADJOURN

5:15 PM **TSRC BUSINESS MEETING:** All attendees are encouraged to attend.

6:30 PM Pre-banquet Reception

Wednesday Morning, September 18, 2019

Combined Session

Materials, Manufacturing and Finished Product

Session Chair: Ian Fearon

9:00 AM **108. ADULT CIGARETTE SMOKERS' EXPECTATIONS, PERCEPTIONS AND REACTIONS TO REDUCED NICOTINE CONTENT CIGARETTE PROTOTYPES.** Andrea VANSICKEL and Jan Angel; Altria Client Services, Richmond. VA USA

9:20 AM **109. FUNCTIONAL FILTER PLUG WRAP PAPER FOR THE CONTROL OF THE THERMAL ENERGY OF THE AEROSOL FROM HEATED TOBACCO PRODUCTS.** Michael LINDNER[1]and Nadine Leichsenring[2]; [1]TANNPAPIER, Traun Austria and [2]Hauni Maschinenbau, Hamburg Germany

9:40 AM **110. WHAT IS ACID ABOUT ACID CIGARS?** John H. LAUTERBACH; Lauterbach & Associates, Macon, GA USA

10:00 AM **111. PREPARATION OF AMINO ACID IONIC LIQUIDS FOR REDUCING HCN AND CROTONALDEHYDE FROM SMOKE AND EVALUATION OF CIGARETTE APPLICATION.** SUN Xuehui[1], Dong Lu[1], Wang Hongwei[2], Yang Song[1], Sun Peijian[1], Guo Ge[1], Pan Lining[1], Sun Zhitao[2], Zhang Xiaobing[1], Tian Haiying[2] and Nie Cong[1]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China and [2]Technology Center, China Tobacco Henan Industrial, Zhengzhou, China

ADJOURN

73rd Tobacco Science Research Conference

Abstracts

1. IS CIVIL DIALOGUE AND ENGAGEMENT BETWEEN DIVERSE STAKE–HOLDERS WITH RESPECT TO TOBACCO HARM REDUCTION FEASIBLE? A REVIEW OF THE PAST. PRESENT AND FUTURE. Scott D. BALLIN; Health Policy Consultant, Washington, DC USA

With a billion smokers in the world and an annual toll of 7 million premature deaths, more can be done to significantly reduce or even eliminate the use of combustible products. Part of that effort involves the development of science- based consumer acceptable lower risk alternative products. The public health community, policy makers, and regulators are very much divided as to whether harm reduction is a viable strategy. Part of these differing views have deep roots in the distrust of the tobacco industry that for decades denied that its products caused serious harms and then developed unregulated products which made implied claims of safety (*i.e.* low tar and nicotine). This personal essay will explore the past, present and the future as means of determining whether there may be a path forward in making 'harm reduction' a part of the solution rather than being seen as part of the problem. The first area reviewed will be starting in the 1950's until about 2000, focusing on tobacco industry tactics and strategies to misuse science and to deny that their products caused cancer, heart disease and other harms. The second period will cover the time from the Master Settlement Agreement (1998), through the passage of the Tobacco Control Act in 2009 and up until the present day. The third area will be on whether it is possible in a regulated environment for diverse stakeholders to engage in a civil dialogue that focuses on making harm reduction a legitimate way of significantly reducing disease and death from cigarettes.

2. FDA REGULATING TOBACCO PRODUCTS ALONG A CONTINUUM OF RISK. Deirdre Lawrence KITTNER; FDA, Center for Tobacco Products, Office of Science, Calverton, MD USA

In 2009, the Family Smoking Prevention and Tobacco Control Act created the Center for Tobacco Products (CTP) and set forth a new public health standard that considers the benefits and risks to both users and non-users of tobacco products. Consistent with this standard, in July 2017, former FDA Commissioner Scott Gottlieb announced FDA's comprehensive plan for tobacco and nicotine regulation. This plan recognized that, while highly addictive, nicotine is delivered through products on a "continuum of risk," with cigarettes being the most harmful. This presentation will: 1) provide a brief overview of FDA statutory requirements for tobacco product regulation; 2) describe CTP activities informed by the continuum of risk paradigm; 3) and provide examples of improved product review processes (*e.g.*, ITP guidance, SE guidance). CTP has cracked down on illegal sales of e-cigarettes to youth, launched tobacco education campaigns, sought input on potential product standards to make combustible products less appealing and addictive, and authorized pre-market tobacco products (*e.g.*, Swedish Match General Snus products, Philip Morris Products S.A.'s IQOS). CTP continues to educate stakeholders to help

improve Pre-Market Tobacco Application (PMTA) and Modified Risk Tobacco Application (MRTPA) processes by sharing expectations for product applications, providing examples of commonly omitted documentation, and encouraging pre-submission meetings and the use of Tobacco Product Master Files (TPMFs). In summary, FDA has used, and will continue to use, available regulatory tools to change the tobacco landscape via strategic regulation in order to improve public health.

3. TOBACCO HARM REDUCTION: WEIGHING THE EVIDENCE. Brian E. ERKKILA; Foundation for a Smoke-Free World, Washington, DC USA

As millions of smokers around the world transition to reduced risk products (RRPs), there is a common refrain often heard from the public health community and media: "We just don't have enough evidence." What they are referring to are the long-term epidemiological studies which have been the cornerstone of the public health for decades. While it is true that most RRPs have gained popularity only in the last decade, that does not mean we do not have evidence of their potential. There is a robust body of literature demonstrating that when smokers move to RRPs their exposure to HPHCs drops significantly. Furthermore, every year we build the body of evidence that these reduced exposures translate into changes in biomarkers of biological effect, compelling evidence that RRPs could yield great benefits to public health. On the behavioral front, researchers have debated the merits of clinical trials examining whether or not electronic cigarettes can help people quit, however when we look at the real-world evidence, we see that millions have already switched. We mainly know the effects of the long-term use of just one type of product, cigarettes, and they are devasting. Stakeholders must not only determine how much evidence around RRPs is enough to develop smart regulations and communication strategies for consumers– but a path to get it done.

4. TOBACCO HARM REDUCTION: ADDRESSING COMPLEXITIES ACROSS THE RISK CONTINUUM. Willie J. MCKINNEY; JUUL Labs, San Francisco, CA USA

Government, industry and public health are learning quickly about the complexities of harm reduction in the new tobacco regulatory universe. Each institution acknowledges the harm caused by smoking cigarettes, that a continuum of risk exists for tobacco products, and that youth should never use tobacco products. Disparities reside in how each institution advances harm reduction and resolves unintended consequences.

In July of 2017, FDA announced its intention to implement a new comprehensive regulatory plan for tobacco and nicotine that would significantly reduce tobacco-related disease and death and protect kids. FDA noted that cigarette smoking is the primary cause of tobacco-induced harm and that a key component to the success of their approach is increasing awareness that nicotine – while highly addictive – is delivered through products that represent a continuum of risk and is most harmful when delivered through smoke particles in combustible cigarettes. Currently, FDA has not launched a public health campaign to help adult smokers understand that nicotine is not the primary source of harm from smoking.

Unfortunately, since the 2017 FDA announcement, adult tobacco users misperceptions about relative risk have increased. Some public health campaigns and statements from notable tobacco control leaders designed to discourage youth from initiating products containing

nicotine perpetuate adult tobacco user misperceptions about relative risk. Adult tobacco user misperception about the continuum of risk inhibit switching from more harmful to less harmful nicotine containing products. Some within industry voluntarily imposed restrictions focused on preventing youth access. FDA guidance for industry addressing this issue shortly followed. Time will tell if the FDA guidance and voluntary industry effort effectively advance harm and address unacceptable unintended consequence.

Industry is continuing to invest significantly in tobacco products that contain technological advances to inhibit youth use and mitigate other unintended consequences. The ability to market these products in the United States is however restricted by a rigorous regulatory process. The US regulatory process presents a significant barrier to quickly leverage product technology to address complex harm reduction challenges.

Tobacco regulation, communication, and availability of product technology are critical components utilized to both advance harm reduction and resolve unintended consequences. More opportunities to discuss barriers and disparate institutional approaches to address the complexities of tobacco harm reduction are needed.

5. INCORPORATING ANALYTICAL VARIANCE INTO A COMPARATIVE QUANTITATIVE RISK ASSESSMENT (QRA) APPROACH FOR TOBACCO PRODUCTS.
Chastain A. ANDERSON, Vanessa Haase, Kimberly D. Ehman, Paige N. Wiecinski and Donna C. Smith; Altria Client Services, Richmond, VA USA

The US Food and Drug Administration (FDA) draft guidance for industry (2012) provides abbreviated lists of harmful and potentially harmful constituents (HPHCs) found in tobacco and cigarette smoke. As FDA considers the abbreviated HPHC list to be representative of the classes of hazardous compounds present in tobacco products or tobacco smoke, it can serve as the basis of a relative "whole-product" toxicological risk comparison for tobacco products (*e.g.*, New Product to Predicate Product). A range of validated analytical techniques exist for quantification of individual HPHCs, which are reported as a mean value with a standard deviation. The objective of this presentation is to propose a method for incorporating analytical variance into a comparative quantitative risk assessment (QRA) framework to provide a more robust representation of relative toxicological risk between whole tobacco products. Using a cigarette example, we provide a general overview of the QRA framework (*i.e.*, hazard assessment, exposure assessment, and risk characterization) and highlight how incorporating the analytical variance in HPHC yields into the exposure assessment and hazard characterization produces an estimate of the range of probable risk as opposed to a single (deterministic) point estimate. The range of risk for each individual HPHC is calculated for both cancer (*i.e.*, Excess Lifetime Cancer Risk – ELCR) and non-cancer (*i.e.*, Hazard Index – HI) effects, and these individual risk estimates are then aggregated into representative estimates of whole-product risk. Additionally, statistical analysis can be performed to assess whether there is a significant difference ($p < 0.05$) in risk between the products.

6. ***IN VITRO*** TESTING OF AN ETHANOL COLLECTION METHOD COMBINING PARTICULATE AND GAS-VAPOR PHASE COMPONENTS: BACTERIAL REVERSION MUTATION (AMES) ASSAY. Sanjay Kumar BHARTI, Bhagyalaxmi Sukka Ganesh, Mariano J. Scian and I. Gene Gillman; Enthalpy Analytical, Henrico, VA USA

Health Canada (HC) guidelines (T-502) for the collection and testing of cigarette smoke are used frequently for *in vitro* testing. Although the guidelines allow the collection and testing of particulate phase (PP), gas-vapor (GVP) phase, and a combination of both (PP+GVP), this method has several limitations. The PP is collected in DMSO and can be tested for cytotoxicity (NRU assay), mutagenicity (Ames assay), and clastogenicity (MN assay), while the GVP phase is collected in PBS and tested only using NRU assay. PBS has limited trapping efficiency of volatile or non-water-soluble compounds and must be used within 60 minutes of collection. These limitations could be overcome with a method allowing collection of PP and GVP together in a solvent providing enhanced trapping and stability of GVP components. We have tested the use of ethanol to collect PP and GVP components using the Ames assay (HC T-501 guideline). Reference 3R4F cigarettes were used. Five strains of Salmonella bacteria (TA98, TA100, TA102, TA1535 and TA1537) were tested in the absence and presence of S9. Results showed that the ethanol condensate collecting PP+GVP together resulted in increased bacterial lawn cytotoxicity in TA98, TA100, TA1535 and TA1537. DMSO-extracted PP showed toxicity in TA98, TA100, TA1537 while PP+GVP in PBS showed toxicity in TA1537. With S9, TPM+GVP in ethanol induced 18-fold and 11-fold revertants in TA98 and TA1537 respectively. In comparison, PP phase induced 16-fold and 8-fold, TPM+GVP in PBS induced 14-fold and 6-fold increase in revertants for the same strains. The ethanol method collects PP+GVP components in a single whole-smoke extract and significantly increase the mutagenic and cytotoxic effects in Ames assay. NRU, MN, and chemistry results are presented separately.

7. CONVERSION OF A PHARMACEUTICAL SALT-SCREENING ROBOT PLATFORM TO AN AUTOMATED STATION FOR UNATTENDED WEIGHING E-CIGARETTES AND SMOKING MACHINE FILTERS. J. Anthony COX and Justin Lu; Sirius Automation Group, Buffalo Grove, IL USA

The authors will demonstrate how to adapt a standardized small footprint biotech laboratory robot originally designed for the screening of pharmaceutical salt & crystallization studies in advanced drug development for use in the high speed analytical weighing of e-cigarettes and smoking machine filter housings, plus the potential addition of other tasks including viscous liquid pipetting/refilling of e-cigarettes to target by weight and resampling of tobacco products for multiple assay types. A modular extension for automated container labeling will further be presented. Data for precision and speed will be tabulated.

8. WORKSHOP SERIES TO IDENTIFY, DISCUSS AND DEVELOP RECOMMENDATIONS FOR THE OPTIMAL GENERATION AND USE OF *IN VITRO* GENOTOXICITY ASSAY DATA FOR TOBACCO AND NICOTINE PRODUCTS. Martha M. MOORE[1] and Rodger Curren[2]; [1]Ramboll US Corporation, Little Rock, AR USA and [2]Institute for *In Vitro* Sciences, Gaithersburg, MD USA

The Institute for *In Vitro* Sciences is sponsoring a series of workshops to identify, discuss and develop recommendations for optimal scientific/technical approaches for utilizing *in*

vitro regulatory genotoxicity assay data within and across tobacco and nicotine product categories. Workshops provide a unique opportunity for invited expert stakeholders to share experiences and to develop recommendations that may serve as a resource for developing optimal data to evaluate the toxicity of tobacco and nicotine products. It is envisioned that some of these recommendations would form the basis for the generation of guidance documents and/or serve as authoritative reference publications for optimal methodologies and data interpretation and to support regulatory submissions. During the first workshop (November 27-28, 2018) workgroup members identified important issues for using *in vitro* genotoxicity assays for evaluating tobacco and nicotine products and issues were triaged into three priority categories based on the amount of available information. This issue list serves as the basis for focused high-priority topics for subsequent workshops. The topic of the second workshop (June 4-5, 2019) is the generation of appropriate test samples for *in vitro* genotoxicity testing and the third workshop (September 2019) addresses optimal methods for cell exposure. Future workshops will tackle issues such as: (1) recommended cell types for *in vitro* cytogenetic evaluations, (2) recommendations for expressing exposure when comparing products within and among product categories, (3) recommendations for comparing toxicological responses within and among product categories, and (4) applying new or existing methods for assessing genotoxicity and other toxicological effects of tobacco and nicotine products using cells in culture. This presentation will provide a summary overview of the workshop series.

9. VOLATILE ORGANIC COMPOUNDS IN ENDS AEROSOL: COLLECTION ON ACTIVATED CARBON. Kathy HUMPHRIES, Mitch Zimmerman, Trevor Lott and Gene Gillman; Enthalpy Analytical, Durham, NC USA

The premarket approval process for ENDS devices requires that a wide range of Harmful and Potentially Harmful Compounds (HPHCs) be determined to support the application. The determination of volatile organic compounds (VOCs) in ENDS aerosol presents unique challenges since many VOCs have exposure limits that are orders of magnitude lower than what is possible to measure with traditional mainstream smoke methods. One possible approach to lower the detection limits is to concentrate the samples via collection on activated carbon media. This approach has been used to determine VOCs from cigarettes and heated tobacco products.

In our approach, ENDS aerosol was collected on a glass fiber filter follow by activated carbon media. Standards were initially prepared in solvent but recovery from the media, especially at low levels, was found to not be quantitative. To overcome media effects, calibration standards were instead prepared by collecting certified gas standards in the same manner as the samples. With this approach we were able to produce linear calibration curves from 0.054µg/mL to 2.5µg/mL with recoveries of 90 to 116% for all compounds. Method detection limits for 1,3-butadiene, acrylonitrile and isoprene are ~4.3ng/puff, benzene and toluene were found to be ~1.8ng/puff assuming collection of 50 puffs.

This process also eliminated the need for cryo trapping, complicated glassware and greatly reduced solvent consumption. Trapping the aerosol onto the active carbon tube has an added benefit of increased sample and standard stability versus collection in solvent.

10. QUALIFICATION OF CELL LINE A549 FOR THE NEUTRAL RED UPTAKE (NRU) ASSAY. Shannon BRUCE, Khushbu Garala, Deepa Srinivasan, Sandra Springer, Douglass Dey, Kayla Campasino and Rohan Kulkarni; MilliporeSigma (BioReliance® Toxicology Testing Services), Rockville, MD USA

As per 3R, *in vitro* cytotoxicity tests such as the Neutral Red Update (NRU) assay are routinely used as alternative toxicity tests to eliminate the use of animals for acute oral toxicity tests. The NRU assay is one of the most commonly used cytotoxicity assays for chemicals, pharmaceuticals, cigarette smoke condensate, plant extracts and medical device extracts. As per the ICCVAM protocol, this test is generally performed in BALB/c 3T3 cells. The modal number of chromosomes in BALB/c 3T3 cells is 78 with a range of 62 to 109. The stem line number is hypo-tetraploid which makes this a karyotypically unstable cell line. We are proposing the use of the A549 cell line which is a hypotriploid human lung adenocarcinoma epithelial cell line. The modal number of chromosomes in A549 cells is 66 with a range of 64 to 67 and can be obtained from American Type Culture Collection (ATCC). Also, as per some of the peer-reviewed literature, this lung cell line may be useful to understand the role of alveolar Type II cells in the drug or chemical delivery at the pulmonary epithelium which makes this work relevant to tobacco research. Since the ICCVAM protocol is designed for the BALB/c 3T3 cells, we have performed a comparative study using A549 cells. We tested nine chemicals, from the NTP database with known different modes of action, using the 96-well plate method. The IC20, IC50, and IC80 values were calculated for each chemical, where possible. Our results indicate that the A549 cells can be used in lieu of the BALB/c 3T3 cells for the Neutral Red Uptake Assay.

11. COMPARISON OF VEHICLE CONTROLS FOR CELL LINE A549 IN THE NEUTRAL RED UPTAKE (NRU) ASSAY. Shannon BRUCE, Khushbu Garala, Deepa Srinivasan, Sandra Springer, Douglass Dey, Kayla Campasino and Rohan Kulkarni; MilliporeSigma (BioReliance® Toxicology Testing Services), Rockville, MD USA

To avoid and minimize acute oral toxicity tests on animals, *in vitro* cytotoxicity tests such the Neutral Red Uptake (NRU) assay are routinely performed. This assay does not have an OECD test guideline but the ICCVAM protocol and OECD guidance document 129 are used to perform this cytotoxicity test in the pharmaceutical, chemical and tobacco industries. In the tobacco industry, since e-liquids are formulated in various vehicles such as propylene glycol and glycerin, we investigated various vehicle controls in the A549 human lung adenocarcinoma epithelial cell line. Using the ICCVAM protocol as a guide, we investigated the use of complete medium, water, dimethyl sulfoxide (DMSO), ethanol (EtOH), propylene glycol (PG), glycerin (VG) and a mixture of PG/VG using the 96-well plate method. The positive control selected was Sodium Lauryl Sulfate (SLS). Our results indicate that test materials can be administered to A549 cells using a variety of vehicles for the Neutral Red Uptake Assay.

12. REAL-TIME MONITORING OF VOLATILE ORGANIC COMPOUNDS AND AEROSOL IN EXHALED BREATH FROM INHALATION OF VAPOR PRODUCTS AND COMBUSTIBLE CIGARETTES. Devon C. O'REGAN[1], Adam M. Ozvald[1], Nadja Heine[1], Rene Gutmann[2] and Bea Rosenkranz[2]; [1]JUUL Labs, San Francisco, CA USA and [2]Ionicon, Innsbruck, Austria

Exhaled breath (EB) analysis is an attractive methodology for clinical studies, due to its non-invasive nature. Analysis of Volatile Organic Compounds (VOCs) in exhaled breath may facilitate diagnosis of pathological conditions, including potential applications in diabetes mellitus, liver, and kidney diseases. Analyzing VOCs in the EB of smokers may unveil biomarkers of smoking-related diseases. A recent study differentially identified exogenous analytes such as furan, acetonitrile, and benzene in the breath of smokers and passive (secondhand) smokers.

Conventional EB studies are based on time-consuming off-line technologies, typically involving sampling into a portable exhaled breath condensate collection device for several breaths, followed by extraction and analysis of the EB sample. These procedures do not allow for real-time analysis and are prone to sample instability. In this study, we present a proof-of-concept for fast real-time chemical profiling setup based on proton-transfer-reaction mass spectrometry (PTR-MS).

For this study, a small group of volunteers was divided into groups of smokers and nonsmokers and mass spectrometric data was collected to analyze the chemical profiles of their EB. Comparison between the baseline EB of smokers and nonsmokers revealed known toxins, including mutagens and carcinogens from biomass burning (*e.g.*, acetonitrile and benzene) in the breath of smokers. Following baseline, exhalation data was monitored and analyzed for smokers and nonsmokers using vapor products and compared to the mainstream aerosol released from the vapor product, enabling tracking of absorption of components, including but not limited to, nicotine, propylene glycol, and menthone. We also compared the aerosol profiles for a broad spectrum of compounds between cigarette smoke and vapor products.

13. SIMULTANEOUS DETERMINATION ANALYSIS OF GLYCEROL, PROPLYLENE GLYCOL, NICOTINE, MENTHOL AND WATER BY DISCHARGE IONIZATION DETECTOR ON GAS CHROMATOGRAPHY. Akihito SHIMAZU and Fumihiro Omori; Japan Tobacco, Tokyo Japan

Several analytical methods for determination of Glycerol (G), Propylene Glycol (PG), Nicotine, Menthol and Water in the aerosol of both e-cigarettes and tobacco vapor products were reported in the past few years. In the methods, those five components were determined by two detector such as Flame Ionization Detector (FID) for G, PG, Nicotine, Menthol and Thermal Conductivity Detector (TCD) for Water respectively. Furthermore, there were several reports about simultaneous determination analysis of those components by TCD. However, the sensitivity of TCD is less than one hundredth compared with that of FID. Thus, it was difficult to determine those five components in common aerosol samples by TCD. Therefore, a new type of detector, the sensitivity of which is comparable with that of FID, is required to determine those five components simultaneously. Discharge Ionization Detector (DID) was found to be an optimal detector for analyzing those components.

The final aim is to develop an analytical method for simultaneously determining G, PG, Nicotine, Menthol and Water in the cigarette smoke and the aerosol of both e-cigarettes and tobacco vapor products by DID. In this study, the applicability of DID was investigated by using standard mixture of those five components. As a result, it was confirmed that peaks of those five components in standard mixture were separated. In addition, it was found that the limit of detection and the limit of quantification were sufficient to determine those five components in this study.

14. DETERMINATION OF DICARBONYLS AND CROTONALDEHYDE IN E-VAPOR PRODUCTS. David ZICH; ITG Brands, Greensboro, NC USA

With the increasing popularity in next generation products (NGPs) such as electronic cigarettes, the need for robust high-throughput methods to accurately quantitate very low analyte values found within these products is growing. Dicarbonyls and monocarbonyls represent a class of compounds which are frequently present within commercial e-cigarette aerosols and often found at trace levels. Methods for the compounds found in literature depend heavily on tedious derivatization of the carbonyl groups with 2,4-Dinitrophenylhydrazine (DNPH), O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine (PFBHA), and other select compounds to improve stability and sensitivity. Other methods in literature use solvents which react with monocarbonyls making combined analysis of mono and dicarbonyls problematic. To address these analytical challenges, a simplified and robust GC-MS/MS method was developed using a PTV (programmable temperature vaporizing) inlet that allows for dual quantitation of both dicarbonyls (diacetyl and acetylpropionyl) and monocarbonyls (crotonaldehyde) without the use of a derivatization step. This method offers advantages over current literature in simplicity and speed for sample preparation and simultaneous quantification of mono and dicarbonyls. The method has a runtime of 22 minutes, LOQ's at 6 ng/mL while maintaining a S/N ratio greater than 10, and recoveries ranging from 90 – 113%.

15. LEVELS OF ENDOTHELIAL PROGENITOR CELLS (EPCS) IN SMOKERS AND MOIST SNUFF CONSUMERS. Subhashini ARIMILLI[1], Peter Chen[2] and G. L. Prasad[2]; [1]Eurofins Professional Scientific Services, Winston-Salem, NC USA and [2]RAI Services Company, Winston-Salem, NC USA.

Endothelial progenitor cells (EPCs) are a key cell type present in circulation and are reported to play a role in the development of cardiovascular diseases. EPCs are derived from the bone marrow and mediate the differentiation, regeneration and maintenance of endothelial cells in response to vascular injury and angiogenesis. EPC levels have been proposed as an important biomarker for endothelial dysfunction. However, the association between levels of EPCs and endothelial dysfunction needs further investigation. Cells with the phenotype of CD34+CD133+CD309+ (VEGR2) are defined as "triple positive" circulating EPCs. To evaluate whether EPC levels can be utilized as a smoking-related biomarker of potential harm, we measured triple positive EPC levels by flow cytometer in peripheral blood mononuclear cells (PBMCs) isolated from three different cohorts: healthy smokers (SMK) (n=40), moist snuff consumers (MSC) (n=40) and non-tobacco consumers (NTC) (n=40). We measured total EPC count, EPC percentage and EPC cumulated percentages of triple positive EPCs from the lymphocyte and monocyte populations of SMK, MSC and NTC. All the measurements showed a significant increase in the EPC levels in the SMK cohort

compared to NTC. The EPC levels in MSC, however, statistically were not significantly different from SMK or NTC. Higher levels of circulating EPCs in healthy smokers may be due to the increased need to repair vascular wall injury due to cigarette smoking. Further work is needed to establish the role of EPCs in understanding endothelial function and to utilize them as biomarkers of potential harm in tobacco consumers.

16. BELIEFS, PERCEPTIONS, AND BEHAVIORAL INTENTIONS RELATED TO NICOTINE, LOW NICOTINE CIGARETTES, AND REDUCED RISK PRODUCTS AMONG U.S. ADULTS. Tiffany PARMS and Kimberly Frost-Pineda; RAI Services Company, Winston-Salem, NC USA

In March 2018, the U.S. Food and Drug Administration (FDA) published an advance notice of proposed rulemaking to obtain information for consideration in developing a tobacco product standard to set the maximum nicotine level for cigarettes. We examined beliefs about nicotine and low nicotine cigarettes (LNC) among U.S. adult current, former, and never smokers. Data from the 2017 Health Information National Trends Survey (HINTS-FDA), a nationally representative cross-sectional probability-based survey, were analyzed using the PROC SURVEYFREQ procedure in SAS 9.4. The majority of respondents believed that nicotine is the main substance in tobacco that makes people want to smoke, and over half of respondents believed that nicotine causes most of the cancer attributed to smoking. More than a third of respondents did not believe that a cigarette could be low nicotine. LNC were frequently viewed as less harmful (29.2%), less addictive (32.8%), and posing a lower risk of causing lung cancer (22.7%) compared to typical cigarettes. Less than one in five respondents indicated that they were likely to use a tobacco product claiming to be less harmful or less addictive. Outcomes were consistent regardless of smoker status. Findings from this analysis highlight the need to educate the public about the effects of nicotine, as well as LNC. In this analysis of nationally representative data, many respondents believed that nicotine is cancer-causing, and some respondents perceived LNC to be less harmful than typical cigarettes.

17. BELIEFS AND PERCEPTIONS ABOUT NICOTINE AND "MINIMALLY ADDICTIVE" CIGARETTES AMONG ADULT CURRENT, FORMER, AND NEVER SMOKERS. Tiffany PARMS, Kimberly Frost-Pineda and Geoffrey Curtin; RAI Services Company, Winston-Salem, NC, USA

In March 2018, the US Food and Drug Administration (FDA) issued an advance notice of proposed rulemaking (ANPRM) seeking information on a tobacco product standard potentially lowering nicotine levels in combustible cigarettes. Understanding consumer perceptions of very low nicotine content (VLNC) cigarettes could inform the potential impact of the proposed standard on public health. In order to assess appeal and perceptions of risk associated with nicotine and a cigarette that presents "minimally addictive" or non-addictive levels of nicotine, a cross-sectional online survey was conducted among adult current, former, and never cigarette smokers recruited from a commercial marketing research panel. Overall, 35% of respondents perceived "minimally addictive" cigarettes to be less harmful, and 45% perceived them as less addictive, compared to typical cigarettes. Eighty-five percent of respondents believed that nicotine causes people to want to smoke, nearly 60% believe nicotine causes most of the cancer caused by smoking, and roughly 70% indicated that addiction to nicotine is something they are concerned about. Differences

in perceptions were seen by smoking status (*i.e.* current, former, and never smokers) and among subgroups (*i.e.* by gender, age, likelihood to quit smoking cigarettes, length of time since quitting smoking, and susceptibility to initiate cigarette smoking). Perceptions of reduced nicotine are associated with reduced perceptions of harm, which could encourage continued use, relapse, or initiation. These findings underscore the need to address unintended consequences of a proposed reduced nicotine product standard.

18. TOBACCO FLASH PYROLYSIS. EFFECT OF THE ADDITION OF MESOPOROUS CATALYSTS. Emilio CALABUIG, Antonio Marcilla and Maria Isabel Beltrán; University Institute of Chemical Process Engineering, San Vicente del Raspeig, Spain

Different studies have been carried out applying microporous and mesoporous catalysts to tobacco, either added to the filter or directly mixed with tobacco, for the reduction of toxic compounds generated by combusting tobacco. The main advantage of mesoporous materials in the catalytic decomposition of tobacco is the easier accessibility of large molecules to active sites through their large pores (2-50 nm).

Relating the smoke generated in tobacco from its components is complex. These relationships are usually established with pyrolysis experiments. Experiments of flash pyrolysis of tobacco references 3R4F from the Reference Cigarette Program from The college of Agriculture of the University of Kentucky and a mixture of reference tobacco with a mesoporous catalyst SBA-15 have been carried out to determine the effect of this type of catalysts in tobacco. A multi-shot pyrolyzer has been used, which was attached directly to a gas chromatography/ mass spectrometer (Py-GC/MS).

Experiments have been run in helium and air atmospheres. In inert atmosphere a reduction is observed in the heavier products and a small increase in some light compounds, leaving to overall reductions around 22%. In oxidizing atmosphere there are larger effects depending on the pyrolysis temperature. Overall reductions of 68% at 500°C, 53% at 300°C or 15% at 700°C have been obtained.

19. SIMULATED LEACHABLES ASSESSMENT FOR CRITICAL COMPONENTS USED IN CARTRIDGES OF ELECTRONIC NICOTINE DELIVERY SYSTEMS. Courtney CULBERT[1] and Randy Weidman[2]; [1]RAI Services Company, Winston-Salem, NC USA and [2]R.J. Reynolds Tobacco Company, Winston-Salem, NC USA

Electronic Nicotine Delivery Systems (ENDS) contain e-liquid formulations that are heated to generate aerosols. The potential for chemicals to leach from the device materials into the e-liquid and transfer into the aerosol has been an area of interest for this type of consumer product. ENDS cartridges are commonly composed of polymers, elastomers, metals, glass fiber, and electronic components. Cartridge components most likely to leach are termed critical components, defined here as components that are in contact with the e-liquid reservoir or aerosol pathway. Previously, critical components were extracted using harsh solvents under exaggerated temperatures and conditions, generating worst case extractables profiles. In this study, the e-liquid formulations themselves were used as the extraction solvent to better simulate what could be expected to leach from the critical components in the ENDS consumer product. For this simulated leachables assessment, the critical components were extracted in e-liquid in a sealed vessel at 60°C for 33 days.

Using the Arrhenius equation, these conditions can be expected to predict extraction at 25°C for 12 months. The resulting e-liquid extracts were characterized via spectroscopic and chromatographic methods to establish the elemental and organic extractable profiles. Characterization of extractables under these simulated leaching conditions supports development of a repository for potential future leachables assessments in aerosol.

20. NEUTRAL RED UPTAKE (NRU) CYTOTOXICITY ANALYSIS OF AEROSOL GENERATED FROM A TEMPERATURE-REGULATED NICOTINE SALT BASED PRODUCT UTILIZING COTTON WICKING MATERIAL. Bryant HIRAKI, David Cook and Manoj Misra; JUUL Labs, San Francisco, CA USA

Background: The JUUL Nicotine Salt Pod System (NSPS) has no user modifiable settings and is temperature regulated to minimize the generation of combustion related byproducts. Aerosol generated from NSPS pods via a cotton wicking material was evaluated for cytotoxicity potential.

Methods: Seven flavors (tobacco, mint/menthol, and fruit flavors; 18mg/mL nicotine) were evaluated. Cytotoxic potential was assessed using the NRU *in vitro* assay (OECD, TG 129; Labstat International ULC, Canada). CHO-WBL cells were treated with NSPS aerosol, positive control, or vehicle control for 24 hours utilizing a dimethyl sulfoxide (DMSO) extraction methodology (method T-502, Health Canada) with modifications. NSPS aerosol exposure occurred via separate dosing of aerosol collected mass in DMSO and gas vapor phase in CMF-PBS across a dose range of 0-1000 µg/mL. Additionally, cells were exposed to the sodium lauryl sulfate positive control at the concentration of 110 µg/mL. Cell viability following NSPS aerosol exposure was compared to the mainstream smoke component from a reference cigarette (3R4F) at dose range of 0-150 µg/mL.

Results: No significant aerosol mediated toxicity was observed at any of the tested NSPS flavors or concentrations. EC50 for the NSPS aerosol and carrier control aerosol could not be calculated because cell viability was greater than 50% for all test conditions.

Conclusions: Under the experimental conditions and based on the criteria for Evaluation of Cytotoxic Response (ISO 10993-5), all NSPS aerosol condensates generated from the 18mg/mL NSPS test articles were found to be non-cytotoxic.

21. INFLUENCE OF THE TIME OF AND STIRRING RATE DURING THE FIRST STEP OF SYNTHESIS OF SBA-15 ON ITS CATALYTIC EFFECT FOR REDUCING TOXICANTS CONCENTRATION IN TOBACCO SMOKE. Nerea JUÁREZ-SERRANO, Javier Asensio-Morant, Isabel Martínez-Catellanos, Desiré Berenguer, Inmaculada Blasco, María Isabel Beltrán and Antonio Marcilla; University of Alicante, Alicante, Spain

Certain mesoporous materials can modify notably the composition of smoke tobacco, since they can act as catalysts and/or adsorbents allowing to reduce the toxic and carcinogenic compounds of the smoke. However, the properties of the catalysts strongly depend on the synthesis conditions affecting their final possible. The main objective of this work is to study the influence of the time and the stirring rate during the first stage of the synthesis on the structure of the SBA-15, and determine the effect of these materials in reducing toxicants of tobacco smoke. The synthesis procedure was based on the method of Zhao modifying the

time and the stirring rate of the first synthesis step. Materials were characterized by X-ray powder diffraction (XRD), nitrogen adsorption isotherms, scanning electron microscopy (SEM) and apparent density. The smoking experiments were performed under ISO3308 conditions using 3R4F tobacco, and the mixtures were prepared adding 4,8% of SBA-15. The composition of the gas fraction and the total particular matter (TMP) were quantified using GC-FID e GC-MS techniques, respectively.

Increasing the time, relevant improvements could be observed in the properties of the materials with significant effects in their activity for reducing the toxic compounds in smoke experiments. Contrarily, increasing the stirring rate adversely affect the porous properties as and its performance in smoking experiments. Short fibres were obtained at the highest stirring rate tested.

22. A SEVEN-MONTH SYSTEMS TOXICOLOGY INHALATION STUDY IN C57BL/6 MICE DEMONSTRATES REDUCED PULMONARY INFLAMMATION AND EMPHYSEMA FOLLOWING SMOKING CESSATION OR SWITCHING TO E-VAPOR AEROSOL EXPOSURES. Ashutosh KUMAR[1], Ulrike Kogel[2], Marja Talikka[2], Julia Hoeng[2], Manuel Peitsch[2], Anthony Skowronek[3] and K. Monica Lee[1]; [1]Altria Client Services, Richmond, VA,USA, [2]Philip Morris International, Neuchâtel, Switzerland and [3]Battelle, West Jefferson, OH USA

Cigarette smoking causes lung cancer, emphysema, and other serious diseases. While cessation remains the most effective approach to minimize smoking-related diseases, alternative nicotine delivery products that limit the generation of combustion by-products may offer reduced risk to those who would otherwise continue to smoke. E-vapor products are one set of such promising nicotine-delivery products. The health risks of long-term inhalation exposures are unknown. We designed a chronic inhalation (4 h/day, 5 d/week, 7 months) study in C57BL/6 mice to evaluate respiratory toxicity of MarkTen® e-vapor aerosols in comparison to the reference 3R4F cigarette smoke (CS). Additional groups were added to explore the impact of CS cessation or switching to e-vapor exposures. There were no significant changes in in-life observations in e-vapor groups compared to the sham control. The CS group had lower body weight and showed transient signs of distress post-exposure and reduced respiratory function during exposure. Following 7 months of exposure, e-vapor resulted in no or minimal increase in pulmonary inflammation, while the CS induced consistently elevated pulmonary inflammation and emphysema. Biological changes in the switching group were similar to those in the cessation group. Transcriptomics analysis showed that the CS exposure elicited a large number of differentially expressed genes (DEG) in the lung and nasal epithelium, while drastically reduced changes were observed in response to e-vapor exposure. Compared with the CS group, the number of DEGs was much smaller in the lungs of the switching or cessation groups, while the reduction in nasal epithelium was less pronounced in the switching or cessation groups. In conclusion, exposures to e-vapor aerosols instead of CS can significantly reduce the respiratory disease risk associated with cigarette smoking.

23. EFFECT OF SBA-15 MORPHOLOGY IN THE COMPOSITION OF THE MAINSTREAM TOBACCO SMOKE. Isabel MARTÍNEZ-CASTELLANOS, Antonio Marcilla, María Isabel Beltrán, Desiré Berenguer, Inmaculada Blasco, Nerea Juarez, Javier Asensio and Emilio Calabuig; University of Alicante, Alicante, Spain

Mesoporous silica materials, such as SBA-15, are attracting increasing interest in the last years. Our research group has focused in their capability for reducing the toxic compounds generated in the smoking process when mixed with tobacco.

By tuning the mixing time and intensity, temperature and other variables, SBA-15 may be prepared with different morphologies. Different SBA-15 have been synthesized with fiber like morphology, spherical particles, platelets and rods. All these materials have been added to 3R4F tobacco and mixed thoroughly. The conditioned mixtures have been rolled in cigarettes and smoked according the ISO 3308 standard. The mainstream tobacco smoke has been collected in two fractions, *i.e.*: the gas and the condensed particulate matter and analyzed by GC/MS. The results have been compared with samples prepared in a similar way but not including any catalysts, in order to study the effect that the structure of the SBA-15 on the reduction of the compounds generated.

SBA-15 is capable of reducing most of the compounds present in the mainstream tobacco smoke. Spherical and rod SBA-15 yield significant reductions in tar and CO, but the greatest reductions were achieved iin the presence of fiber-like and platelet SBA-15: These materials can reduce around 62 and 60% of the condensed fraction collected in Cambridge filters, and 20-25% of CO.

24. DETERMINATION OF AEROSOL PARTICLE SIZE DISTRIBUTION FOR SEVERAL ELECTRONIC NICOTINE DELIVERY SYSTEM PRODUCTS. Chen SONG; R.J. Reynolds Tobacco Company, Winston-Salem, NC USA

Particle size distribution (PSD) measurement is a potentially useful parameter to characterize the emissions of electronic nicotine delivery systems (ENDS) and has been recommended by FDA in their Draft Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems (May 2016). A low flow cascade impactor (MSP 135 Mini-MOUDI) was used to measure the PSD of four different commercial ENDS products. The relatively low sample flow rate (2 LPM) required by the cascade impactor eliminates the need for excessive aerosol dilution as seen in other studies. Dilution promotes particle evaporation and leads to under-sizing of the ENDS product aerosols. Each ENDS product was tested under two different puffing regimens (standard and intense), at two different vaping periods (beginning and end of product cartridge life) and with two different flavor variances (original and mint). Mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and collection efficiency (the total aerosol weight captured by the impactor versus the total aerosol weight lost by the device) were reported. The MMAD of all tested products ranged from 0.7 to 1.6 μm with a GSD ranging from 1.5 to 2.2. The observed MMADs and GSDs were consistent with respect to puffing regimen and flavor variety for each product. With the exception of one product, the measured MMADs and GSDs were also insensitive to the cartridge life sampling timepoint. Finally, the collection efficiency varied from 62% to 100%, indicating that the relative fraction of mass produced in the gas or aerosol phase was dependent on the specific product tested.

25. *IN VITRO* TESTING OF AN ETHANOL COLLECTION METHOD COMBINING PARTICULATE AND GAS-VAPOR PHASE COMPONENTS: *IN VITRO* MICRONUCLEUS ASSAY. Bhagyalaxmi SUKKA GANESH, Sanjay K. Bharti, Mariano J. Scian and I. Gene Gillman; Enthalpy Analytical, Henrico, VA USA

Health Canada (HC) guidelines (T-502) for the collection and testing of cigarette smoke are used frequently for *in vitro* testing and although the guidelines allow for the collection and testing of the particulate phase (PP), the gas-vapor (GVP) phase separately, the method has several limitations. The PP is extracted in DMSO while GVP components are trapped in PBS, which limits the trapping efficiency of volatile or non-water-soluble compounds. Because of this limitation, GVP is only routinely tested for cytotoxicity using the NRU assay, but not for genotoxicity (MN assay) or mutagenicity (Ames assay). We have evaluated the use of ethanol as a trapping solvent for PP and GVP components. ETOH-extracted PP+GVP was compared to PP or PP+GVP collected based on HC methods. CHO-K1 cells were exposed in the absence and presence of S9 metabolic activation for 3 hrs. (21 hrs. recovery) for cytotoxicity and genotoxicity (MN induction) following HC T-503 guidelines. Manual counting of MN was conducted in fixed cells stained with acridine orange. Dose dependent increases in MN were observed in all three types of extracts. Without metabolic activation, HC (PP), HC (PP+GVP) or ETOH (PP+GVP) exposure resulted in a mean fold MN increase in a dose dependent manner from 1.0 to 5.0x for all three extract types. With metabolic activation, HC (PP) and HC (PP+GVP) exposure resulted in a mean fold MN increase in a dose dependent manner from 1.0 to 5.0x while ETOH (PP+GVP) resulted in 1.0 to 3.7x fold MN increase. The ethanol method tested here allows for combined trapping of PP+GVP components yielding a single whole-smoke extract. The ethanol PP+GVP extract tested produces comparable cytotoxicity and %MN results when compared to DMSO extracted PP while allowing the testing of GVP and PP components together. NRU, Ames, and chemistry results are presented separately.

26. NICOTINE DISSOLUTION IN SMOKELESS TOBACCO PRODUCTS. Nancy QIAN, Tracy Derry, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

Nicotine absorption and its levels in smokeless tobacco products have been studied extensively by researchers in recent years. However, there are limited dissolution profiling methods available for the analysis of nicotine in tobacco products. As a result, we developed and validated a method for nicotine dissolution in smokeless tobacco products. The dissolution is carried out in buffered solution on a dissolution apparatus, which is coupled to an automated sampling station using 8-channel filter plate for sample filtration and direct collection into autosampler vials. Dissolved nicotine at different timepoints are subsequently analyzed using a Waters Acquity UPLC with TUV detector. The separation uses Waters Atlantis T3 column with 3 µm particle size, 50 mm length x 3.0 mm diameter under isocratic elution. This validated method has an LOQ of 0.1µg/mL for nicotine. Profiles are generated by plotting % dissolved nicotine versus time. The method validation parameters include specificity, linearity, precision, accuracy, range, robustness, solution stability, and determination of LOD and LOQ. Both the rate of dissolution (profiles) and final level of dissolved nicotine are determined. The ranges of tested nicotine levels (at infinity) are found to vary from 2.0-14.0 mg/g for the smokeless tobacco products that were studied.

27. TOXICITY REFERENCE VALUES: RELEVANCE FOR THE EVALUATION OF TOXICITY AND RISK. Felix AYALA-FIERRO and Ashley Turner; ITG Brands, Greensboro, NC USA

Toxicity Reference Values (TRVs), for cancer and non-cancer biological effects, represent levels below which impact to human health is unlikely to occur. TRVs are derived from long-term (*i.e.* chronic) toxicity studies for the relevant route of exposure identifying the most sensitive endpoints. Alternatively, if weight-of-evidence (WOE) supports it, route-to-route (r-to-r) extrapolation with appropriate dosimetry adjustments may also be used. The quality and relevance of the toxicological studies used to determine benchmark values (*e.g.* BMDL10) to develop the TRV, should be taken into consideration. The selection of the most relevant study (*i.e.* critical effect) for the biological effect of interest, and the type of data-fitting model, often results in conflicting TRVs for the same analyte. Moreover, differences in terminology around TRVs often lead to multiple and differing values published nationally and internationally. The publication of third-party or research-based reviews leading to different TRVs adds debate to this topic.

The evaluation of potential toxicity and risk of tobacco products is conducted by comparing exposure levels of individual analytes (*e.g.* smoke constituents) to their respective TRV. Lifetime exposure is estimated using relevant parameters for tobacco-product specific exposure including inhalation rate, intensity, frequency and duration. The selection of the TRV determines the level of toxicity and risk. TRVs are set for both cancer and non-cancer toxicity evaluations by National (*e.g.* US EPA) and International (*e.g.* Health Canada) agencies. In some instances, TRVs at the state level also exist, including those published by California EPA (Cal/EPA) and Texas Commission of Environmental Quality (TCEQ). This work evaluated available TRVs and their relevance for the evaluation of toxicity and risk of tobacco products. We found that TRVs should be selected on a case-by-case basis and in certain cases TRVs should be confirmed using the appropriate tool such as BMDS.

28. GLYCIDOL IN HEATED TOBACCO PRODUCTS. Norman E FRALEY, Elizabeth Anderson, Chris Almond, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

A method has been developed and validated for the determination of glycidol in heated tobacco products. This trace level compound is particularly difficult in that it is easily mistaken for other commonly occurring compounds, resulting in false positives. This single quad GCMS method harnesses the power of direct, cool, on-column injection to maximize signal. The method validation results will be reported.

29. COMPREHENSIVE INSIGHTS INTO TOBACCO SMOKE USING FLOW-MODULATED GCXGC-TOF MS. Mark LEMONS[1], Laura McGregor[2], Bob Green[2], Anthony Buchanan[2] and Matthew Edwards[3]; [1]Markes International, Sacramento, CA USA, [2]SepSolve Analytical, Peterborough UK and [3]SepSolve Analytical, ON Canada

From an analytical perspective, there is much that remains to be learnt about the composition of cigarette smoke, because of its high degree of complexity – tobacco smoke is thought to contain thousands of components across multiple chemical classes and wide concentration ranges.

Comprehensive two-dimensional gas chromatography (GC×GC), when coupled with time-of-flight mass spectrometry (TOF MS), has been shown to provide improved chemical fingerprinting of complex samples in areas of study as diverse as petrochemical analysis and fragrance profiling. However, commonly-used thermal modulation devices are unable to successfully modulate the most volatile components.

In this study, we use thermal desorption (TD) for collection and analysis of whole cigarette emissions, and couple it with flow-modulated GC×GC–TOF MS, to enable the constituents of whole smoke to be routinely and confidently sampled, separated and identified.

30. IDENTIFICATION OF PREDICTIVE CLINICAL BIOMARKERS FOR DEVELOPING CHRONIC OBSTRUCTIVE PULMONARY DISEASE USING REAL WORLD EVIDENCE DATA. Gang Michael LIU, Patrudu Makena, Kyung Soo Hong, Eric Scott and G. L. Prasad; RAI Services Company, Winston-Salem, NC USA

Identification of predictive biomarkers and quantification of individual risk for developing smoking-associated diseases such as Chronic Obstructive Pulmonary Disease (COPD) aids in evaluating and predicting the health effects from tobacco products. This study aimed to identify predictive biomarker(s) for COPD in U.S. smokers by leveraging a Real World Evidence (RWE) approach. We performed a retrospective analysis of smokers' electronic health records prior to COPD diagnosis dates from the Explorys database available from IBM Watson Health. Electronic health records from 181,250 smokers with COPD and 2.2 million smokers without COPD were analyzed for 75 selected health measures and 900 derived clinical features based on the selected biomarkers at the subject level. A computational model built around RWE data predicted development of COPD with 76% precision (true positive rate) and 0.801 Area Under the Receiver Operating Characteristics Curve on the subject level outcome. A set of 32 biomarkers (*e.g.*, coagulation tissue factor, cholesterol, erythrocytes) and 96 clinical features (different ways a given biomarker is reported or analyzed) were identified to have predictive power in modeling development of COPD. Taken collectively, top clinical biomarkers identified to have high predictability were platelets, cholesterol in HDL, coagulation tissue factor, age and leukocytes. These findings from RWE data help in building an individual risk scoring model to estimate the likelihood of smokers developing COPD.

31. *IN VITRO* TESTING OF AN ETHANOL COLLECTION METHOD COMBINING PARTICULATE AND GAS-VAPOR PHASE COMPONENTS: NEUTRAL RED ASSAY. Mariano J. SCIAN, Bhagyalaxmi Sukka-Ganesh, Sanjay K. Bharti and I. Gene Gillman; Enthalpy Analytical, Henrico, VA USA

The Neutral Red Uptake (NRU) assay Health Canada (HC) guidelines (T-502) for the collection and testing of cigarette smoke are used frequently for *in vitro* testing. Although the guidelines allow for collection of the particulate phase (PP) and the gas-vapor phase (GVP), this method has limitations. GVP is collected in PBS which has limited trapping of volatile or non-water-soluble compounds. The fraction also has limited stability and must be used within 60 minutes of collection. Because of limited stability, GVP is only tested for cytotoxicity using the NRU assay, but not for genotoxicity (MN) or mutagenicity (Ames). These limitations could be overcome with a method that allows collection of the PP and GVP in a solvent with enhanced trapping of GVP components. We evaluated the

use of ethanol to collect PP and GVP components and compared it against the traditional HC collection using the NRU assay and CHO-K1 cells following HC guidelines. Reference 3R4F cigarettes were used. Extraction of PP in ethanol produced comparable results to DMSO-extracted PP (IC50 101.7 μg/mL vs. 87.6 μg/mL), however, GVP in ethanol resulted in higher cytotoxicity when compared to GVP in PBS (IC50 40.2 μg/mL vs. 159.1 μg/mL) likely due to better trapping efficiency of GVP components. The combination of PP+GVP in ethanol showed higher toxicity (IC50 58.2 μg/mL) compared to PP (IC50 87.6 μg/mL) or PP+GVP (IC50 110.3 μg/mL) collected under HC guidelines. The ethanol method allows for combined trapping of PP+GVP yielding a single whole-smoke extract, results in increased trapping of GVP components, and shows comparable or better cytotoxicity results in the NRU assay compared to the HC method. Ames, MN, and chemistry results are presented separately.

32. A SIMPLIFIED METHOD FOR THE ANALYSIS OF MONO-CARBONYL COMPOUNDS IN E-CIGARETTE AEROSOLS BY LC-MS. Jeff ZHU and Aaron Heredia; ITG Brands, Greensboro, NC USA

Mono-carbonyls (formaldehyde, acetaldehyde, acrolein and crotonaldehyde) are present in e-cigarette aerosols, often at trace levels. In recent years, various methods have been reported for the analysis of these compounds at low levels by LC-MS, LC-MS/MS and GC-MS. Herein we wish to present a simplified and robust LC-MS method we have developed to quantitate mono-carbonyls in e-cigarette aerosols. A UPLC system coupled with a single quadrupole mass spectrometer (Waters Acquity UPLC with QDa detector) has been used for the analysis. Chromatography from traditional smoke sample analysis has been modified to improve the separation of these four analytes from the interferences in sample matrices so that the analysis can be performed with good accuracy without internal standards being used. The new method has been validated with good recoveries (85.5 – 121.6%) on e-cigarette aerosols. The overall calibration range is 2 to 400 ng/mL for all four analytes, which covers most of the commercial e-cigarette samples tested without dilution of the sample solutions needed. The LOQ of the method is 2.9 ng/puff for all four analytes. The new method is robust and has been used for the analysis of 30 to 70 e-cigarette samples per day over a period of multiple weeks without any extra instrument maintenance needed.

33. A QUICKER METHOD FOR THE ANALYSIS OF AMMONIA IN E-CIGARETTE AEROSOLS AND E-LIQUIDS BY ION CHROMATOGRAPHY. Jeff ZHU, Brittany Moore and Aaron Heredia; ITG Brands, Greensboro, NC USA

Ammonia has been routinely tested for cigarette smoke for a number of years. Common methods employed are based on ion chromatography using cation exchange columns such as Dionex IonPac CS12 and CS16 columns. These two columns provide good separation of the ammonium peak from the proceeding sodium peak. However, the run times including equilibrating time typically exceed well over 20 minutes because of the high retention of ammonium in these columns. Herein we wish to present a quicker and robust method utilizing Dionex CS18 column with run time of 13 minutes (including equilibrating time) that still provides good separation of ammonium peak from sodium peak. The method LOQ is 0.16 ug/puff, with no quantifiable ammonia determined in the e-cigarette sample matrices we have analyzed. A Dionex ICS 5000 ion chromatography system has been

utilized for the method. The new method has a calibration range of 0.1 to 2 ug/mL for ammonia and has been validated with good recoveries on e-cigarette aerosols and e-liquids.

34. COMPARISON OF A FLAME IONIZATION DETECTOR (GC/FID) TO A NITROGEN-PHOSPHORUS DETECTOR (GC/NPD) FOR GAS CHROMATOGRAPHIC DETERMINATION OF NICOTINE IN CONVENTIONAL AND ULTRA-LOW NICOTINE TOBACCO BLENDS. Darren STEELMAN, Andy Stinson and T. Jeffrey Clark; Liggett Group, Mebane, NC USA

The established CORESTA method to determine nicotine in tobacco uses a gas chromatograph coupled with a flame ionization detector (GC/FID) (CRM 62) or that uses a mass spectrometer (GC/MS) (CRM 87). These methods were developed to evaluate conventional tobacco. Currently, there are no standardized methods available to quantitate the nicotine level in both conventional and in ultra-low nicotine tobacco. Other selective instruments such as a gas chromatograph coupled with a nitrogen-phosphorus detector (GC/NPD), high performance liquid chromatography coupled with an ultraviolet detector (HPLC/UV) and a gas chromatography -mass spectrometer (GC-MS) have been reported for conventional tobacco but have not been widely reported for the evaluation of ultra-low nicotine tobacco.

In the study we will compare the nicotine results between a GC/FID and GC/NPD systems using an ultra-low nicotine tobacco blend (NIST SRM 3222), CRP1.1, conventional mid-level tobacco leaf, and a conventional tobacco blend (1R6F).

We will present instrument precision, method precision, accuracy and LOD & LOQ of both methods. We will also present the advantages and disadvantages of the two detectors.

35. FINGERPRINTING OF E-LIQUIDS BY THE DETERMINATION OF UNTARGETED COMPOUNDS USING TWO MODERN ANALYTICAL APPROACHES. Paulina BIERNACKA, Kenneth Chalcraft, Peter Joza and David Li; Labstat International, Kitchener, ON Canada

To better elucidate characteristic compounds present in e-liquids, and monitor potential changes to a product that occur during storage or between lot preparations, the determination of untargeted analytes is a valuable tool. The aim of this study was to apply two analytical techniques for the untargeted analysis of selected e-liquids using Headspace - Solid Phase Microextraction - Gas Chromatography – Mass Spectrometry (HS-SPME-GCMS) and Liquid Chromatography coupled with Time-of-flight Mass Spectrometry (LC-ToF-MS).

A 1 cm Stableflex DVB/CAR/PDMS fiber was used in the HS-SPME-GCMS analysis, providing the pre-concentration of volatile and semi-volatiles compounds. The mass spectrometer operated in scan mode, monitoring a range of 35-400 m/z. The ToF-MS was operated with electrospray and atmospheric pressure chemical ionization (ESI; APCI), both in positive and negative modes. Operating parameters included an acquisition mass range of 70-750 m/z, typical of most small molecules.

Both techniques used 100 mg of e-liquid. Each method used isotopically labelled compounds as internal standards (or a fiber performance check in the case of SPME). For this study five replicates of four different e-liquids were evaluated.

HS-SPME-GCMS analysis indicated the presence of over 120 different flavour compounds including mono- and sesquiterpenes, higher alcohols, esters, pyrazines, lactones and aldehydes. LC-ToF-MS identified 60 to 400 features, or distinct compounds, depending on the mode of operation, including isomeric compounds typical of modified sugars such as sugar alcohols and acids, as well as, C6 - C18 fatty acids.

The combination of compounds identified in the different e-liquids by both techniques, present at different levels, create a unique and distinguishable fingerprint of each e-liquid.

36. REAL-TIME CHEMICAL PUFF PROFILING OF VAPOR PRODUCT AEROSOL WITH PROTON TRANSFER REACTION - MASS SPECTROMETRY. Luca CAPPELLIN[1] and Nadja Heine[2]; [1]University of Padua and Tofwerk AG, Padova Italy and [2]JUUL Labs, San Francisco, CA USA

The analysis of aerosol and gas phase volatile organic compounds (VOC) emitted by vapor products has relied on a variety of standardized but time-consuming off-line technologies. Conventional methods typically involve the capture of aerosol from multiple sequential puffs (~50) on a filter pad, or in an impinger solution, followed by extraction and derivatization. These procedures are resource intensive and have low time-resolution, prohibiting puff-by-puff analysis, and may lead to sample alteration due to evaporation, water uptake, or chemical reactions on the filter.

In this study, we present a novel methodology which combines a high-resolution PTR-MS with an improved aerosol dilution system and an actuating device to trigger aerosol production from the vapor product and direct it to the dilution system. We employed this methodology to analyze and quantify harmful or potentially harmful constituents (HPHCs), such as acrolein, acetaldehyde, acrylonitrile, crotonaldehyde, and nicotine-n-oxide, in aerosol emitted from vapor products on a puff-by-puff basis in real-time.

Concentrations of several compounds were determined puff-by-puff and validated with results obtained from a contract research laboratory using ISO 17025 accredited methods. The chemical puff profiles of different devices (*e.g.* temperature-regulated, unregulated, and ciga-likes) are presented. The real-time data demonstrates that harmful compounds were not detected for temperature regulated devices for the life of the pod, while in contrast, devices without temperature regulation exhibited significant increase of harmful compounds such as carbonyls and oxides at the end of liquid.

We demonstrate that PTR-MS may be used as an alternative to off-line methods for simultaneous quantification and characterization of most HPHCs found in aerosol as well as VOCs, puff-by-puff, and even intra-puff to enable immediate assessment of new nicotine-containing formulations, changes in device design, and validation of product designs.

37. IMPACT OF DEVICE VARIABILITY ON THE DETERMINATION OF ALDEHYDE COMPOUNDS IN E-CIGARETTE EMISSIONS. I. Gene GILLMAN, Alexander S.C. Pennington and Kathy E. Humphries; Enthalpy Analytical, Durham, NC USA

The determination of aldehyde compounds (formaldehyde, acetaldehyde and acrolein) in e-cigarette emissions has been widely reported in scientific literature. Recent reports have focused on the contribution of flavor compounds in e-liquids and on the production of aldehydes in the resulting aerosol. Contradictory results have been reported on the role that flavors play in the production of aldehydes in e-cigarette emissions. One possible reason for the lack of consensus, is the inherent variability in aldehyde production found in e-cigarettes. In this study, we collected replicate data from a tank-based e-cigarette device using both flavored and matching unflavored liquids. Using 10 unique devices per test condition, we found that typical, across device, variability ranged from 75% to 200% for all measured compounds. With this approach it was not possible to discern the impact of flavor compounds on the production of aldehydes. In an attempt to reduce variability, this study was repeated, except that the same devices were cleaned and reused for all samples. This allowed for a direct comparison of the impact of flavor compounds on the production of aldehydes. With this approach, we were able to determine that all flavored e-liquids that were tested yielded a slight increase in acetaldehyde emissions, two of the six flavored e-liquids tested yielded a slight increase in formaldehyde emissions, and that none of the e-liquids tested yielded an increase in acrolein emissions. Our data suggests that the production of aldehydes in e-cigarettes aerosol is extremely device-dependent and care must be taken to ensure that adequate replicates are collected to determine the impact of device variability on analytical measurements of e-cigarette emissions.

38. OPTIMIZATION AND COMPARISON OF 2,4-DINITROPHENYLHYDRAZINE (DNPH) DERIVATIZATION CONDITIONS FOR THE DETERMINATION OF CARBONYL COMPOUNDS. Lena JEONG[1], John H. Miller IV[2] and Niti Shah[2]; [1]Eurofins Lancaster Laboratories, Richmond, VA USA and [2]Altria Client Services, Richmond, VA USA

FDA's Premarket Tobacco Product Application (PMTA) draft guidance for Electronic Nicotine Delivery Systems (ENDS) recommends analysis of four carbonyls in e-liquids and aerosols - Formaldehyde, Acetaldehyde, Acrolein and Crotonaldhyde. There are two CORESTA recommended methods (CRM) for analysis of carbonyls; CRM No. 74 for mainstream cigarette smoke and CRM No. 86 for tobacco and tobacco products. However, there is currently no CRM for measuring carbonyls in e-vapor products. Carbonyls in e-vapor products are typically lower in concentration compared to those in mainstream smoke. This requires additional method optimization to ensure that the derivatization efficiency and stability of the analytes during the collection process are acceptable to accurately quantify the lower levels of carbonyls.

The specific aim of this study was to evaluate the impact of the DNPH type and concentration, solvents, acid type and pH on the derivatization efficiency for e-liquids. Since the inherent levels of these carbonyls in aerosols from e-vapor products are extremely low and inconsistent, we used fortified e-liquid samples to monitor the recovery. We also evaluated the impact of e-liquid composition on derivatization efficiency. Acrolein can react multiple times with DNPH forming a polyderivatized hydrazone, which can accout for low recoveries. Our results demonstrate that the acidity of the solution has a significant

impact on the derivatization rate, with low pH resulting in rapid decrease in acrolein-DNPH complex. Using buffered solutions to control the pH, coupled with optimizing the concentration of DNPH and solvent, resulted in an improved method, which provided stable recoveries above 85% for all the carbonyls. The learnings from this work could also be applied to the analysis of carbonyls from other tobacco products.

39. GLYCIDOL BEHAVIOR IN GC SYSTEMS. Norman E. FRALEY; Eurofins Professional Scientific Services, Winston-Salem, NC USA

Glycidol is an anlayte of interest in Tobacco Heated Product and analysis of Glycidol can be challenging due to its chemical reactivity. Initial concerns about glycidol being formed as a thermal decomposition product from glycerol was examined. The reaction showing the loss of a water from glycerol could create the conditions for epoxide ring formation and the loss of two water molecules could form acrolein. Initial GC method development examined this by using the heated inlet of the GC and discoveries during development led to curious and unexpected behavior ranging from gas phase ring formation to liquid phase dendritic polymerization.

40. I STEEP MY TEA, SO DO I NEED TO STEEP MY E-LIQUIDS? John H. LAUTERBACH; Lauterbach & Associates, Macon, GA USA

Steeping of tea not only involves extracting the soluble components of the tea from the tea leaves, but also creating conditions where reactions can occur among the various tea components depending on the type of tea, and steeping conditions. The term steeping is now being used to describe treatments that can be applied to e-liquids after they are formulated. Such treatments are believed to improve the hedonic properties of the aerosols generated from the e-liquids. Some websites advertise pre-steeped ready to use e-liquids. Various steeping conditions can be found on the Internet. These range from allowing the e-liquid to remain in the dark for days with or without occasional opening of the container, to high-speed mixing, and to use of ultrasonic baths for rapid steeping (*e.g.* several 30-minute treatments). However, such treatments can also result in heating of the sample depending on the power setting used, the amount of water, and the number and nature of the containers of e-liquid used in the bath. Bath and e-liquid temperatures can exceed 70°C. Consequently we conducted a study to determine chemical changes that occurred after four 30-minute treatments separate by 30-minute cooling periods in a 70-watt DSA Ultrasonic cleaner. Each e-liquid tested was formulated from commercial flavor concentrates (4 mL), 50/50 PG/VG mix (6 mL) and 100 mg/mL nicotine in VG (4 mL) and steeped in capped 1-oz jars. Samples before and after steeping were analyzed by LC using several columns and conditions such as Cogent Phenyl Hydride column with UV detection at 280 nm and gradient elution, 62/38/0.1 ethanol/water/TFA to 33/67/0.1 and the reverse gradient. Excessive steeping with ultrasonic cleaner reduces aromatic aldehydes.

41. ANALYSIS OF E-LIQUIDS OF ELECTRONIC CIGARETTES CONTAINING NICOTINE SALTS. HAN Shulei, Fu Yaning, Liu Tong, Wang Hongjuan, Chen Huan and Hou Hongwei; China National Tobacco Quality Supervision and Test Center, Henan China

Electronic cigarettes containing nicotine salts are gaining in more and more popularity worldwide. However, information about this kind of products is very limited. We developed

a high performance liquid chromatography method for simultaneously determination of seven organic acids & nicotine. To get deep insights into this new and hugely popular type of products, organic acids, nicotine content, nicotine source and pH from 34 e-liquids of prefilled cartridges for nine brands were analyzed. The results showed that: (1) Benzoic acid nicotine salt was detected in about one third samples, nicotine ditartrate was detected in one sample, but the kind of nicotine salts could not be qualified in other samples, indicating that more effective target or non-target methods should be developed for qualitative and quantitative analysis of nicotine salts in e-liquids. (2) Nicotine content of e-liquids containing nicotine salts was significantly higher than that of ordinary e-liquids (not labelled containing nicotine salts) reported in literature report. The claimed concentrations of nicotine labeled on electronic cigarettes containing nicotine salts were often higher than measured values, and the nicotine contents of some samples were not labeled at all. (3) Enantiomeric composition of nicotine showed that relative amount of R-(+)-nicotine was not higher than 2.72%, showing that nicotine in the e-liquids originated from tobacco, rather than from artificial synthesis. (4) The pH of e-liquids containing nicotine salts was significantly lower than that of ordinary e-liquids.

42. DETERMINATION OF AMOUNTS OF HARMFUL SUBSTANCES MIGRATING FROM PLASTIC ASSEMBLIES IN ELECTRONIC CIGARETTES. Fan Meijuan[1], Pan Lining[1], Cui Huapeng[1], Duan Yuanxing[2], Liu Yibo[3], Niu Jiajia[1], Mao Youan[4], Chen Li[1], Guo Junwei[1], Liu Shaofeng[1], Wang Hongbo[1], Liu Huimin[1] and ZHAO Le[1]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China, [2]Technology Center of China Tobacco Yunnan Industrial, Kunming, China, [3]Technology Center of China Tobacco Guangdong Industrial, Guangzhou, China and [4]Technology Center of China Tobacco Hunan Industrial, Changsha, China

Plastic materials are widely used in assemblies of e-cigarettes. Plastic materials shall not transfer harmful substances to the content of e-liquid in quantities which change the composition of the e-liquid or the aerosol and thereby increase the risk for consumers in accordance with the standards published by European, French and Bratish. Depending on the type of plastic materials used, the harmful substances were identified, including formaldehyde, terephthalic acid, isophthalic acid, phenol, bisphenol A, ethylene glycol, vinyl chloride, acrylonitrile, 1,3-butadiene, *etc.* According to the actual conditions of plastic assemblies' contacting with e-liquid, aerosol and mouth, migration tests were established under storage and working conditions. Five analysis methods were developed for identifying those harmful substances in the following two simulants: a) the mixture of propylene glycol, glycerol, nicotine and water; b) water. The migration amounts of harmful substances from plastic assemblies in 30 e-cigarette samples were determined. The results indicated that: 1) ethylene glycol, terephthalic acid, isophthalic acid and vinyl chloride were not detected in any simulants; 2) the migration levels of bisphenol A, formaldehyde, phenol, acrylonitrile and 1,3-butadiene varied between 0.85~291.10, 6.92~52.11, 0.10~11.32, 2.12 and 0.16 mg/kg, respectively. Compared with the regulatory SMLs (specific migration limits) of those harmful substances set by the Commission Regulation (EU) No 10/2011, the migration levels of ethylene glycol, terephthalic acid, isophthalic acid, vinyl chloride, acrylonitrile and 1,3-butadiene were below the SMLs, which may not raise migration risk; however, the migration levels of bisphenol A, formaldehyde and phenol exceeded the SMLs, and the percentages of samples were 83.3%, 30.0% and 23.3%, which may raise migration risk.

43. AN OPEN-LABEL, RANDOMIZED, PARALLEL-GROUP, CONTROLLED STUDY TO EVALUATE CHANGES IN BIOMARKERS OF CIGARETTE SMOKE EXPOSURE AND BIOMARKERS OF POTENTIAL HARM IN ADULT SMOKERS WHO COMPLETELY SWITCH TO USING E-VAPOR PRODUCTS FOR 12 WEEKS. Jeff EDMISTON, Douglas Oliveri, Qiwei Liang and Mohamadi Sarkar; Altria Client Services, Richmond, VA USA

The purpose of this study was to characterize biomarkers of exposure (BOE) to select HPHCs and biomarkers of potential harm (BOPH) in adult smokers (AS) who switched to exclusive use of e-vapor products (EVPs) for 12 weeks.

Method: Generally healthy AS (N = 450, 51% male, 30 – 65 years of age, daily smokers who were not planning to quit, and smoked > 10 cigarettes per day (CPD), for > 10 years) were randomly assigned to either continue to smoke (CC) or switch to one of the EVPs (n=~150 each group). The two EVPs were cartridge-based products containing propylene glycol, glycerol, tobacco derived nicotine (4% by weight), in classic or menthol flavor. Select BOE, BOPH and FEV1/FVC were measured at Baseline and 12 weeks.

Results: Statistically significant differences in absolute change from Baseline ($p < 0.0001$) in BOEs were observed at Week 12 for urinary total NNAL and carboxyhemoglobin (COHb) across both flavor variants. Absolute changes from baseline for nicotine and 5 of its metabolites (nicotine equivalents, NE) were not statistically significantly different between the CC and EVP groups; however, the levels in EVP trended to be higher (0.977 and 1.23 (mg/g Creatinine)). All the BOPH (WBC, HDL-C, 11-dehydrothromboxane B2, 8-epi-prostaglandin F2alpha, soluble intercellular adhesion molecule -1 (sICAM)) were directionally favorable in both EVP groups, as compared to CC smokers, with statistically significant reductions ($p < 0.001$) observed in a biomarker of inflammation (sICAM). Reductions from baseline in FEV1/FVC percent predicted values were statistically significantly less for the two EVP groups compared to the CC smokers ($p < 0.0136$). Average self-reported CPD reduction in EVP subjects was reduced by ~98%.

Conclusions: We demonstrate that significant reduction or complete elimination of many of the HPHCs in EVP aerosol results in significant reductions in BOE and favorable changes in BOPH after switching to EVPs for 12 weeks. The observed biomarker changes, other than nicotine, approached those reported for smoking cessation studies over a comparable time period suggesting that switching to exclusive use of the EVPs tested in this study may be less harmful than continuing smoking.

44. ABUSE LIABILITY OF VERY LOW NICOTINE CONTENT CIGARETTES WITH CHARACTERIZATION OF NICOTINE EXPOSURE PROFILES IN ADULT SMOKERS. Naama LEVY-COOPERMAN[1], Megan J. Shram[1], Debra Kelsh[2], Bradley Vince[2], and Ed Carmines[3]; [1]Altreos Research Partners, Toronto, ON Canada, [2]Altasciences/Vince and Associates, Overland Park, KS USA and [3]Carmines Consulting, Scottsdale, AZ USA

Objectives: In 2017, FDA announced the intention to explore limiting nicotine in cigarettes to minimal/non-addictive levels. Research indicates that reduced nicotine content would potentially limit reinforcing effects of cigarettes. We conducted 2 studies to evaluate abuse liability of very-low-nicotine (VLN™) cigarettes compared with own-brand cigarettes and nicotine gum in current smokers. Methods: In 2 randomized, 2-part, 3-way crossover

studies in smokers of non-menthol (Study 1) or mentholated cigarettes (Study 2), subjects received VLN™ non-mentholated/mentholated cigarette, own-brand non-mentholated/mentholated cigarette and Nicorette® Original Flavor™/White Ice Mint™. Studies included ad libitum product use sessions (Part A) and controlled/uncontrolled product use sessions (Part B). Primary endpoints were maximum reduction on "Urge to Smoke" visual analog scale (VAS) and peak score on "Pleasantness" VAS following controlled use. Pharmacokinetic samples were collected to measure nicotine exposure. Results: In Study 1, VLN™ cigarette had smaller reductions on Urge to Smoke and lower Pleasant VAS scores compared with own-brand cigarette ($P < 0.0001$), and did not differ from nicotine gum on either measure. In Study 2, VLN™ cigarette had lower Pleasant VAS scores compared with own-brand cigarette ($P < 0.0001$), but did not differ on Urge to Smoke. Compared with nicotine gum, VLN™ cigarette had greater reductions in Urge to Smoke ($P < 0.05$), but did not differ on Pleasant VAS. In both studies, nicotine exposure was reduced by 97% for VLN™ cigarettes compared with own-brand cigarette. Conclusions: Results suggest that VLN™ cigarette has lower abuse liability compared with own-brand cigarette and similar abuse liability to nicotine gum. Despite reduced nicotine exposure, VLN™ mentholated cigarettes showed comparable effectiveness in reducing urges to smoke as own-brand cigarette, while also showing greater reductions in urges to smoke compared with nicotine gum.

45. ADVANCING THE COMMERCIAL AND PUBLIC HEALTH GOALS OF POTENTIALLY REDUCED-RISK PRODUCTS THROUGH THE ASSESSMENT OF CONSUMER SATISFACTION. Neil SHERWOOD; Neil Sherwood Consulting, Nyon, Switzerland

Attempts to switch from conventional tobacco products to potentially reduced-risk products (PRRPs) will best succeed if user preferences are fully met by optimizing consumer appeal and consumer satisfaction. While PRRPs are already presented in a variety of attractive forms, manufacturers have paid less attention to the satisfaction consumers derive from their use, with development predicated on the belief that it is simply necessary to approximate nicotine delivery to that of a cigarette and add appropriate flavour combinations. Yet limited penetration of PRRPs into the tobacco marketplace would suggest that this approach is both inefficient and insufficient. When assessed, users of conventional tobacco products report or demonstrate a variety of motivations for their behaviour which range from cognitive enhancement through amelioration of mood to weight control. Such functional effects have often been dismissed as indicative of nicotine dependence, yet many conventional tobacco product users who do not meet dependence criteria still report or demonstrate such motives. Regardless of the underlying mechanisms driving tobacco use, this presentation will seek to show that such functional effects are central to consumer satisfaction with PRRPs. Moreover, it is possible to assess consumer satisfaction at early stages of product development using a variety of methods, the endpoints of which relate closely to several of the motives reported or demonstrated by users of conventional tobacco products. This presentation will illustrate available techniques using relevant data and discuss the possible application of these techniques to future product assessment. Screening PRRPs for consumer satisfaction could save both time and resources for manufacturers and expedite the important public health role that such products could play in reducing the burden of tobacco-related harm.

46. CHARACTERIZATION OF ADULT CIGARETTE SMOKERS' BEHAVIOR DURING SHORT AND LONGER-TERM USE OF REDUCED NICOTINE CONTENT CIGARETTES. Andrea VANSICKEL, Mingda Zhang and Jan Angel; Altria Client Services, Richmond, VA USA

FDA proposed a cigarette nicotine standard to make them "minimally addictive or nonaddictive." Some research has shown reductions in cigarette consumption after switching to SPECTRUM® reduced nicotine content cigarettes (RNC). We do not know whether adult cigarette smokers (AS) would experience similar reductions in smoking with other types of RNC. This study examined AS consumption (N = 70) of their usual brand cigarette (UB), a normal nicotine control cigarette (NNC) prototype, and a RNC prototype (~90% lower than typical marketed cigarettes) under confined short-term (9-hours) usage conditions followed by 17 days of at-home use of the NNC or RNC prototype. A linear mixed model revealed a significantly greater average (±SD) number of RNC smoked relative to UB under short-term usage conditions (13.2±4.7 vs. 12.3±3.9, $p < 0.05$). No significant difference between the number of RNC and NNC (13.2±4.7 vs. 12.9±4.3, $p > 0.05$) smoked was observed during short-term usage. RNC and NNC usage did not differ statistically during longer-term use, with average cigarettes per day of 19.6±11.2 and 18.5±10.2, respectively. Incoming cigarettes per day and stated willingness to use the products again at the end of short-term usage appeared to influence smoking behavior during longer-term use of the RNC and NNC prototypes. Limitations of this study include small sample size, short observation period, and no biochemical verification of study compliance. Results of this study demonstrate an increase in consumption of RNC relative to UB under short-term confined usage conditions and similar consumption to a NNC during longer-term ambulatory use. These findings diverge from previously published ambulatory studies with the SPECTRUM® RNC that reported reduction in smoking behavior relative to NNC and UB conditions.

47. ELECTRONIC NICOTINE DELIVERY SYSTEM PUFFING TOPOGRAPHY CHARACTERISTICS FROM HUMAN STUDIES. Ian M. FEARON[1] and Elaine K. Round[2]; [1]whatIF? Consulting Ltd, Harwell, UK and [2]RAI Services Company, Winston-Salem, NC USA

The manner in which electronic nicotine delivery systems (ENDS) are used is a strong determinant of real-world emissions and, as such, may impact user exposure to chemical constituents. Information on puffing topography is therefore important to facilitate evaluation of machine yields from ENDS to estimate exposure from conditions reflecting actual use. In February 2018, the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) published it's "Technical Guide for the Selection of Appropriate Intense Vaping Regimes for E-Vapour Devices". The review recommended factors to consider when determining puffing parameters to use for aerosol generation under an intense regimen on smoking/vaping machines to facilitate assessment of chemical constituent yields. The literature review which underpinned the CORESTA recommendations was limited to papers published prior to and during 2016. Since that time, a number of studies involving human subjects have been published containing puffing topography data from both ambulatory and laboratory studies, and on a wide range of ENDS types, such as open tank systems and closed systems such as cig-a-like and pod systems. Furthermore, several studies have examined the relationship of ENDS parameters - including power output,

liquid components such as propylene glycol/vegetable glycerin ratios, and the presence of flavour ingredients - and puffing topography.

This presentation will provide a critical review of the literature published since the drafting of the CORESTA review and will discuss how puffing topography varies between different ENDS types and the association of device parameters with puffing topography. Furthermore, the presentation will review the associations of e-liquid components with puffing topography. Recommendations for appropriate puffing parameters to use in aerosol generation for analytical purposes will also be presented.

48. A BLOOD-BASED SMOKING-RELATED GENE EXPRESSION SIGNATURE USING A MACHINE LEARNING APPROACH. Gang Michael LIU and G. L. Prasad; RAI Services Company, Winston-Salem, NC USA

Smoking is a leading risk factor in the onset of multiple forms of cancer, chronic obstructive pulmonary disease, and cardiovascular disease. At present, there is a limited understanding by which changes in gene expression profiles in blood or other tissues can be used to predict smoking status. In this study, we investigated whether a machine learning approach could provide an unbiased method to predict smoking status using microarray expression profiles obtained from the blood. Using multiple feature selection and classification methods, the most optimal algorithm that produced the best predictive model to determine smoking status was a combination of Support Vector Machine (SVM), based on Recursive Feature Elimination (RFE). The 16 gene signature from our machine learning model included not only three previously reported genes (LNNR3, SASH1, and GPR15), but also several newly identified genes including GZMM, which has been reported to be associated with lung adenocarcinoma. In addition, this gene signature has been validated by seven independent publicly available gene expression datasets. In summary, we show that machine learning analysis using expression profiling datasets from blood is useful in ascertaining smoking status and in developing novel Biomarkers of Potential Harm.

49. HIGH THROUGHPUT AIR LIQUID INTERFACE EXPOSURE MODULES: CHARACTERIZATION OF SMOKE/AEROSOL DOSIMETRY AND *IN VITRO* MUTAGENICITY AND CYTOTOXICITY OF TWO TOBACCO PRODUCT TYPES. Robert LEVERETTE[1], Brian Keyser[1], Michael Hollings[2] and Adam Seymour[2]; [1]RAI Services Company, Winston-Salem, NC USA and [2]Covance Laboratories, North Yorkshire UK

The utilization of whole smoke/aerosol exposure systems provides a means to conduct *in vitro* assessments of freshly generated whole smoke/aerosol from combustible cigarettes and tobacco heating products (THPs), as well as electronic nicotine delivery systems (ENDS). One challenge with such systems is ensuring sufficient throughput for *in vitro* toxicological studies in a timely manner. Vitrocell® has developed high throughput whole smoke/aerosol exposure modules designed to concurrently deliver up to seven different doses (six wells per dose) of smoke/aerosol and a clean air control to 48 wells of bacterial (Vitrocell® Ames 48 module) or mammalian (Vitrocell® 6/48 module) cell cultures. These systems were characterized with a series of experiments designed to assess smoke/aerosol delivery and biological responses from a Kentucky Reference 3R4F combustible cigarette or a commercially available THP. Initial dilution airflows consisting of 0.5 – 10 L/min for 3R4F and 0 (undiluted) - 4 L/min for the THP were evaluated. Smoke/aerosol deposition was

measured using fluorescence (Ex 355/Em 485) of captured particulate matter and chemical analysis (*e.g.*, glycerol, nicotine) of either DMSO or PBS traps within the modules. Once the delivery of smoke/aerosol within the modules was deemed satisfactory, the mutagenicity (Ames Assay) and cytotoxicity (Neutral Red Uptake Assay; NRU) of the whole smoke/aerosol delivered from the combustible and THP products were assessed. Results exhibited a dose-dependent deposition of smoke/aerosol constituents for both the combustible and THP test articles, a characteristic dose-dependent increase in revertant counts (Ames) for the combustible cigarette and a dose-dependent decrease in cell viability (NRU) for both test articles. These results demonstrate the Vitrocell® high throughput exposure systems are fit-for-use for the *in vitro* testing of different tobacco product types.

50. CYTOTOXICITY AND ACUTE TOXICITY ASSESSMENT OF SEVERAL TYPICAL NICOTINE SALTS. WANG Hongjuan, Chen Huan, Han Shulei, Fu Yaning, Liu Tong, Hou Hongwei and Hu Qingyuan; China National Tobacco Quality Supervision & Test Center, Henan, China

As a new form of nicotine intake, nicotine salts are increasingly used in new tobacco products such as electronic cigarette. Whether the matrix components of electronic liquids will affect its toxicity, relative to pure nicotine, how to set the concentration limits of nicotine salts in electronic liquids to avoid the risk of misuse, and their inhalation toxicity on lung cell are all important issues deserving attention. Therefore, in this study, the matrix effect of electronic liquid was investigated first; and then 7 representative nicotine salts were selected to test their acute oral toxicity based on rats, and finally their pulmonary cytotoxicity was test based on BEAS-2B cells. The results showed that: (1) The LD50 values of pure nicotine and simulated electronic liquid with the same nicotine equivalent were very close to each other; (2) The LD50 values of acute oral toxicity of 7 nicotine salts were higher than that of nicotine; (3) The IC50 values of the cytotoxicity of 7 nicotine salts were significantly different, partly higher than nicotine and partly lower than nicotine. In conclusion, it can be inferred that the matrix components of electronic liquids have little effect on nicotine toxicity; the general toxicity of these nicotine salts is lower than that of nicotine, which can provide a reference for setting maximum limits of these nicotine salts in electronic liquids; the pulmonary cytotoxicity of different nicotine salts was significantly different, and the pulmonary cytotoxicity of several nicotine salts was much higher than that of nicotine. Further studies on inhalation toxicity of nicotine salts are needed in the future to guide the application of nicotine salts in electronic liquids.

51. THE EFFECT ON TSNAS OF STICK SPACING IN THE BARN. Anne Jack FISHER, Colin Fisher and Huihua Ji; University of Kentucky, Lexington, KY USA

This study was designed to confirm the general consensus that closer packing in the barn (by placing the sticks closer together) results in higher TSNAs (Tobacco Specific Nitrosamines). We needed data to support our recommendation to growers to avoid close packing in the barn. We compared standard stick spacing (8 inches) with close stick spacing (4 inches), using a high converter selection of TN 90. The study was done over three years (2015-2017), with very different weather conditions. In two out of the three years (2016 and 2017), close spacing more than doubled TSNAs. In 2017, TSNAs were particularly high: total TSNAs of 11.3 mg/g in the standard spacing and 29.2 mg/g in the close spacing. The mean of the total TSNAs over the three years was 4.5 mg/g in the standard spacing and

10.8 mg/g in the close spacing. The year with no treatment differences (2015) was very dry during curing, and TSNAs were extremely low: 1.5 – 1.6 mg/g, which is abnormally low for this high converter line. We now have data to confirm that overpacking the barn has the potential to increase TSNAs considerably, especially in humid curing seasons.

52. RELATIONSHIP OF ALKALOIDS, PON, TSNAS, NITRATE AND NITRITE: A TWO-YEAR FIELD ANALYSIS. Ying WU, Huihua Ji, Anne Fisher, Franklin Fannin, Nabanita Chattopadhyay and Lowell Bush; University of Kentucky, Lexington, KY USA

Tobacco-specific nitrosamines (TSNAs) are a class of nitrosamines that are included in the FDA's Harmful and Potentially Harmful Constituents (HPHC) list. Two of these, N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are potent carcinogens. These compounds are formed primarily during tobacco curing and storage, via nitrosation of tobacco alkaloids. The accumulation of TSNAs is influenced by many factors such as weather, nitrogen fertilizer, stalk position, and curing conditions. Pseudooxynicotine (PON) is considered a precursor of NNK and the level of PON is higher in green burley tobacco than in cured leaves. TSNAs occur only in trace amounts in green tobacco leaves although their precursor concentrations are at very high levels. In this study, the burley tobacco variety KT 210LC was grown at the University of Kentucky Agricultural Experimental station Farm in 2016 and 2017. Tobacco plants were harvested 28 days after topping and plants were placed on sticks for curing in a conventional air-curing barn. After curing, leaves were sampled from the top, middle, and bottom stalk positions. The lamina and midrib were separated, then freeze-dried, ground and extracted for alkaloids, nitrite, nitrate, PON and TSNA analysis. The results indicated that the distribution and general trend of these compounds in tobacco during growth and curing were the same between the two years. Alkaloids and PON were higher in the lamina than in the midrib. PON levels increased from topping to harvest. TSNAs were not detected until 14 days after the start of curing. The levels of both TSNAs and nitrite increased significantly in the later stages of curing.

53. OPTIMIZED METHOD FOR DETERMINATION OF SELECTIVE PHENOLIC COMPOUNDS IN CIGARETTE AND CIGAR SMOKE BY UHPLC-FLD. Xiaohong Cathy JIN, Thomas J. Hurst and Karl A. Wagner; Altria Client Services, Richmond, VA USA

Phenolic compounds, including phenol, catechol, and o-, m-, and p-cresol are included in the "Established List of the Chemicals and Chemical Compounds Identified by the FDA as Harmful and Potentially Harmful Constituents [HPHCs] in Tobacco Products and Tobacco Smoke". CORESTA has developed and published a consensus standardized method for the determination of phenolic compounds in cigarette smoke, the CORESTA Recommended Method, CRM 78: "Determination of selected phenolic compounds in mainstream cigarette smoke by HPLC-FLD." CRM 78 has a run time of 34 minutes. We have developed a high throughput method that is based on CRM 78, which has a run time of 10 minutes and uses Ultra-High Pressure Liquid Chromatography (UHPLC) and fluorescence detector (FLD) with a sub-2 μm pentafluoro-phenylpropyl phase analytical column. Data generated with the improved method were consistent with data generated using CRM 78. All requirements for method validation were met including linearity, accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), robustness, and standard and sample extract stability. For example, the repeatability for each analyte was less than 10%, and the linearity was

demonstrated with a coefficient of determination of > 0.995 for the calibration ranges of 0.05 µg/mL - 20 µg/mL for hydroquinone, catechol, and phenol, and 0.01 µg/mL - 4 µg/mL for resorcinol, p-cresol, m-cresol, and o-cresol. This optimized method provides a significant reduction in instrumental run time and larger dynamic range as compared to CRM 78. Furthermore, the method has been demonstrated to be fit-for-purpose for the analysis of cigar smoke where the levels of phenolic compounds are higher than in cigarette smoke. Data from commercial cigars and cigarettes will be presented.

54. VARIATION OF SUGAR LEVELS IN TOBACCO UPON HEATING. Serban C. MOLDOVEANU and Karen Kilby; R.J. Reynolds Tobacco, Winston-Salem, NC USA

Non-polymeric carbohydrates including glucose, fructose, and sucrose are major constituents in flue-cured and Oriental tobaccos. The compounds are also present in burley although at much lower levels. Other small carbohydrates as well as carbohydrate derived compounds such as various sugar acids are also present in tobacco. These compounds start decomposing at relatively low temperatures, around 150-155°C. During tobacco processing, preparation of expanded tobaccos, as well as when tobacco is used in "heat not burn" type cigarettes, the tobacco is exposed to different degrees of heat, frequently higher than the temperatures where carbohydrates start decomposing. A systematic study applied to six types of tobacco was performed regarding the variation of the level of several sugars upon heating. These tobaccos included two flue-cured, two burley, and two Oriental tobaccos. The heating was performed for 5 min at 60°C, 100°C, 150°C, and 200°C. The analysis of sugars was performed using direct silylation followed by a GC/MS analysis. Together with sugars, other compounds were detected in the chromatograms. Since burleys have low levels of sugars and most of other tobacco components are more stable up to 200°C, the burley composition appears to change very little with heating. On the other hand, the flue-cured and Oriental tobaccos show some decrease in sugars for heating up to 150°C and a significant decrease when tobacco is heated at 200°C.

55. VARIATION OF TSNAS LEVELS IN TOBACCO UPON HEATING. Serban C. MOLDOVEANU and Marlene Adams; R.J. Reynolds Tobacco, Winston-Salem, NC USA

Tobacco specific nitrosamines including nitrosoanabasine (NAB), nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and nitrosonornicotine (NNN) are naturally present at trace levels in tobacco. During tobacco processing, preparation of expanded tobaccos, as well as when tobacco is used in "heat not burn" type cigarettes, the tobacco is exposed to different degrees of heat. Very limited information is available in the literature regarding the variation of TSNAs when tobacco is heated (at relatively low temperatures), only with indications that TSNAs increase during tobacco heating. For this reason, a systematic study applied to six types of tobacco was performed regarding the variation in the levels of TSNAs upon heating. The TSNAs levels were measured using a LC/MS/MS method. The evaluated tobaccos included two flue-cured, two burleys, and two Orientals. The heating was performed for 2 min and for 5 min at 100°C, 150°C, 200°C, and 250°C. The levels of TSNAs in various tobacco types varies considerably, but the rate of TSNAs increase upon heating was somewhat similar. For 2 min heating, the increase in TSNAs up to about 200°C was relatively small but the levels become almost double when the temperature increases to 250°C. For 5 min heating, the increase in the levels of TSNAs

starts at about 150°C with a maximum at 200°C, but the heating at 250°C produces TSNAs decomposition and the levels are reduced.

56. PREDICTION OF TOTAL AMOUNT OF TOBACCO-SPECIFIC NITROSAMINES IN TOBACCO LEAVES BY FLUORESCENCE EXCITATION-EMISSION MATRIX. Hirotaka NAITO and Takumi Koike; Japan Tobacco, Kanagawa Japan

The amount of tobacco specific nitoroamines (TSNAs) in tobacco leaves is determined by LC-MSMS in the official method (CORESTA Recommended Method 72). More rapid and simple method seems to be required for saving time and easy procedure because it takes long time to conduct the official method. We applied fluorescence excitation and emission matrix (F-EEM) spectroscopy known as a rapid mesurement to estimate the total amount of TSNAs in tobacco leaves. F-EEM spectroscopy records fluorescence spectrum that emitted from compounds, mainly electronic conjugation system, at various excitation wavelengths. TSNAs would be suitable for F-EEM spectroscopy due to their electronic conjugation system in chemical structures.

The mixture of 4 kinds of TSNAs (1.28 μg/g in ethanol) showed 350 nm fluorescence peak at 280 nm excitation by adjusting sensitivity of detector, photomultiplier tube, at 950 V. We detected fluorescence of powdered tobacco around 350 nm, applying an optical filter to attenuate fluorescence intensity above 400 nm derived from other compounds.

In the same way, 71 powdered tobacco samples were served to F-EEM and obtained data were applied to a partial least squares (PLS) regression analysis to generate regression model. We estimated the total amount of TSNAs of different 36 tobacco samples from their fluorescence data with regression model. The correlation between the estimated values and measured values by an official method was R2 = 0.96 with 0.18 μg/g standard error. These results suggest the availability of fluorescence spectroscopy to estimate the total TSNAs rapidly but not individuals.

57. CTP SUBMISSIONS – TIPS AND INSIGHT FOR PREPARING AN ELECTRONIC SUBMISSION. Jeffrey K. SMITH[1], Deborah Sholtes[1], Seth Glatstein[1] and Glenn Angermeier[2]; [1]US FDA, Silver Spring, MD USA and [2]WiseDesign, McLean, VA USA

Currently, tobacco product submissions are organized differently across application types and often, across applicants, and are received in a format that is not amenable for reviewers to organize and easily find what they need. This "unstructured" format is not conducive to automation and complicates analysis across applications and products. Familiarity with FDA's supported technical file formats, data specifications and standards will aid in the creation of an electronic submission of sufficient functionality to facilitate its processing and review by CTP.

CTP intends to be consistent, wherever possible, with existing paradigms, file formats, and data standards developed by other FDA Centers. CTP strives to leverage existing electronic standards and participate in existing standards bodies. Both FDA and industry have benefited from the use of common technical standards including searchability, reliability, accuracy, and harmonization of submission structure across application types. Such

standards also facilitate the development of supporting tools by the commercial market for regulatory submission creation, review, and analysis.

FDA regulations avoid technical details on submission format and organization as technologies change over time. These details are outlined in technical specifications and guidance documents and adopted industry standards. Historically, independent standards associations have helped drive eSubmission standards and FDA has participated as a member. FDA can endorse such standards through Federal Register notice.

FDA is currently evolving towards using the Health Level Seven (HL7) Regulated Product Submission (RPS) standard for its submissions. For CTP the structure is named the electronic Tobacco Technical Document or eTTD. CTP is testing a draft eTTD specification with software companies that provide eCTD solutions to regulated industry to ensure technical viability. Once validated, the eTTD will proceed for public comment.

58. USP ELEMENTAL IMPURITIES: LIMIT TEST FOR METALS IN NICOTINE BY ICP-MS. Hamid LOTFI and Margaret Arroyo; Global Laboratory Services, Wilson, NC USA

Nicotine included in e-cigarette formulations usually originates as a 90%+ solution, which is purified further prior to use. While the FDA oversees the tobacco industry, there is currently little regulation regarding the quality of raw materials or mandated testing. However, manufactures have a duty of care to ensure the quality of their products, and to practice good product stewardship, and in the absence of such guidelines have turned to the USP monograph for nicotine as the gold standard to assess its quality.

Although the USP nicotine monograph specifies several analytical tests to assess its quality, this presentation will focus solely on the evaluation of elemental impurities, which is a limit test, and the challenges encountered.

Quantitation of metals by ICP-MS can be subject to both spectral interferences and matrix effects resulting in suppression or enhancement of the signal response and error in quantitation. These interferences were eliminated or negated through the use of advanced analytical techniques such as dynamic reaction cell (DRC) and kinetic energy discrimination (KED) as well as the judicious selection of each metal isotope to monitor and choice of internal standards used.

The developed method was validated according to ICH and FDA guidelines. The validated method is both accurate and precise and is able to analyze the mandated Class 1 metals (cadmium, lead, arsenic, and mercury) and the optional Class 2a and Class 3 metals in nicotine samples to ensure that the product meets USP requirements.

61. HPHC MARKET MAP STUDY FOR US MACHINE-MADE CIGARS – PART 1 PHYSICAL PROPERTIES, FILLER AND SMOKE HPHC VARIABILITY. Karl A. WAGNER, Michael J. Morton, Raquel M. Olegario, Lara L. Baker and Jennifer H. Smith; Altria Client Services, Richmond, VA USA

Market map studies have been used in the cigarette industry for many years to aid in the characterization of the marketplace. These studies provide comparative values and predictive models for aiding in the assessment of other products. However, the characterization of the physical properties and smoke and filler HPHCs of cigars has been much more limited than with cigarettes.

We examined the physical properties and the filler and smoke HPHCs of 24 machine-made cigars from the US marketplace. The goal was to establish HPHC ranges for filler and smoke yields and to develop predictive relationships to estimate smoke yields of cigars not included in this study.

Products were smoked using the CORESTA, ISO, and Intense smoking regimes for the constituents on the FDA abbreviated HPHC list for cigarettes. The cigars were also tested

for each of the filler constituents on the FDA abbreviated HPHC list for cigarettes. Cigars show much greater variability in weight and resistance to draw than cigarettes and that variation is reflected in much greater variability in smoke yields than is seen with cigarettes.

The relative variability of smoke HPHCs and the product yield orderings are similar with all three smoking regimes. Filler HPHCs are less variable than smoke HPHCs. The smoke HPHCs are correlated to the overall yields of the products as measured by TPM, tar, or carbon monoxide yields. Many of the smoke yield correlations are further improved by taking the tobacco filler HPHCs into account.

This work will be discussed in a two-part presentation. Part 1 will focus on the description of the products and the inherent variability of cigars. Part 2 will focus on predictive models.

62. HPHC MARKET MAP STUDY FOR US MACHINE-MADE CIGARS – PART 2 PREDICTIVE MODELS. Michael J. MORTON, Karl A. Wagner, Raquel M. Olegario, Lara L. Baker and Jennifer H. Smith; Altria Client Services, Richmond, VA USA

Market map studies have been used in the cigarette industry for many years to aid in the characterization of the marketplace. These studies provide comparative values and predictive models for aiding in the assessment of other products. However, the characterization of the physical properties and smoke and filler HPHCs of cigars has been much more limited than with cigarettes.

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63. TOBACCO HEATING PRODUCTS (THP) : EXPERIMENTAL CONSIDERATIONS FOR ACHIEVEING REPRESENTATIVE YIELDS. Ian TINDALL[1], Linda Crumpler[2] and James Okpeh[1]; [1]Cerulean, Milton Keynes UK and [2]Cerulean, Richmond, VA USA

Performing "traditional" TNC (Tar Nicotine carbon monoxide) analysis of THP products present significant challenges to the scientist. Creating, capturing and transporting the aerosol formed for analysis is not as straightforward as for conventional burn down products. These factors that ultimately influence experimental success must be understood and considered when developing standards for test and analysis.

A challenging aspect is the complete capture of all aerosol formed. It is proposed that significant aerosol may be lost as dead volume increases which gives rise to concerns regarding exposure or capture studies. This paper shows the relationship between dead volume and capture efficiency and measures that can be taken to maximize total aerosol capture.

Through manipulation of the length and topography of the path to the capture pad the effect on system capture efficiency is demonstrated via simple mass balance experiments for a variety of THP devices and smoking topography. A further complication is the conditions underwhich samples are stored and condition can be shown to imact the yield of the product.

The relationship between capture efficiency and dead volume is shown not to be a simple one with the majority of aerosol (40%) being lost from a capture system within the first 10mm of the butt end of the THP. Reducing the dead volume path length to 4mm brings the majority of the aerosol within the capture envelope.

Overcoming the condensation of aerosol before capture is one of practical engineering as the study shows. The implications for exposure (*in vivo* or *in vitro*) are less clear and should give some pause for thought in experimental design.

64. COMPARISON OF TWO DIFFERENT HIGH-RESOLUTION MASS SPECTROMETERS FOR UNTARGETED LC/MS ANALYSIS OF CIGARETTE SMOKE EXTRACTS. Yuichiro TAKANAMI, Nobumasa Kitamura, Norimichi Orikata and Tetsuya Tobita; Japan Tobacco, Yokohama, Kanagawa Japan

Cigarette smoke contains at least 6000 compounds. Comprehensive chemical analysis of this complex mixture is often achieved by untargeted liquid chromatography (LC)/mass spectrometry (MS) analysis, which requires the use of several state-of-art instruments such as high-resolution mass spectrometers (HRMS). However, the technical aspects of the analysis are poorly understood, especially for cigarette smoke analysis. To identify key techniques for untargeted analysis, we analyzed cigarette smoke using two different HRMS and compared the results.

Total particulate matter from 3R4F cigarette smoke was extracted with methanol and injected into Orbitrap (QE = Q Exactive, Thermo Fisher Scientific) and time-of-flight (QTOF = Xevo Qtof, Waters) LC-HRMS systems. The same type of C18 column was installed in each LC system, and they were operated under the same conditions, including

the gradient elution. Spectra were acquired by MS and tandem MS (MS/MS) in data-dependent acquisition mode for the QE system or MSE mode for the QTOF system. The obtained data were processed by Progenesis QI software (Waters). Several peaks from the two instruments were manually aligned. The QE showed higher sensitivity for molecules with lower molecular weights than the QTOF. There was a non-linear correlation between the retention times obtained with the two systems. A comparison of MS/MS data from the two instruments showed that spectra obtained by the QE provided more information to identify compounds than the QTOF. The QE system acquires spectra using multiple collision energy, which produces fragments for both fragile (nicotine) and solid (norharman) compounds. This feature is advantageous for estimation of compound identities by MS/MS. In the untargeted analysis, it is important to understand the characteristics of acquired MS/MS when comparing them with known spectra.

65. EFFECT OF SBA-15 MORPHOLOGY ON THE THERMAL DECOMPOSITION OF NICOTINE. Javier ASENSIO, Antonio Marcilla, Maria Isabel Beltrán and Nera Juárez; Alicante University, Alicante Spain

Nicotine is the most abundant alkaloid both in tobacco and tobacco smoke, main responsible of the addictive character of tobacco. SBA-15 is a mesoporous silicate that has proven to be very effective in reducing tars and nicotine from tobacco smoke when added to tobacco under smoking conditions. The present work presents preliminary results on the thermal degradation, using a pyrolizer (Py/EGA-GC/MS) online with a gas chromatographer coupled with mass selective detector, of nicotine under different conditions (atmospheres and temperatures), and in the presence of two SBA-15 catalysts of different morphologies. The two SBA-15 mesoporous catalysts with fibre like and platelets morphologies, were synthetized in our laboratory and characterized by N2 adsorption isotherms, scanning electron micrographs (SEM) and transmission electron micrographs (TEM) and X-ray diffraction.

Nicotine decomposes at lower temperatures under inert atmosphere than in air. The presence of fibre like SBA-15 slightly modifies the thermal decomposition of nicotine under inert atmosphere. Nevertheless, the platelet shaped SBA-15 strongly affects the thermal behaviour nicotine favouring its decomposition, occurring at lower temperatures with high yield of compound such as quinolone and 2-methyl-1H-Indole, among others. Under oxidative atmosphere, both catalysts have a less marked effect on the thermal degradation of nicotine.

67. CHARACTERIZATION OF SMOKELESS TOBACCO PRODUCTS EXTRACTED WITH DIFFERENT SOLVENTS FOR *IN VITRO* TESTING. Jingjie ZHANG[1], Doshi Utkarsh[1], Jyoti Thaikoottathil[1], Monica K. Lee[1] and Russell L. Wolz[2]; [1]Altria Client Services, Richmond, VA USA and [2]Enthalpy Analytical, Richmond, VA USA

Unlike cigarettes, there are no standardized methods available for preparing extracts from smokeless tobacco (SLT) products for *in vitro* toxicological evaluation. Methods are available for Harmful and Potentially Harmful constituents (HPHCs) characterization but they often differ from methods for *in vitro* studies in which limited types of solvents can be used. Additionally, the extracts tested *in vitro* are not typically characterized for constituent levels, making it difficult to interpret the observed response. The purpose of this study was to characterize extracts from two CORESTA reference SLT products: CRP1.1 (Swedish style snus pouch) and CRP 2.1 (American-style loose moist snuff) using solvents that are routinely used for *in vitro* testing (ethanol, DMSO, and artificial saliva). We compared the extraction efficiency of each solvent based on selected analytes (nicotine, tobacco-specific nitrosamines (TSNAs), nitrate, and benzo[a]pyrene (B[a]P)). Reference products were first characterized with analyte-specific methods. Nicotine and TSNAs in CRP 1.1 and CRP 2.1 and B[a]P in CRP 2.1 were generally comparable with literature values, while B[a]P in CRP 1.1 (0.5 ng/g) was lower than the CORESTA-reported value of 0.7 ng/g. The reference products were then extracted at up to 20% w/v concentration in each solvent for 2 hours at 37°C or 24 hours at ambient temperature. TPleasehe extraction efficiency was reported as percent recovery compared to the analytical reference values. In general, the percent recovery of analytes ranged from 70-103% for different solvents. This study suggests that characteristics of extracts prepared for *in vitro* studies are dependent on the extraction method. Therefore, it is imperative that appropriate test article characterization should accompany any *in vitro* toxicological evaluation of SLT products.

68. TRACE METALS ANALYSIS OF TOBACCO HEATED PRODUCT BY ICP-MS. Danielle BENNER, Donald Stogner, Jamil Gray, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

As new tobacco heated products are developed, there is a need to improve or develop methods to determine if the products contain trace metals. A method was developed and validated to determine the trace metal content in THP by ICP-MS for arsenic, cadmium, chromium, nickel, and lead. The samples are smoked on a rotary smoke machine and the subsequent aerosol is captured using an electrostatic precipitation tube (EP tube). The aerosol is then extracted with methanol and placed on a heating block to evaporate the methanol. It is then further digested with nitric acid after the methanol has evaporated. Samples are analyzed using a NexION 300S ICP-MS. The results of the validation will be reported.

69. APPLICATION OF ISOTHERMAL MICROCALORIMETRY TO MST PRODUCTS -- A NEW METHOD TO MONITOR AGING. Robert B. RAGLAND and Mark J. Rusyniak; Altria Client Services, Richmond, VA USA

Isothermal microcalorimetry can be used to measure heat conduction as a function of time as a tool to rapidly measure chemical and physical changes that can occur during aging. This can be particularly useful when trying to determine a product's stability over its shelf life which typically involves the collection of a large amount of data at designated time intervals to assess changes in the finished product, as a function of time and environmental conditions. For tobacco products such as moist smokeless tobacco (MST), it can be difficult to attribute chemical or physical changes to a singular mechanism due to chemical reactions and microbial activity occurring concurrently.

Commercially available MST products were tested to determine characteristic heat flows relative to tobacco particle size and flavor variants. Also, samples of the unfinished MST tobacco base and finished product were treated using electron beam irradiation for the purpose of cold pasteurizing the material. The pasteurized samples were analyzed against non-pasteurized variants to assess heat flow contribution due to microbial activity and lignin oxidation. Results showed that products pasteurized prior to fermentation had a reduced heat flow signal similar to products pasteurized after fermentation indicating the microbes used for fermentation do not negatively impact product stability. Samples were also tested in ampoules pressurized with nitrogen and oxygen to measure heat flow relative to oxygen consumption and transitions to anoxic conditions. Based on pressure calorimetry, oxygen consumption appears to constitute a large portion of the baseline heat flow measurements where relatively small heat flows were observed in an inert atmosphere.

70. COMPARATIVE LEVELS OF CARBONYL DELIVERY BETWEEN MASS-MARKET CIGARS AND CIGARETTES. Joseph J. JABLONSKI, J. Hunter Maines, Andrew G. Cheetham, Alexandra M. Martin and I. Gene Gillman; Enthalpy Analytical, Richmond, VA USA

The recent 2016 deeming of cigars by the US Food and Drug Administration (FDA) has led to an increased interest in cigar science, including ways to accurately measure the harmful and potentially harmful constituents (HPHCs) found within mainstream cigar smoke. At present, there are a limited number of standardized methods available for the evaluation of HPHCs in mainstream cigar smoke, except for nicotine and carbon monoxide. This study sought to investigate carbonyl delivery in commercially available cigars and cigarillos and compare them to levels found in cigarettes. First the standard cigarette method, CORESTA recommended method 74 (CRM-74), was optimized for cigar smoking, including evaluation of the trapping efficiency and the stability of the carbonyl-hydrazone adducts due to the increased smoke time required for cigar collection. The optimized trapping solution was then applied in a survey of the carbonyl delivery in commercially available cigars and cigarillos for comparison to published cigarette data. Smoked under CRM-64 conditions, cigars were found to yield similar levels of formaldehyde to those found in commercially available cigarettes (20.2 ± 11.7 vs. 22.1 ± 13.5 μg/cig respectively). Greater levels of acetaldehyde (2133 ± 470 vs. 365 ± 176.5 μg/cig), acrolein (52.7 ± 23.7 vs. 33.4 ± 17.0 μg/cig) and crotonaldehyde (42.4 ± 14.7 vs. 14.7 ± 6.8 μg/cig) were observed in cigar mainstream smoke when compared to cigarettes collected under conditions prescribed

by ISO standard 3308. Furthermore, cigarettes smoked under the Health Canada Intense smoking regime delivered higher levels of formaldehyde (20.2 ± 11.7 vs. 74.6 ± 24.0 µg/cig), acrolein (52.7 ± 23.7 vs. 120.5 ± 14.9 µg/cig) and crotonaldehyde (42.4 ± 14.7 vs. 51.5 ± 8.7 µg/cig) emissions as compared to cigars smoked under the CORESTA regime, while acetaldehyde was found to be higher in cigar emissions (2133 ± 470 vs. 1234 ± 147 µg/cig).

71. NICOTINE VARIABILITY OF LOW NICOTINE CULTIVARS VERSUS NORMAL NICOTINE CULTIVARS. Kenny LION, Andrew Adams, Whit Morris, Emily Brown, Brittany Irving, Marcos Lusso and Dongmei Xu; Altria Client Services, Richmond, VA USA

Nicotine is the most abundant alkaloid in cultivated tobacco (Nicotiana tabacum), typically constituting more than 90% of total alkaloids. Recently, the U.S. Food and Drug Administration (FDA) issued an Advance Notice of Proposed Rulemaking (ANPRM) to obtain information for consideration in developing a tobacco product standard to set the maximum nicotine level in cigarette filler to "minimally addictive or non-addictive" levels. Nicotine levels are highly variable across different years and locations as data collected over decades in the Minimum Standards Programs show. Analysis of the Burley check varieties in the Minimum Standards Program showed relative standard deviations ranging from 20% to 28% for individual varieties across locations from 2012 to 2018. Though there are decades of nicotine analysis from the Minimum Standards Program, there is not much data collected and publicly available on nicotine variability in low nicotine cultivars. The purpose of this study is to determine variability in nicotine content across multiple years and locations from low nicotine cultivars and compare it to normal nicotine cultivars. To initiate this long term study we analyzed low nicotine cultivars alongside normal nicotine cultivars at the same locations from 2012 to 2018. Assuming the variability in nicotine content for low alkaloid cultivars is similar to or greater than the variability in normal nicotine cultivars, it will have major impacts on meeting any regulatory standard. This could suggest that to meet a standard of 0.3-0.5 mg/g nicotine content the cultivars themselves may need to have much lower nicotine levels to consistently meet the proposed standard year after year. This will have major implications on the technical achievability of such a standard.

72. A COMPARISON OF QUARTZ FILTER COLLECTION VERSUS ELECTROSTATIC PRECIPITATION COLLECTION IN E-CIGARETTE AEROSOL SAMPLES. I. Gene GILLMAN, Alexandra Martin, Samuel Hochstetler, Justin Lata, Nicholas Race, Darybelle Collins And Patrick Kelly; Enthalpy Analytical, Richmond, VA USA

The determination of metals in e-cigarette aerosol has been routinely performed by collecting the aerosol on quartz fiber filter pads. The trapped aerosols are digested using acid dilution and analyzed using ICP-MS. The quartz filters have similar performance to standard Cambridge filters. The major problem with using quartz filters, is they contain detectable levels of many of the metals of interest and, even more problematic, the amount of metals varies between filters and filter lots. Each new lot of filters must be evaluated for background levels to establish method limit of detection (LOD). Even with background subtraction, the quartz filter method has elevated LODs and increased variability near the method LOD.

In order to reduce background levels and improve method LOD we evaluated the use of an electrostatic precipitation (EP) unit to collect the aerosol. The EP unit has long been established in the collection of cigarette smoke (Health Canada Official Method T-109).

In this study we validated the use of a 20-port EP system for the determination of metals in e-cigarette aerosol; focusing on trapping efficiency, analyte recovery, and background contamination. We will present findings that show a decrease in background contamination and an improvement in the detection limits of the EP method over the quartz filter method. The method LODs were lowered by an average of 72%. For example, Chromium for the filter collection method had an average method LOD of 103 ng/collection while the LOD for the EP method was 5 ng/collection. The EP system offers an additional improvement since each EP unit has the ability to collect up to 3.5 g of aerosol collected mass (ACM) while the filters are limited to maximum of ~0.8 grams of ACM. The ability to collect additional ACM significantly reduces method LOD on a per gram basis.

73. COLLECTION AND CHARACTERIZATION OF MAINSTREAM CIGARETTE SMOKE CONDENSATES USING A GLASS FIBER FILTER AND ETHANOL CONTAINING IMPINGER. I. Gene GILLMAN, Jacob P. Hilldrup, Sarah R. Packett and Jacqueline M. Collins; Enthalpy Analytical, Henrico, VA USA

Traditional smoke collections for *in vitro* toxicology assays, including regulatory Health Canada (HC) testing, have utilized a multi-phase setup that traps particulate matter with a glass fiber filter followed by a liquid filled impinger for gas phase compounds. Dimethyl sulfoxide (DMSO) is used to extract particulate matter from the filter while the gas phase is collected in phosphate buffer saline (PBS). These solvents have been extensively used for the assays mentioned above but may not effectively trap the full range of compounds produced during cigarette combustion. Additionally, the separate collection of particulate and gas phases divides mainstream smoke into two samples. However, many compounds are present in both the particulate and gas phases so separation may not be ideal.

This study investigates an alternate smoke collection procedure that combines the particulate and gas phases together using ethanol instead of the traditional DMSO/PBS solvents. This alternate procedure traps particulate matter with a glass fiber filter in series with an ethanol filled impinger for gas phase compounds. The filter is extracted with the impinger contents to yield a single whole smoke condensate.

Mainstream 3R4F smoke was collected using ethanol and traditional HC methods. The filter pad and impinger contents were analyzed separately for the FDA abbreviated list of HPHCs. Particulate phase compounds like nicotine, benzo[a]pyrene, and TSNAs were similar in both methods. However, large differences were seen when comparing the trapping efficiency of ethanol versus PBS for volatile organic compounds (VOCs). The VOC content of PBS was found to be 6µg/mL while ethanol was found to be 48µg/mL, an 800% increase. Ammonia, formaldehyde, and acetaldehyde increased by approximately 15% when using the alternate trapping procedure. The stability of the extracts was also determined for both methods. The impact of the condensate collection method on the HC toxicology assays will be presented separately.

74. DETERMINATION OF PRIMARY AROMATIC AMINES IN SMOKELESS TOBACCO PRODUCTS. Andrew G. CHEETHAM and Alexandra M. Martin; Enthalpy Analytical, Richmond, VA USA

The list of harmful and potentially harmful constituents (HPHCs) published by the FDA in 2012 features six primary aromatic amine (PAA) compounds—1-aminonaphthalene (1-ANP), 2 aminonaphthalene (2 ANP), 4-aminobiphenyl (4-ABP), o-anisidine (o-AND), 2,6-dimethylaniline (DMA), and o-toluidine (o TOL). Of these six PAAs, 2-ANP, 4-ABP, and o-TOL have been deemed to be Group 1 carcinogens by the International Association for Research on Cancer (IARC). PAAs in mainstream smoke are thought to be combustion products formed from the tobacco's nitrogen-containing constituents. Smokeless tobacco products (STPs) would, therefore, be expected to be relatively free from PAAs, but some tobacco curing processes may introduce PAA contaminants through exposure to smoke and heat. In this study, we sought to determine if PAAs are present in a range of STPs and if there is any correlation to the tobacco types used in the product. Using a procedure adapted from a draft CORESTA method for the analysis of PAAs in mainstream cigarette smoke by GC-MS, we screened a variety of reference and market products for their PAA content. For many products, the levels were below either the verified quantitation or detection limits for each analyte. However, elevated and quantifiable levels of the Group 1 carcinogens, o TOL and 2-ANP, were observed in products containing significant proportions of dark-fired tobacco. The CORESTA research product CRP3.1, for instance, is a dry snuff product for which the tobacco blend is 62% dark-fired tobacco, and was found to contain approximately 4.9 ng/g o-TOL and 0.17 ng/g 2 ANP. Elevated levels of the Group 2B carcinogens DMA and o-AND were also found (0.48 and 0.57 ng/g, respectively). For comparison, a Kentucky 3R4F cigarette smoked under ISO conditions generates an average of 45 ng/cig o-TOL, 5.8 ng/cig 2-ANP, 3.2 ng/cig DMA, and 2.5 ng/cig o-AND. Results from our market product survey will be presented and discussed.

75. ANALYSIS OF NICOTINE, MENTHOL, WATER, PROPYLENE GLYCOL, AND GLYCERIN IN TOBACCO HEATED PRODUCT. Tiffany LANDINGHAM, Michael Siernos, Corey Posten, Chris Almond, David D. Mickey, Carl J. Adams and Salem Chouchane; Eurofins Professional Scientific Services, Winston-Salem, NC USA

As the Tobacco Heated Product (THP) industry is expanding, the accurate analysis of nicotine, water, menthol, glycerin, and propylene glycol has become increasingly important with regard to regulatory submissions. To evaluate these products, an aliquot of the aerosolized THP device was collected on a 44 mm Cambridge filter pad, and transferred to a Kimble glass tube. The sample was then extracted in isopropyl alcohol, shaken for 30 minutes and analyzed on an Agilent 7890 GC Flame Ionization Detector (FID) using a method specified to identify and quantify the analytes of interest. Sample results were quantified by preparing the samples against a freshly prepared calibration curve. All results met the acceptance criteria of the method with the low spiked recovery samples yielding results of 100-114% recovery; the high spike recovery yielded results of 95-125%. While all other analytes are relatively stable, water content can be dynamic when exposed to the testing environment. Great care was taken to ensure the Cambridge pads are placed quickly into the Kimble glass tube and capped to ensure the amount of water found was from the sample and not the testing environment. This analysis proves that this method is suitable

for the accurate identification and quantification of analytes present in Tobacco Heated Products. The validation results will be presented.

76. NICOTINE *IN VIVO* EXTRACTION AND PHARMACOKINETICS OF NON-TOBACCO-BASED NICOTINE POUCHES (ZYN®) COMPARED WITH TOBACCO-BASED SWEDISH SNUS AND AMERICAN MOIST SNUFF. Mikael STAAF, Tryggve Ljung and Robert Pendrill; Swedish Match, Stockholm, Sweden

Objectives

- To compare *in vivo* extracted dose and pharmacokinetics of nicotine from ZYN® with Swedish snus (General) and U.S moist snuff (Longhorn)
- To evaluate the effect of the flavor component methyl salicylate on nicotine extraction and uptake

Methods

Three clinical studies were conducted as open randomized multiple-way cross-over trials. The treatments were administered as single doses during 60 minutes. Blood levels of nicotine were followed over 6 hours after administration.

Results

The extraction of nicotine in the ZYN® products were approx. 50-60%, compared to ca 30% and 20% in Swedish snus and U.S. moist snuff, respectively.

The peak plasma concentrations (Cmax) of ZYN® (6 mg/unit), Longhorn (18 mg/unit), ZYN (8 mg/unit) and General (16 mg/unit) were approx. 14, 17, 18 and 21 ng/ml.

Conclusion

The higher rate of extraction for the ZYN® pouch could be explained by the different geometry, which would lead to a more efficient saliva penetration of the pouch.

The evaluation of pharmacokinetics showed that, the ZYN® products gave rise to significantly larger nicotine uptake than conventional, tobacco-based products with equivalent nicotine content. However, more important, the studies also showed that the ZYN® products do not entail a higher nicotine exposure compared with some commercially available tobacco-based snus or moist snuff products that are currently common on the Scandinavian and U.S. markets.

The assessment of the effect of methyl salicylate on nicotine extraction and plasma concentrations showed no significant difference for neither ZYN® or tobacco-based moist snuff products.

77. COMPARISON OF METHODS FOR MEASURING THE PARTICLE SIZE DISTRIBUTION OF SMOKELESS TOBACCO PRODUCTS. Sean P. PLATT, Charnise Jackson, Kathryn Dill and Mark Rusyniak; Altria Client Services, Richmond, VA USA

Tobacco cut size is one of the product properties for smokeless tobacco products, which the Food and Drug Administration has proposed as a requirement for a Substantial Equivalence (SE) submission. (21 CFR Parts 16 and 1107). However, no guidance has been provided for a

standard method to perform these measurements. Sieve analysis can been used to determine particle size distribution (PSD) for smokeless tobacco products; however, this technique is time consuming, labor intensive, and requires that moist products be dried prior to sieving. Dynamic image analysis (DIA) is a method used to measure particle size that incorporates a high-speed camera to capture images of particles, as they flow through a cuvette. Image analysis software is used to compute the PSD. DIA present distinct advantages over sieve analysis. DIA has greater resolution, since the bin sizes for the distribution can be set much more narrowly than with sieves. DIA offers a wide particle size range, limited only by the camera and optics in use, which for this work ranged from 1 μm to 20 mm. The image analysis algorithms allow for a variety of metrics to be applied to the distribution. In this work, the particle size is calculated as the diameter of a circle of equal projection (EQPC), and length of fiber through a direct connection of the two most distant points (LEFI). The EQPC method allows us to easily transform the data from a length mode to surface area or volume weighted distributions. We examine differences in PSD of four MST products including snus, snuff, fine cut, and long cut. A direct comparison between the weight-based sieve method and optical DIA methods is presented.

78. PROBABILISTIC RISK ASSESSMENT TO COMPARE HEALTH RISKS OF ORAL TOBACCO PRODUCTS. Annette B. SANTAMARIA[1], Marshall E. Krotenberg[2], Scott M. Drouin[1], Kimberly D. Ehman[3], Chastain A. Anderson[3], Vanessa Haase[3] and Donna C. Smith[3]; [1]Rimkus Consulting Group, Houston, TX USA, [2]Rimkus Consulting Group, Phoenix, AZ USA and [3]Altria Client Services, Richmond, VA USA

In the evaluation of substantial equivalence (SE) for tobacco products for submission to the US Food and Drug Administration (FDA), differences in harmful and potentially harmful constituents (HPHCs) may necessitate the determination of whether a new tobacco product raises different questions of public health. Quantitative risk assessment (QRA) is a useful tool for evaluating public health concerns, informing regulatory decisions, and developing approaches for risk-benefit analyses. Traditional health-risk QRA approaches typically begin by screening available data in a deterministic QRA intended to be protective of human health. This approach uses mathematical models to produce point estimates of risk (*e.g.*, average or reasonable worst-case) from which risk estimates may then be compared for individual chemicals, chemical mixtures, consumer products, or remediation approaches. If questions about comparative risks remain, a probabilistic risk assessment (PRA) may be conducted. PRA utilizes tools to identify, characterize, and quantify the key input factors that impact risk estimates. As such, PRA provides information regarding uncertainties and variabilities, and ultimately informs the risk management decision-making process. PRA uses mathematical modeling approaches that rely on distributions of data as inputs, resulting in calculated probability distributions of the relationship between exposure and risk. This presentation will include an example of a PRA conducted on a smokeless tobacco product to estimate and compare noncancer and cancer health risks between two products. The PRA will focus on the 8 HPHCs required to be reported to FDA for smokeless tobacco products. The presentation will include a description of the data and methods available for conducting the PRA, the potential uncertainties/challenges with each step, and how variability in the toxicity and exposure parameters impact the comparative risk estimates.

79. COMPARISON OF THE ACETALDEHYDE, FORMALDEHYDE, AND ACROLEIN YIELDS IN CIGARS UNDER DIFFERENT SMOKING REGIMENS USING A LINEAR CIGARETTE SMOKING MACHINE. Mimy YOUNG, Todd L. Cecil, Tricia Johnson and Shixia Feng; Food and Drug Administration, Calverton, MD USA

Currently, there is very little information on the mainstream smoke yields of carbonyl compounds (*e.g.*, formaldehyde, acetaldehyde, and acrolein) in cigar tobacco products. This study used a standard linear smoking machine and puffing protocols to provide information on the carbonyl yields in mainstream smoke from different commercial cigar tobacco products. Specifically, this study compared the variability of the carbonyl yields of ten small sheet-wrapped cigars (cigarillos), five leaf-wrapped cigars, and two reference cigarettes (3R4F and 1R6F) using three different smoking regimens: two standard cigarette smoking regimens (ISO and Canadian Intense [CI]) and one cigar smoking regimen (CORESTA Recommended Method [CRM] No. 64). The mainstream tobacco smoke from each smoking regimen was analyzed for carbonyls using CRM No. 74, in which the mainstream tobacco smoke is collected using a smoking machine fitted with an impinger containing 2,4-dinitrophenylhydrazine (DNPH) and analyzed using liquid chromatography with an ultraviolet detector. Results demonstrate that carbonyl yields in the mainstream smoke of cigar tobacco products using the three different smoking regimens resulted in high levels of variability across the cigar products. For example, under the three smoking regimens, the formaldehyde yields of commercial cigarillos ranged from 9.4-28 µg/cigar under ISO, 8.2-43 µg/cigar under CI, and 8.6-13 µg/cigar under CRM No. 64. Leaf-wrapped cigars resulted in formaldehyde yields that ranged from 11-13 µg/cigar under ISO, 11-22 µg/cigar under CI, and 16-21 µg/cigar under CRM No. 64. In contrast to the study cigar products, reference cigarettes resulted in higher formaldehyde yields using the standard cigarette smoking regimens but comparable formaldehyde yields using CRM No. 64 (21-28 µg/cigarette under ISO, 76-96 µg/cigarette under CI, and 12-14 µg/cigarette under CRM No. 64).

80. USE OF THE BENCHMARK DOSE APPROACH IN CARCINOGENIC RISK ASSESSMENT FOR NNK. Ashley TURNER and Felix Ayala-Fierro; ITG Brands, Greensboro NC USA

The Benchmark Dose (BMD) approach for Carcinogen Risk Assessment was implemented by modeling tumor data and exposure to establish a dose-response relationship. First, the Inhalation Unit Risk (IUR) was derived using linear extrapolation and then a benchmark dose lower bound (BMDL1.0) was established as the Point of Departure (POD). The Lifetime Average Daily Intake (LADI, Exposure Concentration) was calculated for NNK (Nicotine-derived nitrosamine ketone) in two commercial cigarette products under ISO and HCI smoking conditions and converted to daily doses. The excess cancer risk was calculated as the Incremental Lifetime Cancer Risk (ILCR) and compared to the IUR and POD.

The maximum exposure levels, under HCI smoking conditions, resulted in exposures approximately 1/10th the IUR and below the POD. The difference in excess cancer risk in the two products was very small. At ISO smoking conditions exposure levels were approximately 1/20th the IUR and also below POD. There was a slight difference in excess cancer risk between the two products but the change in this region of the dose-response curve indicates no impact to human health. Margin-of-Exposure (MOE) for NNK were

calculated at the BMDL1.0 and compared to literature values. At the BMDL1.0 the MOE could be interpreted as the Margin-of-Safety (MOS). The use of the POD as the reference for health effects can be expanded to other analytes with debatable IURs published by different agencies or in the public literature.

81. REVIEW OF RELEVANT TOXICOLOGICAL DATA USING BMDS TO ESTIMATE INHALATION UNIT RISK FOR RISK ASSESSMENT PRACTICES. Ashley TURNER and Felix Ayala-Fierro; ITG Brands, Greensboro NC USA

Carcinogen Risk Assessment relies in the use of inhalation unit risk (IUR) values often published by National or International government agencies. However, many smoke analytes lack IURs or have conflicting values due to different interpretation of the tumorigenic data (critical effect) or the model used to fit the data. This leads to incomplete risk assessment and increase uncertainty in the risk assessment process. The objective of this work is to review the availability of toxicological data for selective smoke analytes, determine data limitations, re-calculate IUR's in case of conflicting values, or estimate IUR's for selective smoke analytes when they have not been previously reported.

The Benchmark Dose (BMD) approach was implemented by modeling tumor data and exposure to establish a dose-response relationship. For mutagenic substances the practice is to use linear extrapolation to low doses as a conservative approach. The IUR was derived following this extrapolation and the benchmark dose lower bound (BMDL) at different response levels was also estimated. The BMDL at the relevant response level, *e.g.* 10% (BMDL10) could be used as the Point of Departure (POD).

The use of the BMD approach confirmed previously published IUR for a strong nitrosamine carcinogen. Although assumptions on critical effects at low doses, referenced in published literature, could increase the IUR. In the case of a weak carcinogen with limited information and no known published IUR, the approach resulted in values that could be used as the IUR for risk assessment practices. In summary, the use of the BMD approach to determine POD as the reference for health effects can be expanded to other analytes with debatable IURs published by different agencies or in public literature.

82. HPHC ANALYSIS OF SEVEN FLAVORS OF A TEMPERATURE-REGULATED NICOTINE SALT-BASED POD SYSTEM. David COOK, Bryant Hiraki and Manoj Misra; JUUL Labs, San Francisco, CA USA

Background
The JUUL Nicotine Salt Pod System (NSPS) has no user modifiable settings and is temperature regulated to minimize the generation of combustion related byproducts. Aerosol generated from NSPS pods via a cotton wicking material was evaluated for harmful and potentially harmful constituents (HPHCs).

Methods
Seven flavors (tobacco, mint/menthol, and fruit flavors; 18mg/mL nicotine) were analyzed for HPHCs listed in the US FDA PMTA draft guidance document for ENDS. Testing was conducted by an accredited ISO 17025 laboratory using validated methods (Labstat International ULC, Canada). Machine topography was 70mL volume, 3 second puff duration,

and 30 second inter-puff interval. Each analytical result was generated using a unique pod with 10 replicates collected for each assay. A panel of 22 analytes from six categories of HPHCs was assessed: tobacco specific nitrosamines (TSNAs), polyaromatic amines (PAAs), polyaromatic hydrocarbons (PAHs), carbonyls, volatile organic compounds (VOCs), and metals. Comparator reference combustible cigarettes (3R4F) were also evaluated.

Results
HPHCs were reduced by 99% in NSPS aerosol vs. mainstream smoke of the 3R4F comparator cigarette. The majority (95%) of NSPS aerosol analytes were below the level of quantification. Notably, VOCs (acrylonitrile, benzene, 1,3-butadiene, isoprene, and toluene) and select carbonyls (diacetyl, acetyl propionyl, and crotonaldehyde) in the aerosol were uniformly below the level of detection in all seven flavors.

Conclusions
Consistent with prior research, NSPS pods using a cotton wicking material demonstrated significant reductions in HPHCs on a puff-for-puff basis compared to reference combustible cigarettes.

Limitations
This was a preliminary assessment of ten replicates of each analytical method for each formulation, using one puffing regime on a smoking machine under laboratory conditions. Comprehensive characterization of human HPHC exposure requires user topography data and biomarker analyses.

83. TOBACCO EMISSIONS FOR CANADIAN CIGARETTES: A LOOK BACK ON 10 YEARS. Dilara JAKUPOVIC, Huda Masoud, Nemanja Mladjenovic and Trevor Mischki; Health Canada, Ottawa, ON Canada

With an estimated 45,000 deaths attributable to smoking in Canada in 2012, it remains the leading preventable cause of disease and premature death in Canada. Since 2000, the Canadian Tobacco Reporting Regulations require cigarette manufacturers to test and submit levels of certain chemicals found in tobacco smoke for brands sold in Canada. This study examined the trends for 40 analytes found in Canadian cigarette emissions over the past decade. Descriptive statistics were computed by method and/or year, and mean concentrations by method were plotted over time for each of the 40 smoke constituents. One-way analysis of variances (ANOVAs) were used to compare means across years, using the Tukey-Kramer adjustment for multiple comparisons. Two-way ANOVA models were constructed to examine the effect of smoking method, year, as well as the interaction of smoking method and year, on each smoke constituent. While it was observed that nicotine levels have remained consistent in Canadian cigarettes, other smoke constituents have seen significant variability. In particular, overall downward trends were observed in levels of some known carcinogens, including tobacco specific nitrosamines and benzene. In addition, cigarettes labelled as "light/mild" had a chemical emissions profile significantly different than those labelled "bold/regular" under both ISO and HCI smoking methods. This study will contribute to Health Canada's understanding of cigarette smoke emissions and how they have changed over time. This data will be made publically available, consistent with the WHO Framework Convention on Tobacco Control, providing access to public health researchers for further evaluation.

84. ALTERED LUNG BARRIER FUNCTION IS A PHYSIOLOGICALLY-RELEVANT BIOMARKER OF POTENTIAL HARM. Patrudu MAKENA, Sarah Baxter-Wright, Peter Chen and G. L. Prasad; RAI Services Company, Winston-Salem, NC USA

Cigarette smoking is a major risk factor for diseases including cardiovascular disease, lung cancer, and COPD. One of the key functions of lung epithelial cells is to serve as a physical barrier against insults including cigarette smoke. Chronic smoking induces a state of chronic inflammation and oxidative stress, resulting in epithelial barrier disruption, which leads to increased lung permeability and culminates in tissue damage and remodeling, contributing to smoking-induced lung diseases. We evaluated lung barrier function in a single-center, ambulatory, clinical study. Lung permeability, measured as the half-life (T1/2) of inhaled 99mTC-DTPA, was assessed in seventeen subjects consisting of six Smokers (SMK), five Moist Snuff Consumers (MSC), and six Non-Tobacco Consumers (NTC). Half time clearance of 99mTC-DTPA from the lungs was measured at baseline, and approximately 7 and 14 days later. Right lung images were captured with a gamma-camera after inhalation of 99mTC-DTPA. Smokers, relative to MSC and NTC, exhibited significantly faster clearance of the inhaled probe (shorter T1/2), indicating increased lung permeability. NTC and MSC had similar clearance times of the probe (longer T1/2), suggesting that moist snuff use does not perturb lung permeability. The altered lung barrier function in smokers is a contributing factor to smoking-related diseases such as lung cancer and COPD. Altered lung barrier function may serve as a biomarker of potential harm related to tobacco use.

86. DETERMINATION OF TOBACCO ALKALOIDS IN CONSUMER PRODUCTS BY UPLC-MS/MS USING A MODIFIED QUECHERS METHOD. John R. SHIFFLETT, James B. Wittenberg and Dawit Z. Bezabeh; Alcohol and Tobacco Tax and Trade Bureau, Beltsville, MD USA

The U.S. Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for collecting Federal excise taxes on tobacco products. Tobacco products in the U.S. may fall into several taxable categories including cigars, cigarettes, snuff, chewing tobacco, pipe tobacco and roll-your-own. The existence of these taxable categories means that the TTB is also responsible for the determination of proper tax classification. Since a product must contain tobacco to be subject to the Federal excise tax, laboratory methods that test for phytochemicals suggestive of the presence of tobacco can provide useful information to determine the taxable status of a product.

An analytical method has been developed for the simultaneous quantitative determination of nicotine and the minor alkaloids cotinine, nornicotine, anatabine, anabasine, and isonicoteine in tobacco leaf and product fill material. This method uses QuEChERS for sample extraction and ultra-performance liquid chromatography with electrospray ionization – tandem mass spectrometric detection (UPLC-ESI-MS/MS) for analysis of the extracts. QuEChERS is an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe sample preparation and is a technique that is frequently used in the analysis of pesticide residues from consumer products. Results demonstrate the utility of this method for the analysis of a diverse set of consumer products including filler from cigarettes and cigars as well as pipe, roll-your-own, smokeless, and shisha tobaccos.

87. ASSESSMENT OF *IN VITRO* TOXICITIES DEMONSTRATED BY TOTAL PARTICULATE MATTER (TPM) AND GAS VAPOR PHASE (GVP) SAMPLES GENERATED FROM TOBACCO HEATING PRODUCTS (THPS) COMPARED WITH A COMBUSTIBLE CIGARETTE. Thomas J. SHUTSKY, Casandra K. West, Kristen G. Jordan and Christopher S. Junker; RAI Services Company, Winston-Salem, NC USA

Eclipse is a brand family of tobacco heating products (THPs). Primarily heating rather than burning tobacco reduces combustion-related toxicant emissions compared to conventional cigarettes. *In vitro* test batteries have commonly been used to support the toxicological evaluation of chemical and complex mixtures, including cigarette smoke. The series of studies presented here assessed *in vitro* toxicities of mainstream total particulate matter (TPM) and mainstream gas vapor phase (GVP) test samples generated from two Eclipse THPs and a marketed combustible cigarette. The THP test items (Eclipse and Eclipse Menthol) and the combustible cigarette (Newport 100 Box Menthol) were puffed according to Health Canada Intense (HCI) regimen parameters (Health Canada official method T-115). Samples were tested using the following *in vitro* toxicology assays following Health Canada official methods: Ames bacterial mutagenicity (T-501), Neutral Red Uptake (NRU) cytotoxicity (T-502), and *in vitro* Micronucleus genotoxicity (T-503). TPM and GVP generated from both the combustible and THP cigarettes were positive in the Ames, with revertant induction (mutagenicity) greater for the combustible cigarette TPM than either THP; however, there was no difference in the GVP results. In the NRU assay, the mean log (IC50) was significantly lower for the combustible cigarette TPM and GVP compared to the THPs, indicating higher cytotoxicity with the combustible product.

Results from the micronucleus assay demonstrated higher genotoxicity for the combustible cigarette TPM and GVP test items when compared to the THPs. Notably, there were no significant differences in response between the two Eclipse THP test samples in any of the assays. Collectively, these results demonstrate that the Eclipse THPs (Menthol and Non-menthol) are less mutagenic, cytotoxic and genotoxic when assessed alongside a commercial combustible cigarette.

88. HOW DID WE MOVE FROM SMOKING TOPOGRAPHY TO VAPING TOPOGRAPHY? Elise POULIN-DELORME, Kevin Menager and Florian Lozano; SODIM, Saint Jean de Braye, France

The principle of measurement of the Smoking Puff Analyser – Manual (SPA-M) device is based on Bernoulli's Law applied through the thin layer coupled with the Perfect Gas Law. The issue with e-cigarettes is that the flow that comes through the hole is smaller than the diaphragm. That is why it is not possible to detect the difference of pressure (ΔP) through the cigarette holder. The easiest solution proposed was to "disturb" the flow in order to get a deflected flow and a non-zero ΔP between entry and exit of the cigarette holder's insert.

The aim of this study is to validate an adapter, which ensures that the vaping topography is possible with a SPA-M device.

Three different models of obstacles were designed and manufactured to perform validation tests (a simple metallic bar, a holes ring and a ball).

The first test consisted in checking the volume of the puff to three different levels (42 ml, 55 ml and 80 ml) and to analyze the results of each obstacle in comparison with a soap bubble volumeter. The second test was to measure the pressure drop of e-cigarettes and to compare the results obtained by three adapters.

For the first test, no significant volume difference has been recorded, even if for bigger volumes the holes ring adapter caused a slight decrease in volume. For the second test, the holes ring obstacle generated a significant difference, in contrast to the simple metallic bar and a ball, where the results were unchanged and remained in the acceptable tolerance range.

A special adapter design for e-cigarettes including a simple metallic bar allows SPA-M device to be used for vaping topography.

89. DETERMINATION OF NICOTINE, TOBACCO-SPECIFIC NITROSAMINES, AND POLYCYCLIC AROMATIC HYDROCARBONS IN CANDIDATE REFERENCE MATERIAL 8112 TOBACCO SMOKE CONDENSATE SOLUTION. Walter B. WILSON and Lane C. Sander; National Institute of Standards and Technology, Gaithersburg, MD USA

The National Institute of Standards and Technology (NIST) has recently developed a new low-nicotine and tobacco-specific nitrosamines (TSNAs) cigarette tobacco filler Reference Material (SRM 3222) to help analytical testing laboratories evaluate current and new analytical methods. Recently, NIST efforts have focused on the development of

a new candidate Reference Material (RM) 8112 (Tobacco Smoke Condensate Solution). Analytical laboratories continuously measure targeted analytes in their cigarette smoke. For quality control, reference cigarettes from the University of Kentucky Center of Tobacco Reference Products are analyzed congruently with unknown samples under specific ISO or HCI smoking regimes. The certificate of analysis (COA) for the reference cigarettes include certified values and associated uncertainties allowing the analyst to assess the performance of their analytical procedure. If the values do not agree within the measurement uncertainties, the analyst must determine the source of the disagreement. Candidate RM 8112 is under development for use as a cigarette smoke condensate control material. Because analyte levels are associated to the solution material and not a specific tobacco or smoking regime, measurement issues can be isolated to either the analytical method or smoking machine. Additionally, the new reference material will have the capability to easily be adopted for method development studies. RM 8112 will include smoke condensate samples including two ampoules of a low-nicotine and two ampoules of a high-nicotine smoke condensate solution. The condensate solutions have been characterized for nicotine, TSNAs, and polycyclic aromatic hydrocarbons by either LC-MS/MS and/or GC-MS. The concentration levels are approximately two orders of magnitude lower for nicotine, which allows for analytical laboratories to have a RM for current cigarette products (high-nicotine) and potential future low-nicotine cigarettes.

90. DIFFERENTIAL REGULATION OF ION CHANNEL FUNCTION FROM EXPOSURES TO CIGARETTE SMOKE AND ENDS PREPARATIONS. Rachael E. RAYNER[1], Patrudu Makena[2], G. L. Prasad[2] and Estelle Cormet-Boyaka[1]; [1]The Ohio State University, Columbus, OH USA and [2]RAI Services Company, Winston-Salem, NC USA

Cigarette smoking is known to disrupt the normal mucociliary function of the lungs, whereas the effect of Electronic Nicotine Delivery Systems (ENDS) is incompletely understood. This study aimed to compare the effects of acute exposure of primary normal human bronchial epithelial (NHBE) 3D cultures at air-liquid interface to combustible cigarette and ENDS preparations on mucociliary function including ion channel function, ciliary beat frequency (CBF) and airway surface liquid (ASL) height. Differentiated NHBE cultures were exposed to whole smoke conditioned media (WS-CM) or total particulate matter (TPM) prepared from 3R4F reference cigarettes, whole aerosol conditioned media (ACM) or e-TPM generated from a marketed ENDS product, or nicotine alone. We found that a dose of 7μg/ml equi-nicotine units of cigarette TPM and WS-CM significantly decreased Cystic Fibrosis Transmembrane conductance Regulator (CFTR) and the epithelial sodium channel (ENaC) function which regulate fluid homeostasis in the lung. Conversely, higher (56μg/mL) equi-nicotine units of ENDS preparations or nicotine alone had no effect on CFTR and ENaC function. Despite a significant decrease in ion channel function, cigarette smoke preparations did not alter CBF and ASL. Similarly, ENDS preparations and nicotine alone had no effect on ASL and CBF. This study demonstrates that acute exposures of cigarette smoke preparations exert a notable inhibitory effect on the CFTR and ENaC function compared to ENDS preparations. In summary, the functional assays described herein are useful for tobacco product evaluations.

91. TOBACCO-SPECIFIC NITROSAMINES IN THE MAINSTREAM SMOKE OF COMMERCIAL LITTLE CIGARS. Selvin H. EDWARDS[1], Matthew Hassink[1], Kenneth M. Taylor[1], Cliff Watson[2], Peter Kuklenyik[2], Ruth Wang[2], Patrick Chen[2], Liza Valentin-Blasini[2] and Brett Kimbrell[2]; [1]Food and Drug Administration, Silver Spring, MD USA and [2]Centers for Disease Control and Prevention, Atlanta, GA USA

Cigars are among the broad variety of deemed tobacco products that have been less extensively studied and characterized than cigarettes. Small sheet-wrapped cigars, often called little cigars, are a subcategory of cigars that are similar to conventional cigarettes but have been previously determined to have higher tobacco-specific nitrosamine (TSNA) levels in the whole product by comparison. To understand the smoke delivery of TSNAs by little cigars, the mainstream smoke of 60 commercial little cigar products was measured for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) according to the International Organization of Standardization (ISO) and Canadian Intense (CI) smoking regimens using a validated LC/MS/MS method. NNK and NNN by ISO smoking regimen ranged from 89 – 879 ng/cigar and 201 -1540 ng/cigar, respectively; by the CI regimen, NNK and NNN ranged from 138 – 1571 ng/cigar and 445 – 2780 ng/cigar, respectively. The average transfer ratio for NNK and NNN from tobacco filler to mainstream smoke is 24% and 36% by the ISO and CI smoking regimens, respectively. By the ISO smoking regimen, mainstream smoke NNK and NNN yields exhibit a moderate correlation ($R2 = 0.60 – 0.68$; $p < 0.0001$) with tobacco filler NNK and NNN. The mainstream smoke NNK and NNN yields of little cigars were also determined to be 3- to 5-fold higher compared to previously tested commercial cigarettes. The mainstream smoke NNK and NNN yields have wide variation among commercial little cigar products and suggest that, despite their similarities to cigarettes, little cigars deliver more of these carcinogenic TSNAs.

92. WITHIN BRAND AND ACROSS BRAND CONTENT AND VARIABILITY OF NICOTINE AND TOBACCO-SPECIFIC NITROSAMINES IN 8 COMMERCIAL US CIGAR BRANDS. Jacob P. HILLDRUP, I. Gene Gillman and Katilyn N. L. Brooks; Enthalpy Analytical, Henrico, VA USA

The recent 2016 deeming of cigars by the US Food and Drug Administration (FDA) has led to an increased interest in cigar science, including ways to accurately measure the harmful and potentially harmful constituents (HPHCs) found within cigar tobacco. However, there are few published studies on HPHCs in cigar filler and even less is known about the cigar to cigar variability of HPHCs in cigar filler tobacco. In this study we attempted to quantify the variability of nicotine and TSNAs (NNN, NNK, NAT, NAB) in cigar filler on a per cigar basis, from both machine-made and premium cigars. Eight total brands were analyzed.

Cigars were individually conditioned, weighed, and ground. Tobacco from each cigar was analyzed in triplicate for nicotine and NNN, NNK, NAT, and NAB content (by GC-FID and LC-MS/MS, respectively). Analytical results were reported on a per gram basis and corrected to a per cigar basis using the original cigar weight.

On a per gram basis, the level of nicotine present in the products ranged from 0.75 to 1.80% with an average variability of 9.5% for the analysis of 10 cigars per brand. The amount

of nicotine per cigar ranged from 17.9 to 232.6 mg with an average variability of 12.3%. Premium (long-leaf) cigars showed the highest amount of nicotine variability at 20.2%.

On a per gram basis, the total amount of TSNA present in the products ranged from 7.8 to 55.9 μg/g with an average per brand variability of 23.7%, for the analysis of 10 cigars per brand. The amount of total TSNA per cigar ranged from 18.4 to 585.5 μg with an average per brand variability of 24.2%. Individual TSNA variation ranged from 14.4 to 61.3% across all brands tested.

93. CIGAR USAGE PATTERNS AMONG ADULT TOBACCO USERS: RESULTS OF A LARGE, NATIONALLY REPRESENTATIVE SURVEY. Susan MORRIS; Altria Client Services, Richmond, VA USA

We present results of a large, cross-sectional survey showing detailed information about usage behavior among self-reported cigarillo users who are legal-age to purchase tobacco. Furthering previous work, such as PATH, we designed the Cigar Landscape Study (CLS) to improve survey capture of cigarillo usage among a nationally representative, probability-based web panel. We enrolled an online sample of adult tobacco users (ATUs; n =12,516) to assess first tobacco product ever used, consumption on days used and time to first cigarillo among adult cigarillo users (ACU; n= 3,362). In line with other studies and government data sources, patterns of use for cigarillo consumers vary significantly from those of cigarette consumers. Overall, the majority (72%) of ACU "Ever Users" smoked less than 20 lifetime cigarillos. These data suggest a high degree of trial without repeated use within the cigarillo category. Additionally, among ACU who have smoked cigarillos every day or somedays in the past 30 days, 11% smoked just one or two puffs on days smoked while another 23% smoked less than half a cigarillo. These numbers diverge from average daily cigarette consumption estimated by CDC (14.1 cigarettes per day). ACUs that use cigarillos exclusively every day or somedays have an average time to first cigarillo of 467 minutes (relative to 96 minutes among current adult cigarette smokers in PATH Wave 3), suggesting very low dependence on cigarillos. Additionally, of the ATUs that ever used cigarillos, only 5% reported that cigarillos were the first product they ever used. Patterns of cigarillo use vary considerably to those reported for cigarettes, with cigarillo consumption generally being far fewer and more intermittent than cigarette use.

97. METHODOLOGY TO DETERMINE THE PARTICLE SIZE DISTRIBUTION, MEAN, AND STANDARD DEVIATION FROM SIEVE DATA. F. Kelley ST. CHARLES[1] and Walter T. Morgan[2]; [1]St. Charles Consultancy, Lewisville, NC USA and [2]RAI Services Company, Winston-Salem, NC USA

A method is described which allows the mean and standard deviation of particle size to be estimated from sieve data. The results can be used to calculate and plot the particle size distribution, the cumulative distribution, percentiles, and guide sieve selection for optimal separation of similar samples. Both normal and log-normal distributions are described. The methodology is demonstrated using oral tobacco products, but it can also be applied to any similar distribution where the data are compartmentalized into ranges (*e.g.* histograms) or truncated with an undetermined upper limit (*e.g.* measurements are stopped at some upper limit) such as soil samples or pack seals. The root-mean-square difference between calculated and measured sieve results for the percent (%) of product on a given sieve is similar to the standard deviation of multiple sieving replicates of the same sample. This gives confidence that the mean and standard deviation calculated by this technique is sufficiently correct to characterize the product accurately. Chi-square goodness of fit tests demonstrate the observed distributions are consistent with the estimated distributions.

98. *IN VITRO* DISSOLUTION TESTING OF NICOTINE RELEASE FROM SMOKELESS TOBACCO PRODUCTS. Fadi ALDEEK, John H. Miller IV, Tim L. Danielson, Yezdi B. Pithawalla, Celeste T. Wilkinson, Anthony P. Brown and Karl A. Wagner; Altria Client Services, Richmond, VA USA

Developing dissolution testing methods to measure the nicotine release profiles from smokeless tobacco products is valuable for product assessment and for product-to-product comparisons. Furthermore, it can allow for understanding which physical or chemical parameters have an impact on nicotine release from smokeless tobacco products.

In this work, we developed a robust dissolution method to study the *in vitro* release of nicotine from smokeless tobacco products using the U.S. Pharmacopeia flow-through cell dissolution apparatus 4 (USP-4). We further developed and validated a sensitive UPLC-PDA method for the accurate quantitation of the released nicotine into artificial saliva, which is our selected dissolution medium. We have successfully shown the applicability of the validated method by investigating the release profiles of nicotine from various commercial and CORESTA reference smokeless tobacco products [CRP 1.1 (Swedish style snus pouch), CRP 2.1 (American style loose moist snuff), CRP 4 (loose-leaf chewing tobacco) and CRP 4.1 (chopped loose-leaf chewing tobacco)].

Nicotine release profiles were analyzed by calculating the difference factor (f1) and similarity factor (f2) by adopting methodology referenced in Guidance for Industry from FDA's Center for Drug Evaluation and Research (CDER) and also by fitting the release profile curves to first order kinetics models. Nicotine release was found to be dependent on the form and cut of the smokeless tobacco products, with a slower release observed for Swedish snus and loose leaf, compared to chopped and loose smokeless tobacco. This dissolution methodology can be extended to measure and compare release of other constituents from smokeless tobacco and novel oral tobacco products, and has the potential for method standardization.

99. EXTRACTION OF BAP IN WATER FROM MOIST SNUFF. Serban C. MOLDOVEANU and Andrew Harrison; R.J. Reynolds Tobacco, Winston-Salem, NC USA

Moist snuff contains traces of several polycyclic aromatic hydrocarbons including benzo[a] pyrene (BaP). The transfer of BaP from moist snuff to a human user, is likely to involve an extraction process from the snuff matrix into saliva. A previous *in vitro* study was performed to evaluate the extraction of BaP into water or into artificial saliva and reported at 72nd TSRC. It was found that around 100% of the initial BaP remains in the moist snuff matrix when this extraction is performed. As a result, only a very small amount of BaP is likely to be extracted, but the precise level of BaP in the water (or saliva) was not measured. Present study continued the effort to understand the extraction of BaP from moist snuff by measuring the BaP levels in the water extract. Nine types of moist snuff were evaluated for the extraction with water. The moist snuff samples were commercially available and were purchased from the market. They included moist snuff of multiple widths and flavors. The extraction was performed from 2 g of moist snuff (as is) with 100 mL water at 37°C, using continuous mild agitation for 30 min. To the extract were added 20 uL of a solution of internal standard containing 4 ug/mL indeno[1,2,3-cd]pyrene and the solution was filtered. 5 mL saturated solution of NaCl were added to the filtrate which was further extracted with 5 mL cyclohexane. The cyclohexane was evaporated under a stream of air, and the residual was dissolved in 1 mL methanol. The methanol solution was analyzed by HPLC with fluorescence detection. The results indicate very low levels of BaP in the water solution.

100. EVALUATION OF CONSTITUENTS RELEASED FROM SMOKELESS TOBACCO PRODUCTS TO HUMAN SALIVA. Siqi GUAN, Huihua Ji and Lowell Bush; University of Kentucky, Lexington, KY USA

Nine harmful and potentially harmful constituents (HPHCs) are listed in the US Food and Drug Administration (FDA) abbreviated HPHCs list for smokeless tobacco products, including nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosonornicotine (NNN), and benzo[a]pyrene (B[a]P). Nicotine is primarily metabolized to cotinine in humans. Releasing these HPHCs from smokeless tobacco products to saliva is important for understanding how these compounds are transferred to humans. The certified reference smokeless tobacco products, including loose leaf (3S1), snus (1S5), Swedish style snus (1S4), and moist snuff (3S3), were used to evaluate these compounds released into human saliva. Smokeless products were produced in 2016 by the Center for Tobacco Reference Products (CTRP) of the University of Kentucky under a Cooperative Agreement with the FDA. The objective of this study was to evaluate the amount of NNN, NNK, nicotine, cotinine, and B[a]P released from smokeless tobacco reference products

into human saliva *in vitro* with increasing incubation time at 37°C. The incubation time ranged from 1 min to 1 hour and shaking intensity was set at 80 tilts per minute and +/- 20 degrees. B[a]P released from 3S1, 1S5, and 1S4 was below LOQ (0.5 ng/mL). In contrast, B[a]P was released from 3S3 into human saliva from 2% at 1 min to 3.5% at 1 hour. 20~50% of NNN, NNK, nicotine, and cotinine was released from 3S1, 1S4, and 1S5 at 1 minute, which was dependent on constituents and product type, then significantly increased over an hour. NNN, NNK, nicotine, and cotinine initially were released very quickly from 3S3 (50-65% released at 1 minute) and then slightly increased over an hour.

101. CHARACTERIZATION OF THE BACTERIAL COMMUNITY ASSOCIATED WITH SMOKELESS TOBACCO REFERENCE PRODUCTS UNDER DIFFERENT STORAGE CONDITIONS. Shuang LIU, Isaac Greenhut and Luke Moe; University of Kentucky, Lexington, KY USA

In this study we characterize the bacterial communities associated with smokeless tobacco reference products (STRP), including loose leaf chewing tobacco (3S1), moist snuff (3S3), American snus (1S5), and Swedish snus (1S4) during a 1-year storage period. All products were stored under three temperatures: frozen (FR -20°C), cold room (CR 4°C), and room temperature (RT 21°C), and were sampled seven times (initial, 1-month, 2-month, 3-month, 6-month, 9-month, and 12-month). We performed culture-dependent colony counting to assess the bacterial and fungal load and used Illumina Miseq sequencing of 16S rRNA gene V4 region to profile the bacterial community structures of our tobacco samples. The results showed that bacteria, not fungi, were the dominant microbes and the bacterial loads were different among products: 3S3 > 3S1 > 1S5 > 1S4. The culturable populations significantly decreased at the 12-month sampling point for all products. Diversity and composition of the bacterial communities shifted under different sampling time and different storage condition. The most abundant phyla were Firmicutes and Proteobacteria for all products and the top 10 operational taxonomic units (OTUs) of 3S1 and 3S3 were the same. The top 10 OTUs for snus samples were different within 1S4 and 1S5, as well as between snus samples and moist snuff and loose leaf. Our results will be correlated with physiochemical parameters (*e.g.*, moisture, pH, nicotine, and tobacco-specific nitrosamines).

102. NOVEL APPLICATION OF DIFFERENTIAL ION MOBILITY SPECTROMETRY-TANDEM MASS SPECTROMETRY FOR IMPROVED ASSAY SELECTIVITY AND SENSITIVITY IN THE QUANTITATIVE DETERMINATION OF TOTAL NNN, TOTAL NNAL AND 2- / 3-HPMA IN HUMAN URINE. Jeff PLOMLEY; Altasciences, Laval, Canada

Differential Ion Mobility Spectrometry (DMS) has recently emerged as an orthogonal gas-phase ion separation technique which, when interfaced between liquid chromatography (LC) and Tandem Mass Spectrometry (MS/MS), promises improvements in both assay selectivity and sensitivity by differentiating analyte from interference based upon physical cross-section. In the current research, we have established the potential for DMS technology to address existing LC-MS/MS assay limitations associated with the quantitation of the urinary biomarkers 2-/3-Hydroxypropylmercapturic acid (HPMA), total N-nitrosonornicotine (NNN), and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).

Leveraging a SCIEX TripleQuad 6500+ with SelexION differentail mobility separation device in an LC-DMS-MS/MS workflow, total NNN method detection limits could be lowered tenfold from those previously reported in the literature, to 0.20 pg/mL. This represents a relevant improvement in sensitivity since 25-30% of smoker urine contains baseline NNN concentrations < 2.00 pg/mL. Achieving a 0.20 pg/mL detection limit required only a fivefold concentration of extract, accomplished without concomitant matrix effect or interference. Further, the additional selectivity offered by the LC-DMS-MS/MS approach suggested that previous reports of augmented NNN response in urine left at room temperature were due to a chromatographically unresolved isobaric interference, which proved separable from NNN when leveraging ion mobility.

For total NNAL, a fivefold improvement in signal-to-noise ratio (SNR) using LC-DMS-MS/MS allowed extracts to be reconstituted without concentration, resulting in an absence of ionization suppression and increased assay robustness for a 5.0 pg/mL detection limit (SNR 40:1). In the determination of 2-/3-HPMA, the sensitivity for 2-HPMA by LC-DMS-MS/MS was increased twofold compared to LC-MS/MS, whilst an interference at the retention time of 3-HPMA could be eliminated by ion mobility, reducing complex and prolonged sample preparation and chromatographic separation.

Each of the three LC-DMS-MS/MS methods was successfully validated according to the criteria established by the U.S. FDA in the 2018 Bioanalytical Method Validation Guidance document for small molecule quantitation.

103. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 1: SELECTION OF REPRESENTATIVE FLAVOR MIXTURES FOR TOXICOLOGICAL EVALUATIONS USING A STRUCTURAL GROUPING APPROACH. Kimberly D. EHMAN[1], Timothy B. Langston[1], Ashutosh Kumar[1], Monica Lee[1], Davide Sciuscio[2], Patrick Vanscheeuwijck[2] and Julia Hoeng[2]; [1]Altria Client Services, Richmond, VA USA and [2]PMI R&D, Neuchâtel, Switzerland

A variety of flavor ingredients are used in potentially reduced-risk tobacco or nicotine products, including e-vapor products. For this work, the ingredients were initially evaluated for quality and purity, which included assessment of food grade and GRAS (Generally Recognized as Safe) status, followed by a comprehensive review of the available toxicological data. Considering the number of available flavors and the numerous potential flavor combinations, toxicity testing of each individual compound or formulation may not always be feasible. This presentation outlines a pragmatic approach to selecting representative compounds and flavor mixtures that reflect a range of more than 200 commonly used flavors (*e.g.*, a flavor "toolbox"). To develop a representative mixture, each individual flavor in our toolbox was allocated to one of 34 structural groups defined in the European Commission (EC) Regulation No 1565/2000 (European Commission, 2000), and broader structural groups were further subdivided to better reflect the range of structural differences. Flavors within a given structural group are expected to exhibit similar metabolic and biological properties. Following this approach, 38 groups were defined, encompassing 27 of the original 34 EC groups. We then ranked each flavor within a group based on a toxicological evaluation (literature, in silico predictive analysis, and internal data). Lastly, using an objective computational procedure and scoring system, we selected representative flavors (*i.e.*, predicted worst-case based on the toxicological profile) from each structural

group, which were combined to create a full "toolbox" flavor mixture. The final flavor mixture created by this approach could be tested in preclinical (*in vitro* and *in vivo*) toxicity studies, in part, to support the range of flavors in a flavor toolbox.

104. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 2: PREPARATION AND STABILITY CHARACTERIZATION OF REPRESENTATIVE FLAVOR MIXTURES. Cameron R. SMITH[1], John H. Miller IV[1], Niti Shah[1], Ashutosh Kumar[1], Monica Lee[1], Felix Frauendorfer[2], Philippe Guy[2], Pierrick Diana[2] and Anneke Glabasnia[2]; [1]Altria Client Services, Richmond, VA USA and [2]PMI R&D, Neuchâtel, Switzerland

GLP guidelines require the characterization of test articles for preclinical biological studies to ensure the identity and composition are consistent from batch to batch and throughout the testing period. For e-vapor products, this would suggest that the analysis of individual ingredients, including various flavors and carriers, be conducted each time a formulation is made. In this study, we explored a unique approach to create concentrated mixtures ("pre-blends") prior to making a final formulation (containing 38 flavors) to maximize stability and simplify the preparation procedure. The 38 flavor compounds were sub-divided into five (5) groups based on structural moiety, solubility and chemical reactivity (*i.e.*, unreactive, electrophilic, nucleophilic, basic, acidic). These groups were used to make a total of 6 pre-blends with their long-term stability confirmed up to 4 weeks. At the same time, the pre-blends were mixed to make two types of final formulations containing all 38 flavor compounds, with and without nicotine. The final formulation was stable up to 3 days in the presence of nicotine and 10 days without nicotine. Our work demonstrates the benefit of creating stable and concentrated pre-blends to avoid daily formulation making thus reducing the frequency of batch characterization especially for repeated long-term dosing studies.

105. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 3: *IN VITRO* CYTOTOXICITY AND GENOTOXICITY OF REPRESENTATIVE FLAVOR MIXTURES. Utkarsh B. DOSHI, Jingjie Zhang, Ashutosh Kumar and K. Monica Lee; Altria Client Services, Richmond, VA USA

Flavor compounds "generally recognized as safe" for oral consumption are commonly used in inhalable e-vapor products for which insufficient safety data exists. The hazard characterization of each flavor and various flavor mixtures is resource and time-demanding. Here we explored a pragmatic approach, where we selected representative flavors and tested for *in vitro* cytotoxicity and genotoxicity. From a group of commonly used individual flavors (> 200) in e-vapor products, 38 flavors were selected using structural grouping and available toxicological data. These flavors were mixed in a carrier (PG/VG/water) to prepare a test mixture (prototype flavor mixture with up to 18% flavor load), with and without nicotine, and were subjected to a standard CORESTA battery of *in vitro* cytotoxicity (Neutral Red Uptake [NRU]) and genotoxicity (Ames and micronucleus [MN]) assays. Test mixtures (with and without nicotine) were negative in the Ames mutagenicity assay but showed cytotoxicity in all three assays including NRU assay. In the MN genotoxicity assay, the test mixture with nicotine was negative but the test mixture without nicotine provided equivocal results. To further identify the potentially responsible flavor(s) for the cytotoxicity response in the NRU assay of the test mixtures, we divided the 38 flavors

into 5 subgroup mixtures according to their solubility and chemical reactivity, and tested them using NRU cytotoxicity assay. Results suggested that subgroup mixtures containing certain flavors – for example, ethyl maltol, furaneol and isopulegol – were more cytotoxic, consistent to literature findings as *in vitro* cytotoxicant/irritant. The results align with the overall systematic toxicity evaluation approach, deconstructing mixtures into subsets of flavors, ultimately in support of flavor read-across assessment.

106. PRECLINICAL TESTING OF FLAVORS IN E-VAPOR PRODUCTS, PART 4: FLAVOR TRANSFER FROM THE LIQUID TO THE AEROSOL FOR INHALATION EXPOSURE. Jingjie ZHANG, Cameron Smith, Chase Anderson, Nicholas McCutcheon, John Miller and K. Monica Lee; Altria Client Services, Richmond, VA USA

Many flavor compounds used in e-liquids are generally recognized as safe (GRAS) for oral consumption. However, the respiratory effects of flavors in e-vapor aerosols are unknown. Preclinical inhalation studies can provide toxicity data to assess the inhalation risk of flavors in e-vapor aerosols. The purpose of this study is to generate and characterize the aerosols from e-liquids formulated with selected flavor mixtures as well as confirm the flavor transfers from the e-liquid to the aerosol. The flavor mixtures containing 38 flavor ingredients, with and without nicotine, in a carrier matrix of propylene glycol (PG), glycerin, and water were aerosolized by a capillary aerosol generator. The generated aerosol was collected with a Cambridge filter pad with a liquid impinger containing ethanol in series for flavor analysis. The aerosol mass was determined gravimetrically. 22 out of 38 flavors were analyzed with a GC/MS method. In addition, the major components of the carrier matrix (PG and glycerin), nicotine, selected carbonyls, pH, as well as the particle size distribution of the aerosol were measured. Results showed that 1) all the 22 monitored flavors were found in the aerosol for both formulations with or without nicotine; 2) PG, glycerin, and nicotine content, as well as pH of the aerosol were consistent with those of the e-liquid; 3) levels of selected carbonyls were low and comparable to the ranges reported on e-vapor aerosols in literatures; 4) mass median aerodynamic diameter (MMAD) of the aerosol was around 1 μm with the geometric standard deviation (GSD) < 2 for both formulations. This study provides a preliminary characterization of the e-vapor aerosol and demonstrated the transfer of flavors from the e-liquid to the aerosol.

107. PREDICTED IMPACTS OF E-CIGARETTES ON US MORTALITY AND HEALTH CARE COSTS. Bill POLAND[1] and Sylvain Larroque[2]; [1]Certara USA, Menlo Park, CA USA and [2]JT International, Geneva, Switzerland

Introduction
The US FDA seeks to ensure that new tobacco products are appropriate for the protectionof the public health and recommends assessment of tobacco-related morbidity as well as mortality. A simulation model that predicts the effect of e-cigarettes on tobacco use and mortality was extended to approximate effects on morbidity, as measured by US health care costs.

Methods
The US adult population was simulated through year 2100, using randomly generated tobacco product use histories including initiation, cessation, and switching between products. Smokers and e-cigarette users also transitioned to and from dual use, which

affected average cigarettes smoked per day and cigarette quit rates. The model predicted premature deaths avoided by e-cigarettes. Although e-cigarettes may benefit smokers who switch completely to them, benefits are less certain for other groups. The net effect depends on uncertain transition rates and the Excess Relative Risk (ERR) experienced by e-cigarette users relative to cigarette smokers. Sensitivity to each input was tested systematically, and a hypothetical "break-even" ERR, which would reduce a product's net population benefit to zero in terms of avoided premature deaths, was calculated. Tobacco-attributable morbidity costs were assumed proportional to tobacco-attributable mortality and were scaled from recent US estimates.

Results
Simulations suggested that e-cigarettes would avoid 2.5 million premature deaths and an average of $15.6 billion/year health care costs in the US population through 2100 in a base scenario, and provide a net benefit in all except extreme scenarios. The base-case break-even ERR was over 50% relative to cigarettes.

Conclusions
These simulations show that e-cigarettes would very likely benefit the overall health and health care costs of US adults, despite uncertainties in ERR and other inputs.

108. ADULT CIGARETTE SMOKERS' EXPECTATIONS, PERCEPTIONS AND REACTIONS TO REDUCED NICOTINE CONTENT CIGARETTE PROTOTYPES.
Andrea VANSICKEL and Jan Angel; Altria Client Services, Richmond, VA USA

FDA announced the intention for a nicotine standard for cigarettes to make them "minimally addictive or nonaddictive." Published research has primarily gathered perceptions/behavior data in response to reduced nicotine content cigarettes (RNC) without providing participants with background information on the proposed RNC standard. This study provides a qualitative assessment of adult cigarette smoker (AS) responses to RNC prototypes at varying nicotine levels, achieved through tobacco blending. We conducted 29 in-context focus groups (5 to 12 AS of legal age to 64) with various cigarette nicotine contents (approximately 70 to 90% lower than typical marketed cigarettes). Each group started by discussing FDA's proposed RNC standard, participants then used the RNC prototype ad lib in a naturalistic setting for 45-60 minutes before discussing their experience and potential future behaviors if FDA implements the RNC standard. Findings indicate that some AS associate RNC with lower harm than normal nicotine cigarettes. During ad lib use, the majority of AS experienced sensory deficits, citing weak "airy" smoke and lack of strength/impact along with a lack of tobacco satisfaction. AS raised concerns that they might smoke more cigarettes and that, less experienced smokers may prefer the smoother, easier to smoke aspects of RNC. Common potential future behaviors if the FDA enacted the RNC standard included purchasing the most sensorially acceptable cigarette available, switching to higher tar cigarettes, considering illicit cigarette sources, and trying to quit/cut down while using other tobacco products to replace their cigarettes. Results from this study consistently indicate a breadth of potential unintended consequences associated with a RNC standard.

109. FUNCTIONAL FILTER PLUG WRAP PAPER FOR THE CONTROL OF THE THERMAL ENERGY OF THE AEROSOL FROM HEATED TOBACCO PRODUCTS. Michael LINDNER[1] and Nadine Leichsenring[2]; [1]TANNPAPIER, Traun Austria and [2]Hauni Maschinenbau, Hamburg Germany

Next generation products, which include all kinds of e-vapor items as well as heated tobacco products (HTPs or "heat sticks"), have successfully entered the tobacco market in addition to conventionally combustible cigarettes. From the basic geometrical concept, HTPs resemble traditional filter cigarettes with various formats, and therefore, heat sticks require filter wrapping materials. Filter wrapping materials are thin and flexible sheets made of paper or other web substrates in order to enable specific quality and technical properties. In general, filter wrapping materials can be separated into Tipping Paper and filter plug wrap paper. The purpose of the present study is to focus on filter plug wrap paper ("plug wrap paper") and to describe individual technical functionalities of the same. Hereby, the application of a physically active substance on the plug wrap paper will be demonstrated to lower the temperature of the aerosol generated by HTPs. As some heat stick versions are geometrically distinctively shorter than combustibles, the cooling rate of the aerosol is comparably low yielding high remaining temperature of the inhaled evaporation and distillation result. Selective surface treatment of the plug wrap paper trough coating generates enhanced thermal absorptivity, and its efficiency will be quantitatively investigated under pilot plant test conditions as well as evaluated with heat absorption calculations and phase transition aspects. Moreover, mechanical embossing of the plug wrap paper is subjected to increase the effective area of the coated surface thus providing a stronger contribution to the temperature reduction.

110. WHAT IS ACID ABOUT ACID CIGARS? John H. LAUTERBACH; Lauterbach & Associates, Macon, GA USA

The so-called ACID cigars are reportedly popular among cigar smokers who want different smoking experience. The cigars are not acid as the pH-values of slurries of the filler, binder, and wrapper of two brand-styles of ACID cigars (Blondie and Kuba Kuba, both from Drew Estates) were not atypical of such values reported in the literature for cigar tobaccos. According to one of the Drew Estates web pages, the ACID came from the nickname of a person associated with the business. Samples of each brand-style were dissected into wrapper, binder, and filler portions for the main part of the cigar. The wrapper on the mouth end was a separate piece of tobacco and some have reported it to have a sweet taste. It was keep separate from the remainder of the fractions as was the tobacco underneath that wrapper. Each fraction was placed in a glass jar. The initial aroma of the cigar when it was removed from the wrapper was similar to a man's cologne, the aroma rapidly dissipated from the different fractions once they were removed from the entire cigar. Each fraction was first extracted with hexane. The hexane extracted tobacco was further extracted with acetone, and then with 95% ethanol. The wrapper on the mouth end was extracted with water and the aqueous extract was analyzed by LC using a Cogent Amide column with RI detection. The organic extracts were analyzed on a Cogent Phenyl Hydride column using UV detection at 280 nm and 254 nm. As with other flavored cigars, the aroma is nice, but there is not much there besides constituents normally found in cigar tobaccos.

111. PREPARATION OF AMINO ACID IONIC LIQUIDS FOR REDUCING HCN AND CROTONALDEHYDE FROM SMOKE AND EVALUATION OF CIGARETTE APPLICATION. SUN Xuehui[1], Dong Lu[1], Wang Hongwei[2], Yang Song[1], Sun Peijian[1], Guo Ge[1], Pan Lining[1], Sun Zhitao[2], Zhang Xiaobing[1], Tian Haiying[2] and Nie Cong[1]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China and [2]Technology Center, China Tobacco Henan Industrial, Zhengzhou, China

To selectively remove HCN and crotonaldehyde from cigarette mainstream smoke, a series of tetrabutyl ammonium hydroxide-amino acid ionic liquids (TBAILs) were synthesized. The ionic liquids were characterized by FT-IR, NMR and TG. The results showed that TBAILs were successfully obtained and had good thermal stability below 150°C. The adsorption of HCN and crotonaldehyde from cigarette smoke was evaluated by a homemade device and the preparation conditions were optimized. The optimum amino acids of TBAILs are threonine, serine, asparagine and phenylalanine while the reaction time is 6 h. The tetrabutylammonium hydroxide-threonine ionic liquid was applied in cigarettes in the form of paper-cellulose acetate dual filter. The yields of HCN and crotonaldehyde in mainstream smoke were reduced by 50.8% and 27.7% respectively at 10-mg loading, and 81.4% and 39.9% respectively at 20-mg loading, compared with the reference cigarettes. The removal performance was stable in 3 months. It can be seen that TBAILs have remarkable adsorption effect on harmful components in cigarette smoke, and good industrial applicability with simple preparation and lower cost.

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