

PROGRAM BOOKLET AND ABSTRACTS

Volume 69

69th Tobacco Science Research Conference



September 20-23, 2015
Naples, Florida USA

Host:
ITG Brands

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NAPLES
MARCO ISLAND
EVERGLADES

FLORIDA'S PARADISE COAST

September 20, 2015

Welcome 69th Tobacco Science Research Conference...

... to Florida's Paradise Coast. On behalf of the Naples, Marco Island, Everglades Convention & Visitors Bureau (CVB), we warmly welcome you to our area.

We know you are here to learn and network during your conference, and there are several special activities planned during your stay. We hope you will also have an opportunity to explore the beauty and charm of our little slice of Paradise.

We encourage you to visit our restaurants and attractions, delight in our nightlife and historic sites, stroll our avenues, shop our boutiques and discover our unique arts and culture scene. We hope you will also have time to explore the pristine Gulf Coast Everglades ecosystem, with endless adventures awaiting you on foot, paddling a kayak, or exploring this pristine watery treasure by boat.

Most of all we invite you to come back and visit Florida's Paradise Coast again very soon.

Sincerely,



Jack Wert
Executive Director

Convention
& Visitors Bureau
2660 N. Horseshoe Dr. #105
Naples, FL 34104
P (239) 252-2384
F (239) 252-2404
ParadiseCoast.com
f TheParadiseCoast
t @paradisecoast



GENERAL PROGRAM

Sunday, September 20, 2015

2:00 pm – 6:00 pm	Registration.....	Royal Palm Foyer
2:00 pm – 6:00 pm	Speaker Ready Room.....	Acacia 1
6:30 pm – 9:30 pm	Welcome Reception.....	Sunset Veranda

Hosted by – ITG Brands

Monday, September 21, 2015

7:30 am – 8:30 am	Session Chairs Breakfast.....	Cypress
7:30 am – 8:30 am	U.S. TAG: ISO/TC 126 Breakfast	Hibiscus
7:30 am – 5:00 pm	Registration.....	Royal Palm Foyer
7:30 am – 5:30 pm	Speaker Ready Room.....	Acacia 1
8:00 am – 8:45 am	Morning Coffee	Royal Palm Foyer
8:45 am – 11:50 am	Symposium	Royal Palm Ballroom
	“The Scientific Basis of Harm Reduction and the Risk Continuum”	
	Chair: Clarissa Tatum, Eastman Chemical Company	
10:00 am – 10:30 am	Coffee Break.....	Royal Palm Foyer
11:50 pm – 1:00 pm	Lunch	Sunset Veranda
11:50 pm – 1:00 pm	Tobacco Science Council Meeting.....	Chill Out Lounge
1:00 pm – 2:20 pm	Poster Session	Orchid Foyer
2:20 pm – 5:30 pm	Session A: <i>Methods Development</i>	Royal Palm Ballroom
	Session B: <i>E-Cigarettes</i>	Royal Palm Ballroom
3:20 pm – 3:50 pm	Coffee Break.....	Royal Palm Foyer

Tuesday, September 22, 2015

7:30 am – 5:00 pm	Registration.....	Royal Palm Foyer
7:30 am – 5:00 pm	Speaker Ready Room.....	Acacia 1
7:30 am – 8:00 am	Morning Coffee	Royal Palm Foyer
8:00 am – 11:50 am	Session A: <i>Methods Development</i>	Royal Palm Ballroom
	Session B: <i>Regulations & Quality</i>	Royal Palm Ballroom
9:40 am – 10:10 am	Coffee Break.....	Royal Palm Foyer
11:50 am – 1:15 pm	Lunch	Sunset Veranda
12:00 pm – 1:15 pm	Policy Committee Lunch	Mangrove
1:15 pm – 2:20 pm	Poster Session	Orchid Foyer
2:20 pm – 5:10 pm	Session A: <i>Agronomy</i>	Royal Palm Ballroom
	Session B: <i>E-Cigarettes</i>	Royal Palm Ballroom
3:40 pm – 4:10 pm	Coffee Break.....	Royal Palm Foyer
5:15 pm – 6:00 pm	TSRC Business Meeting	Acacia 4-5
6:30 pm – 7:15 pm	Social Hour	Royal Palm Foyer
7:30 pm – 10:00 pm	Award Banquet	Royal Palm Ballroom

Wednesday, September 23, 2015

8:00 am – 8:30 am	Morning Coffee	Royal Palm Foyer
8:00 am – 11:20 am	Speaker Ready Room.....	Acacia 1
8:30 am – 11:20 am	Session A: <i>Agronomy</i>	Royal Palm Ballroom
	Session B: <i>Toxicology & Materials</i>	Royal Palm Ballroom
9:50 am – 10:20 am	Coffee Break.....	Royal Palm Foyer
11:20 am	Adjourn	

LIFETIME ACHIEVEMENT AWARD

Michael F. Borgerding



Michael F. (Mike) Borgerding, raised in Fairfax, Virginia, received degrees from Virginia Tech (B.S., Biochemistry; M.S., Chemistry) and Wake Forest University (Ph.D., Chemistry) during his academic training. In an undergraduate course taught by Dr. Harold McNair, he was first introduced to gas and liquid chromatography, and thereafter developed a keen interest in instrumental analysis techniques involving separation science. After completing his M.S. under the direction of Dr. McNair, Dr. Borgerding joined R.J. Reynolds Tobacco Company in 1980 as a junior research chemist, recognizing that complex mixtures, such as tobacco and tobacco smoke, provided an excellent opportunity to apply his training in chromatographic science.

Dr. Borgerding's initial work focused on the development of new qualitative separation techniques to evaluate tobacco and tobacco smoke composition, the development and validation of analytical methods for the quantitative measurements of selected smoke constituents (many now known as "HPHCs") and the design of a product evaluation strategy to characterize smoke chemistry from new technology cigarettes. Early in his career, Mike led team efforts to evaluate the mainstream and sidestream smoke from Premier, a cigarette that heated rather than burned tobacco, and Eclipse, a cigarette that primarily heats rather than burns tobacco. Results of those studies have been shared broadly during presentations at TSRC, in the peer-reviewed literature and with government agencies. Presentation of the Premier cigarette chemistry during a then-unique, joint Wednesday morning session of TSRC appeared to set an attendance record for a post-banquet session, as interest in a cigarette that did not burn tobacco was quite high!

Within R.J. Reynolds, Mike held various scientific positions in his first twenty-one years, rising to Sr. Principal Scientist in 2001. In 2007 he became a Sr. Director in the Research and Development Department, responsible for leading the Reynolds clinical studies team. Recently, he has joined the RAI Services Company as a Sr. Director in Regulatory Oversight.

Mike's career has been defined by opportunities for collaboration. He has had the good fortune to lead and contribute to large scientific efforts within R.J. Reynolds, across the tobacco industry, with contract laboratories and with state, federal and international government bodies. Notable examples include the FTC Multiplier Equation Study, the Massachusetts Benchmark Study and the first Canadian Benchmark Study. He has also conducted studies to understand the effects of "non-standard puffing regimens" on cigarette smoke yields. Results of those efforts contributed to the final puff volumes and puff frequencies designated for use today as the "Massachusetts" and "Health Canada" smoking regimens. In 2005, Mike was designated as one of two scientists from the United States to participate in an international expert working group, ISO TC126 Working Group 9, charged with identifying a smoking regime that is more representative of the ways in which people smoke; a first step in establishing a new, international standard for testing cigarettes.

During much of the last decade, Dr. Borgerding's research has focused on the conduct of clinical studies. Together with a team of talented colleagues, he has led clinical study efforts to evaluate new and existing tobacco products; including, moist snuff, dissolvable tobacco, Swedish-style snus and cigarettes, among others. Such studies have included measurements of tobacco product use behaviors, quality of life changes, biomarkers of exposure and biomarkers of potential harm. Results of such studies provide a means of placing tobacco products on a risk continuum. Research has also been conducted towards the development and validation of new, tobacco-relevant biomarkers.

An adjunct professor in the Virginia Tech Chemistry Department since 1995, Dr. Borgerding has mentored post-doctoral research fellows in the area of Analytical Chemistry. Those rewarding research efforts have been in collaboration with Drs. Harold McNair and Larry Taylor, true pioneers in the area of chromatographic and instrumental analysis techniques.

During his career, Dr. Borgerding has been a frequent speaker at the Tobacco Science Research Conference and other scientific meetings and he has been an active participant in CORESTA, both as a speaker and as a task force participant. He has authored or co-authored over 150 scientific papers and presentations.

Significant mentors have included Harold M. McNair, Willie L. Hinze, Joseph N. Schumacher, Brenda T. Hodge and Robert A. Lloyd, Jr.

Mike and his wife, Teresa, reside in Winston-Salem, NC. Their immediate family includes three daughters, Jennifer, Erika and Mary Patricia; one daughter-in-law, Mary Elizabeth; two sons, Nicholas and Stephen; one son-in-law, Mike; and four grandchildren, Johnathan, Christopher, Hannah and Wells.

69th TOBACCO SCIENCE RESEARCH CONFERENCE

MONDAY MORNING, SEPTEMBER 21, 2015 COMBINED SESSION

- 8:45 WELCOME: Rob Stevens, ITG Brands, 69th TSRC Chair
- 8:55 SYMPOSIUM: “The Scientific Basis of Harm Reduction and the Risk Continuum”
Chair: Clarissa Tatum, Eastman Chemical Company
- 9:00 1. HISTORICAL, CURRENT, AND FUTURE PERSPECTIVES OF HARM REDUCTION IN THE TOBACCO INDUSTRY. Michael W. OGDEN and Summer N. Hanna; RAI Services Company, Winston-Salem, NC USA
- 9:30 2. A FRAMEWORK FOR THE ASSESSMENT OF REDUCED RISK TOBACCO AND NICOTINE PRODUCTS. Frazer Lowe, Ian M. Fearon, Oscar M. Camacho, Emmanuel Minet and James MURPHY; British American Tobacco (Investments) Limited, Southampton, UK
- 10:00 *Break*
- 10:30 3. VERY LOW NICOTINE CIGARETTES AND LOW-TAR-TO-NICOTINE CIGARETTES AS POTENTIAL REDUCED EXPOSURE TOBACCO PRODUCTS. Michael R. MOYNIHAN; 22nd Century Group, Inc, Clarence, NY USA
- 11:00 4. PRODUCTS FOR TOBACCO HARM REDUCTION – IS THE LIGHT AT THE END OF THE TUNNEL A LED? Carl D. D’RUIZ; ITG Brands, Greensboro, NC USA
- 11:30 Panel discussion with all symposium speakers
- 11:50 *Lunch*
- 1:00 Posters
5. DETERMINATION OF THE MOST SUITABLE RACK, AMOUNT OF TOBACCO AND CURING REGIME FOR BASMA TOBACCO IN SEMI BULK CURING. Reza MOJIBSENZADEH; Iranian Tobacco Company, Behshahr, Mazandaran, Iran

6. DETERMINATION OF THE MOST SUITABLE RACK TYPE AND TOBACCO AMOUNT IN COLLECTOR AND COMPARISON IT'S QUANTITY AND QUALITY CHARACTERISTICS WITH METHOD OF BASMA TOBACCO STRINGING. Reza MOHSENZADEH; Iranian Tobacco Company, Behshahr, Mazandaran, Iran
7. COMPARISON OF THE CERULEAN E-CIGARETTE PRESSURE DROP TESTING INSTRUMENT TO THE CES 508 CIGARETTE PRESSURE DROP AND VENTILATION TESTER UNIT FOR ELECTRONIC CIGARETTES, CONVENTIONAL TOBACCO BURNING CIGARETTES, AND FILTER TIPS. Karen W. AVANTS¹, Steven A. Wilson² and T. Jeffrey Clark¹; ¹Liggett Group, LLC, Mebane, NC USA and ²Eastman Chemical Company, Kingsport, TN USA
8. TERMINATION SYSTEMS IN ROUTINE ANALYTICAL SMOKING: NON-CONTACT ALTERNATIVES TO COTTON. Ian TINDALL, James Vincent and Linda Crumpler; Cerulean Milton Keynes, UK
9. SYSTEMATIC ERRORS IN VENTILATION MEASUREMENT AND THEIR RESOLUTION. Ian TINDALL, James Vincent and Tim Mason; Cerulean Milton Keynes, UK
10. MEASUREMENT OF E-CIGARETTE AEROSOL PARTICLE SIZE WITH A LOW FLOW CASCADE IMPACTOR. David B. KANE and Mark J. Rusyniak; Altria Client Services, Richmond, VA USA
11. EFFECT OF LABORATORY CONDITIONS ON E-CIGARETTE AEROSOL COLLECTION. Anthony BROWN, Karl Wagner, John Miller and Jason W. Flora; Altria Client Services, Richmond, VA USA
12. METHOD FOR ALKALOID DETERMINATION IN TOBACCO THAT IS HIGHLY SELECTIVE, SENSITIVE AND SUITABLE FOR REGULATORY REPORTING. Anthony P. BROWN, Michael Morton, David Self, Jason W. Flora and Karl Wagner; Altria Client Services, Richmond, VA USA
13. DETERMINATION OF GAS-PHASE CARBONYLS IN E-CIGARETTE AEROSOL USING A SORBENT TUBE (EPA TO-11A) VS. AN IMPINGER COLLECTION. Celeste WILKINSON, James Wilkinson, John Miller and Jason W. Flora; Altria Client Services, Richmond, VA USA
14. PARTICLE BREAKTHROUGH COMPARISON OF COMMERCIAL CARBON FILTERS WITH CELFX™ CARBON/ACETATE FILTERS. R. M. ROBERTSON, J. N. Suthar and S. Basu; Celanese, Narrows, VA USA

15. CELFX™ CARBON TECHNOLOGY: PUFF-BY-PUFF PROFILE. R. M. ROBERTSON, J. N. Suthar and S. Basu; Celanese, Narrows, VA USA
16. FDA COMPLIANT ANALYSIS OF NICOTINE PLUS NINE METABOLITES IN HUMAN URINE BY LC-MS/MS. Patrick MILLER, Ridha Nachi, G. Paul Brown, Veniamin Lapko, Christine Kafonek and Kirk Newland; Celerion, Lincoln, NE USA
17. AN APPLICATION METHOD OF BACTERIOPHAGES AGAINST *RALSTONIA SOLANACEARUM* FOR PREVENTION TOBACCO BACTERIAL WILT. Qin Li, Li Sanjie and Shi Junxiong; Guizhou Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China
18. IDENTIFICATION OF NEW PHENYLPROPANOID GLYCOSIDES IN TOBACCO USING SOLID-PHASE EXTRACTION-GAS CHROMATOGRAPHY/MASS SPECTROMETRY WITH TRIFLUOROACETYLATION. Qin Ka, Li Sanjie, Xiang Zhangmin, Cai Bin, Pan Wenjie and Lei Bo; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China
19. ANALYSIS OF AROMA COMPONENTS FROM MAINSTREAM CIGARETTE SMOKE USING LOW TEMPERATURE SOLVENT EXTRACTION FOLLOWED BY GC×GC×HR-TOFMS. XIANG Zhangmin, Geng Li, Li Sanjie, Zhai, Zhou Shuping and Pan Wenjie; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China
20. APPLICATION OF LINEAR MODELS TO LINK CIGARETTE YIELDS DELIVERED UNDER ISO AND INTENSE REGIMES. Rémi JULIEN, Thomas Verron, Xavier Cahours and Stéphane Colard; SEITA - Imperial Tobacco Group, Fleury-Les-Aubrais, France
21. DIVERSITY AND DISTRIBUTION OF FUNGAL ENDOPHYTES IN *NICOTINANA TABACUM*. WANG Dong Sheng; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China
22. THE DETERMINATION AND COMPARISON OF AMMONIA CONTENT IN TOBACCO PRODUCTS BY ION CHROMATOGRAPHY AND CONTINUOUS FLOW ANALYSIS. Andy STINSON¹, Sherry Gilliland¹, Amelia M Paolantonio², Thomas D. Lockhart² and I. Gene Gillman²; ¹Liggett Group LLC, Mebane, NC USA and ²Enthalpy Analytical Inc., Durham, NC USA

23. THE DETERMINATION AND COMPARISON OF AMMONIA CONTENT IN MAINSTREAM TOBACCO SMOKE BY ION CHROMATOGRAPHY AND SPECTROPHOTOMETRIC METHODS. Andrew J. HUCKINS and Andy Stinson; Liggett Group LLC, Mebane, NC USA
24. NRF2 RESPONSE TO WHOLE SMOKE IN THREE DIMENSIONAL (3D) AIRWAY CULTURES. Wanda R. FIELDS, Brian M. Keyser and Betsy R. Bombick; R. J. Reynolds Tobacco Co., Winston-Salem, NC USA
25. ENGINEERING TOBACCO FOR RESISTANCE TO TOMATO SPOTTED WILT VIRUS. Muqiang GAO and David Zaitlin; KTRDC, University of Kentucky, Lexington, KY USA
26. CONSIDERATIONS FOR AUTOMATION OF STANDARDIZED METHODS FOR TOBACCO/SMOKE INVESTIGATIONS. Julian A. COX and Justin Lu; Sirius Automation Inc, Buffalo Grove, IL USA
27. INTEGRATE MEASURES BIO-CONTROL TOBACCO BACTERIAL WILT AND REMEDIATE TOBACCO MONO-CROPPING LAND. LIU Yanxia, Li Ming, Ca Guo and Shi Junxiong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

MONDAY AFTERNOON, SEPTEMBER 21, 2015

SESSION A

METHODS DEVELOPMENT

Session Chair: Steve Wilson

2:20 PM

28. DETERMINATION OF NINE VOLATILE NITROSAMINES AND HYDROXY-NITROSAMINES IN CIGARETTE FILLER AND MAINSTREAM TOBACCO SMOKE USING ISOTOPE-DILUTION LC-MS/MS. Mehran SHARIFI, Peter Joza and William Rickert; Labstat International ULC, Kitchener, ON, Canada

2:40 PM

29. A SINGLE STEP SOLID-PHASE EXTRACTION METHOD FOR GC/MS ANALYSIS OF AROMATIC AMINES IN MAINSTREAM CIGARETTE SMOKE. Chorng B. HUANG, Jason W. Flora and Karl Wagner; Altria Client Services, Richmond, VA USA

3:00 PM

30. APPLICATION OF NEAR INFRARED SPECTROSCOPY TO DETECT MOLD CONTAMINATION IN TOBACCO.

YANG Lei^{1,3}, Hou Ying¹, Li Jing-Jing¹, Li Wei¹, Wang Jia-Jun², Yang Qian-Xu², Wang Bao-Xing² and Pan Xue-Jun³; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China, ²China Tobacco Yunnan Industrial Co., Ltd., Kunming, Yunnan, China and ³Kunming University of Science and Technology, Kunming, Yunnan, China

SESSION A

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29. A SINGLE STEP SOLID-PHASE EXTRACTION METHOD FOR GC/MS ANALYSIS OF AROMATIC AMINES IN MAINSTREAM CIGARETTE SMOKE. Chorng B. HUANG, Jason W. Flora and Karl Wagner; Altria Client Services, Richmond, VA USA

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YANG Lei^{1,3}, Hou Ying¹, Li Jing-Jing¹, Li Wei¹, Wang Jia-Jun², Yang Qian-Xu², Wang Bao-Xing² and Pan Xue-Jun³; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China, ²China Tobacco Yunnan Industrial Co., Ltd., Kunming, Yunnan, China and ³Kunming University of Science and Technology, Kunming, Yunnan, China

3:20 PM BREAK

MONDAY AFTERNOON, SEPTEMBER 21, 2015

3:50 PM

31. CARBON MONOXIDE AND NITROGEN OXIDE DIFFUSION THROUGH CIGARETTE PAPER II. Joseph WANNA; SWM Intl., Alpharetta, GA USA

3:50 PM

39. COMPUTATIONAL TOOL FOR ESTIMATING INDOOR AEROSOL/ VAPOR CONCENTRATION. Ali A. ROSTAMI, Yezdi Pithawalla, Mohamadi Sarkar and Jianmin Liu, Altria Client Services Inc., Richmond, VA USA

4:10 PM

32. IGNITION PROPENSITY OF CIGARETTES ACCORDING TO ISO 12863 USING TWO DIFFERENT SUBSTRATES. Mario MAYR; delfortgroup / Papierfabrik Wattens GmbH & Co KG, Austria

4:10 PM

40. TOXICANTS IN BIOFLUIDS ORIGINATED FROM STABLE ISOTOPES OF PROPYLENE GLYCOL IN ECIGARETTES. Raymond H. FARMEN, Kirk E. Newland, Ridha Nachi and Chris J. Kafonek; Celerion, Lincoln, NE USA

4:30 PM

33. THE HEAT OF VAPORIZATION OF NICOTINE FROM TOBACCO. F. Kelley ST.CHARLES¹ and Serban Moldoveanu²; ²R. J. Reynolds Tobacco Co., ¹Lewisville, NC USA and ²Winston-Salem, NC USA

4:30 PM

41. A NOVEL NICOTINE SUBLINGUAL TABLET WHICH MIMICS CIGARETTE SMOKING NICOTINE PHARMACOKINETICS. John MCCARTY¹, Frank J. Vocci² and Matt Torrington²; ¹IntraTab Labs Inc., Miami, FL USA and ²Friends Research Institute Inc., Baltimore, MD USA

4:50 PM

34. A QUANTITATIVE ANALYSIS OF TERTIARY AMINES IN TOBACCO LEAVES. Kei KOBAYASHI, Atsushi Nagai and Shinsuke Sato; Japan Tobacco Inc., Yokohama, Japan

4:50 PM

42. ASSESSING THE HEALTH EFFECTS OF LAUNCHING E-CIGARETTES USING SYSTEM DYNAMICS: A UK CASE STUDY. Ian M. Fearon¹, Oscar M. CAMACHO² and Andrew Hill²; ¹British American Tobacco (Investments) Limited, Southampton, UK and ²Ventana Systems, Salisbury, UK

MONDAY AFTERNOON, SEPTEMBER 21, 2015

5:10 PM

Not Presented

35. QUICK MOISTURE MEASUREMENT
IN THE LABORATORY WITH THE
MICROWAVE RESONANCE METHOD.

André TEWS; TEWS Elektronik GmbH &
Co. KG, Hamburg, Germany

ADJOURN

TUESDAY MORNING, SEPTEMBER 22, 2015

SESSION A

METHODS DEVELOPMENT

Session Chair: Thaddeus Jackson

8:00 AM

43. DEAD VOLUME AND IMPINGER CAPTURE: WILL MACHINE DESIGN CHANGE PUFFING CONDITIONS? Ian TINDALL, Linda Crumpler and Akinwande Cole; Cerulean, Milton Keynes, UK

8:20 AM

44. DIRECT EXTRACTION OF CAMBRIDGE FILTER PAD HOLDERS. Thomas SCHMIDT¹, Nils Rose¹ and Beata Kowalski²; ¹Borgwaldt KC GmbH, Hamburg, Germany and ²BKC Hamburg, Germany

8:40 AM

45. ABBREVIATED METHOD FOR TNCO ANALYSIS - MODIFIED ISO SMOKING. Rana TAYYARAH; ITG Brands, Greensboro, NC USA

SESSION B

REGULATIONS & QUALITY

Session Chair: Ian Fearon

8:00 AM

53. INFLUENCE OF CIGARETTE FILTER VENTILATION ON SMOKERS' MOUTH LEVEL EXPOSURE TO TAR AND NICOTINE: A RETROSPECTIVE META-ANALYSIS OF 11 STUDIES IN 9 COUNTRIES. Ian M. FEARON¹, Sheri A. Bowman², John W. Caraway², Peter Chen², Paul R. Nelson², Madeleine Ashley¹, Christopher J. Shepperd¹ and Graham Errington¹; ¹British American Tobacco (Investments) Limited, Southampton, UK and ²R. J. Reynolds Tobacco Company, Winston-Salem, NC USA

8:20 AM

54. HARMFUL AND POTENTIALLY HARMFUL CONSTITUENTS (HPHC) LEVELS IN TOBACCOS AND MAINSTREAM SMOKE FROM CIGARILLOS AND FILTERED CIGARS. John H. LAUTERBACH; Lauterbach & Associates, LLC, Macon, GA USA

8:40 AM

55. RELATIONSHIPS BETWEEN HPHC IN CIGARETTE SMOKE. Beatrice TEILLET, Thomas Verron, Xavier Cahours and Stéphane Colard; SEITA - Imperial Tobacco Group, Fleury-Les-Aubrais, France

TUESDAY MORNING, SEPTEMBER 22, 2015**9:00 AM**

46. QUALIFICATION OF THE JB2090 SMOKING MACHINE FOR E-CIGARETTE TESTING ACCORDING TO CORESTA AND TOBACCO CIGARETTE TESTING ACCORDING TO ISO 3308. Dritan XHILLARI, Daniel Bachman, Henry DeFord and Rudolph Jaeger; CH Technologies, Westwood, NJ USA

9:20 AM

47. INFLUENCE OF MEASUREMENT UNCERTAINTY ON RECOMMENDED REGULATION FOR CIGARETTE SMOKE CONTENTS. Li Zhonghao, Hu QingYuan, Tang GangLing, Yang Fei, Pang YongQiang, Fan ZiYan, Zhang HongFei, Liu ShanShan, Bian Zhaoyang, Jiang Xingyi, Zhang Wei, Chen Huan and Chen ZaiGen; China National Tobacco Quality Supervision & Test Center, Zhengzhou, Henan, China

9:00 AM

56. ROUTINE AND DETAILED ANALYSES OF SOME TRADITIONAL PIPE TOBACCOS ON THE US MARKET. John H. LAUTERBACH¹ and Mark Ryan²; ¹Lauterbach & Associates, LLC, Macon, GA USA and ²Daughters & Ryan, Inc., Kenly, NC USA

9:20 AM

57. U.S. PHARMACOPEIA DISSOLUTION TECHNIQUE FOR THE DETERMINATION OF NICOTINE AND FLAVOR RELEASE FROM SMOKELESS TOBACCO PRODUCTS. John MILLER, Helen Miller, Richard Schibetta, Anthony Brown, Tim Danielson, Karl Wagner and Jason W. Flora; Altria Client Services, Richmond, VA USA

9:40 AM BREAK

10:10 AM

48. METHOD DEVELOPMENT AND VALIDATION OF SELECTED METALS IN E-LIQUID SAMPLES. Jeremy BROWN; Global Laboratory Services, Inc., Wilson, NC USA

10:10 AM

58. STABILITY OF REFERENCE CIGARETTES IN DIFFERENT STORAGE CONDITIONS. Huihua Ji, Ying Wu and Neil Fannin; University of Kentucky, Lexington, KY USA

10:30 AM**59. CENTER FOR TOBACCO**

TUESDAY MORNING, SEPTEMBER 22, 2015

10:30 AM

49. ON-LINE REAL-TIME ANALYSIS OF E-CIGARETTE VAPOURS AND HEAT-NOT-BURN TOBACCO PRODUCTS BY PHOTO-IONIZATION MASS SPECTROMETRY. Ralf ZIMMERMANN¹, Sven Ehlert², Thorsten Streibel¹, Jan Heide¹, Jan Wolter¹ and Andreas Walte²; ¹University of Rostock, Germany and ²Photonion GmbH, Schwerin, Germany

10:50 AM

50. DETERMINATION OF ALLYL ALCOHOL IN ELECTRONIC CIGARETTE (E-CIG) AEROSOL AND LIQUIDS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY. Xinyu LIU¹, Peter Joza and Bill Rickert; Labstat International ULC, Kitchener, ON, Canada

11:10 AM

51. INVESTIGATE PARTICLE SIZE DISTRIBUTIONS OF ELECTRONIC CIGARETTE AEROSOLS USING A MULTI-ANGLE LIGHT SCATTERING INSTRUMENT. Chen SONG¹, Don Holve² and Steve L. Alderman¹; ¹R. J. Reynolds Tobacco Company, Winston Salem, NC USA and ²EnviroMetrix Instruments LLC, Berkeley, CA USA

11:30 AM

52. NEW HIGHLY SENSITIVE AND SELECTIVE METHOD FOR CARBONYL DETERMINATION IN E-CIGARETTE AEROSOLS. Jason W. FLORA, Celeste Wilkinson, James Wilkinson and John Miller; Altria Client Services, Richmond, VA USA

REFERENCE PRODUCTS UPDATE ON 1R6F PRODUCTION AND PROFICIENCY TESTING PROGRAM.

Orlando CHAMBERS, Socrates Canete and Huihua Ji; Center for Tobacco Reference Products, University of Kentucky, Lexington, KY USA

10:50 AM

60. WAS THERE A TEMPORAL INCREASE IN CIGARETTE NICOTINE YIELD-TO-CONTENT RATIOS IN PRODUCTS REPORTED TO THE MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH? Michael J. MORTON, David A. Self, Raquel M. Olegario and Scott Appleton; Altria Client Services, Richmond, VA USA

11:10 AM

61. CAN US FDA SUBSTANTIALLY EQUIVALENT PREDICATES BE DEVELOPED WITHOUT KNOWLEDGE OF AND A SAMPLE OF THE PREDICATE PRODUCT? John H. LAUTERBACH; Lauterbach & Associates, LLC, Macon, GA USA

11:30 AM

62. A COMPARISON OF STATISTICAL AND MATHEMATICAL METHODS IN SUPPORT OF EQUIVALENCE TESTING. Rana TAYYARAH; ITG Brands, Greensboro, NC USA

LUNCH

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

1:15

Posters

63. EFFECT OF ELECTRICITY OFF AMOUNT ON THE CURING DIFFERENT STAGES ON QUALITY AND YIELD OF FLUE-CURED TOBACCO. Reza MOHSENZADEH; Iranian Tobacco Company, Behshahr, Mazandaran, Iran
64. STUDYING RESEARCH AND EXTENSION TOPPING HEIGHT (LEAF NUMBER) AND TIMING ON QUALITATIVE AND QUANTITATIVE YIELD IN FLUE-CURED TOBACCO. Reza MOHSENZADEH and M. Reza; Iranian Tobacco Company, Behshahr, Mazandaran, Iran
65. COMPARISON OF INITIAL AND NEAR END-OF-LIFE ELECTRONIC CIGARETTE AEROSOL YIELDS. J. A. BODNAR¹, S. L. Alderman², S. K. Pike² and R. J. Potts¹; ¹R. J. Reynolds Tobacco Company, Winston-Salem, NC USA and ²RJR Vapor Company, Winston-Salem, NC USA
66. STUDY OF THE DECOMPOSITION OF TOBACCO/CITRATE/SBA-15 MIXTURES IN NITROGEN AND AIR ATMOSPHERES. A. MARCILLA, M. I. Beltrán, A. Gomez-Siurana, I. Martinez and D. Berenguer; Alicante University, Alicante, Spain
67. STUDY OF THE EFFECT OF DIFFERENT CATALYSTS IN THE DECOMPOSITION OF NICOTINE. A. MARCILLA, M. I. Beltrán, A. Gomez-Siurana, I. Martinez and D. Berenguer; Alicante University, Alicante, Spain
68. CHEMICAL FINGERPRINTING OF TOBACCO AND RELATED PRODUCTS BY TD-GC-TOF MS. Laura McGregor¹, Bob GREEN¹, Caroline Widdowson¹, Kevin Collins¹, Chris Hall² and Pete Grosshans²; ¹Markes International, Llantrisant, South Wales, UK and ²Markes International Inc., Cincinnati, OH USA
69. EFFECT OF PUFF DURATION AND PUFF VOLUME ON E-CIGARETTE AEROSOL COLLECTION. John MILLER and Jason W. Flora; Altria Client Services, Richmond, VA USA
70. UPLC-MS/MS FOR HIGH-THROUGHPUT ANALYSIS OF AROMATIC AMINES IN CIGARETTE SMOKE. Xiaohong JIN, Chorong B. Huang, Karen Avery, Karl Wagner and Jason W. Flora; Altria Client Services, Richmond, VA USA

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

71. DETERMINATION OF DIACETYL IN E-VAPOR PRODUCTS USING GAS CHROMATOGRAPHY AND MASS SPECTROMETRY. Chong B. HUANG, Karl Wagner and Jason W. Flora; Altria Client Services, Richmond, VA USA
72. QUANTITATIVE SCREENING OF POTENTIAL CONTAMINANTS IN E-CIGARETTE FORMULATIONS: ETHYLENE GLYCOL AND DIETHYLENE GLYCOL. Niti H. SHAH, Karl Wagner and Jason Flora; Altria Client Services, Richmond, VA USA
73. A VERSATILE METHOD FOR THE ANALYSIS OF TSNAS IN TOBACCO PRODUCTS AND CIGARETTE SMOKE BY LC-MS-MS. Jeff ZHU¹, Nancy Qian¹, Shalina Jones¹ and Serban Moldoveanu²; ¹Eurofins Lancaster Laboratories, Winston-Salem, NC USA and ²R. J. Reynolds Tobacco Co., Winston-Salem, NC USA
74. METHYLATION PROFILES IN CHRONIC SMOKERS AND MOIST SNUFF CONSUMERS. G. L. PRASAD and Michael F. Borgerding; R. J. Reynolds Tobacco Co., Winston-Salem, NC USA
75. IMPACTS OF TOTAL PARTICULATE MATTER FROM CIGARETTE SMOKE ON EARLY DEVELOPMENT OF ZEBRAFISH (*DANIO RERIO*). G.L. PRASAD¹; Andrey Massarsky², Nishad Jayasundara², Richard T. DiGiulio², Jordan Bailey³ and Ed Levin³; ¹R. J. Reynolds Tobacco Co., Winston-Salem, NC USA, ²Duke University, Durham, NC USA and ³Duke University Medical Center, Durham, NC USA
76. EFFECT OF MATURITY ON THE ENZYMES AND CHEMICAL COMPOSITIONS RELATED TO CARBON-NITROGEN METABOLISM IN FLUE-CURED TOBACCO DURING CURING STAGE. WEI Kesu, Wu Sheng Jiang, Pan Wen-jie, Li De-lun, Jiang Jun, Li Guo-bin and Xie Yi-shu; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China
77. ACTIVITIES OF AZOXYSTROBIN AND DIFENOCONAZOLE AGAINST *ALTERNARIA ALTERNATA* AND THEIR EFFICACY FOR THE CONTROL OF TOBACCO BROWN SPOT. WANG Hancheng, Wang Maosheng, Chen Xinjiang, Wang Jin and Shang Shenghua; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China
78. INTEGRATE MEASURES BIO-CONTROL TOBACCO BACTERIAL WILT AND REMEDIATE TOBACCO MONO-CROPPING LAND. LIU Yanxia, Li Xiang, Cai Lulu and Shi Junxiong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

79. DEVELOPMENT OF USER-FRIENDLY MARKER FOR NIC2 IN TOBACCO. Qiulin QIN, Dandan Li, Robert Miller, Anne Jack and Shengming Yang; University of Kentucky, Lexington, KY USA
80. IMPACT OF DIFFERENT PARAMETERS ON THE COLLECTION AND GENERATION OF E-CIGARETTE AEROSOL FOR NICOTINE DELIVERY ANALYSES. David K. COOK, Aaren N. Routh, Ryan C. Mills and Daniel G. Morgan; ITG Brands, Greensboro, NC USA
81. FORMATION OF NNK FROM PSEUDOOXYNICOTINE (PON). Ying WU, Huihua Ji, Neil Fannin and Lowell Bush; University of Kentucky, Lexington, KY USA
82. ASSESSMENT OF TWO HIGH THROUGHPUT *IN VITRO* METHODS FOR THE QUANTIFICATION OF CIGARETTE SMOKE INDUCED MICRONUCLEI. Bethany COOPER¹, Manoj Misra¹ and Robert Leverette²; ¹ITG Brands, Greensboro, NC USA and ²R. J. Reynolds Tobacco Co., Winston-Salem, NC USA
83. *IN VITRO* TOXICITY SCREENING OF BLU ELECTRONIC CIGARETTE LIQUIDS AND IMPLICATIONS FOR HUMAN EXPOSURE. Manoj MISRA¹, R. D. Leverette², B. T Cooper¹ and M. B. Bennett¹; ¹ITG Brands, Greensboro, NC USA and ²R. J. Reynolds Tobacco Co., Winston-Salem, NC USA
84. THE PREPARATION OF ADDITIVES TO REDUCE PHENOL AND ITS APPLICATION IN CIGARETTE. XIONG Shan-Shan¹, Wu Jing-Qiang¹, Xu Jian-Rong², Shen Jing-Xuan¹, Xiao Wei-Yi¹, Yao Zhen-Yu¹ and Xu Lan-lan¹; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China and ²China Tobacco Fujian Industrial Co., Ltd., Xiamen, China
85. DISCUSSIONS ON HARM REDUCTION MECHANISM OF BIOMASS STEM GRANULE MATERIAL IN CIGARETTES. ZI Wenhua, Long Minghai, Yang Lei, Shen Yan and Li Biao; Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China
86. STUDY ON INFLUENCE OF PROCESSING STRENGTH ON FLUE-CURED TOBACCO LEAF QUALITY IN THE THRESHING AND REDRYING PROCESS. LONG Minghai¹, Hua Yikun², Wang Xianguo², Lin Nan¹ and Zi Wenhua¹; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China and ²Hongyun Honghe (Group) Co., Ltd., Kunming, Yunnan, China

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

SESSION A

AGRONOMY

Session Chair: Darlene Lawson

2:20 PM

87. NICOTINE ANALYSIS IN SEVERAL NON-TOBACCO PLANT MATERIALS.

Serban C. MOLDOVEANU, Scott A.

Wayne and Darlene M. Lawson;

R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

2:40 PM

88. THE DEMETHYLASE MUTANTS – PANACEA OR NEW PROBLEMS?

Anne JACK, Huihua Ji, Neil Fannin, Colin Fisher and Angela Schoergendorfer; University of Kentucky, Lexington, KY USA

3:00 PM

89. EVALUATION OF ANDROGENIC AND GYNOGENIC DOUBLED HAPLOID LINES FOR USE AS PARENTAL LINES FOR HYBRID BURLEY TOBACCO VARIETIES.

R.D. MILLER and Ezequiel Deoliveira; University Of Kentucky, Lexington, KY USA

SESSION B

E-CIGARETTES

Session Chair: Chris Russell

2:20 PM

94. EFFECT OF POWER LEVEL ON THE YIELD OF TOTAL AEROSOL MASS AND FORMATION OF ALDEHYDES

IN E-CIGARETTE AEROSOLS. I. Gene

GILLMAN, Emil W. Stewart and Amelia

R. Paolantonio; Enthalpy Analytical Inc, Durham, NC USA

2:40 PM

95. INFLUENCE OF RELATIVE HUMIDITY CONDITIONING ON AEROSOL AND LIQUID CHEMISTRIES

IN ELECTRONIC CIGARETTES. Candice

K. CUNNINGHAM¹, Steven L. Alderman²

and Doug Brown²; ¹R. J. Reynolds Tobacco

Company, Winston Salem, NC USA and

²R. J. Reynolds Vapor Company, Winston Salem, NC USA

3:00 PM

96. Withdrawn

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3:20 PM

90. EFFECT OF ARTIFICIAL ORDERING ON TSNAS DURING SHORT TERM STORAGE. Colin FISHER¹, Anne Jack¹, Lowell Bush² and Bob Pearce²; ¹KTRDC and ²Plant and Soil Sciences, University of Kentucky, Lexington, KY USA

3:20 PM

97. NON-TARGETED ANALYSIS OF EMISSIONS FROM TOBACCO HEATING PRODUCTS. Kelly REES, Justin Frosina, Michal Brokl, Christopher Rawlinson and Chris Wright; British American Tobacco, Southampton, UK

 3:40 PM BREAK

4:10 PM

91. TRANSCRIPTOME-ANALYSIS ENABLED THE DEVELOPMENT OF CO-DOMINANT MARKERS FOR NIC1 AND NIC2 IN TOBACCO. Shengming YANG, Qiulin Qin, Dandan Li, Robert Miller and Anne Jack; University of Kentucky, Lexington, KY USA

4:10 PM

98. EVALUATION OF BIOMARKERS OF SMOKE EXPOSURE IN ADULT SMOKERS FOLLOWING DUAL USE OF CIGARETTES WITH ELECTRONIC CIGARETTES. Carl D. D'RUIZ; ITG Brands, Greensboro, NC USA

4:30 PM

92. METABONOMICS STUDY OF TWO NICOTIANA GENOTYPES UNDER CADMIUM STRESS. ZHANG Yanling, Guo Yuanyuan, Zhou Huina and Zhai Niu; Zhengzhou Tobacco Research Institute, Zhengzhou, Henan, China

4:30 PM

99. REDUCTIONS IN BIOMARKERS OF SMOKE EXPOSURE IN FOLLOWING COMPLETE SUBSTITUTION OF CIGARETTES WITH ELECTRONIC CIGARETTES. Carl D. D'RUIZ; ITG Brands, Greensboro, NC USA

4:50 PM

93. TRANSCRIPTION FACTOR NTF1 INTERACTED WITH NTRAP2.2 TO NEGATIVELY REGULATE EXPRESSION OF NTPSY. LEI Bo, Ding Fuzhang, Zhao Huina, Cai Kai, Pan Wenjie and Cai Bin; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

4:50 PM

100. AN ONLINE SURVEY OF 5,000 VAPERS' PERCEPTIONS AND EXPERIENCES OF USING ELECTRONIC CIGARETTES AS AN AID TO SMOKING CESSATION. Chris RUSSELL, Neil McKeganey and Tiffany Hamilton-Barclay; Centre for Drug Misuse Research, Glasgow, Scotland, UK

 ADJOURN

5:15 PM

TSRC BUSINESS MEETING: All attendees are encouraged to attend.

WEDNESDAY MORNING, SEPTEMBER 23, 2015

SESSION A

AGRONOMY

Session Chair: Anne Jack

8:30 AM

101. QUANTITATIVE PROTEOMICS ANALYSIS THE TOBACCO TRICHOMES UNDER CADMIUM STRESS. FU Qiang, Lin Shifeng, Zou Jie, Yu Jing, Zhang Xiaolian and Ren Xueliang; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

8:50 AM *Not Presented*

102. ISOLATION AND IDENTIFICATION OF TOBACCO SPECIFIC PLANT GROWTH-PROMOTING RHIZOBACTERIA AND ITS FIELD APPLICATION. LI Xiang, Liu Yanxia and Shi Junxiong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

9:10 AM *Not Presented*

103. THE SOLID-STATE-FERMENTATION OF THE DISCARDED TOBACCO LEAVES TO MAKE ORGANIC FERTILIZER AND ITS EFFECT ON TOBACCO GROWTH. LI Xiang, Liu Yanxia and Shi Junxiong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

SESSION B

TOXICOLOGY & MATERIALS

Session Chair: Wanda Fields

8:30 AM

108. *IN VITRO* CYTOTOXICITY OF ABORIGINAL AUSTRALIAN SMOKELESS TOBACCO PRODUCT 'PITURI' COMPARED TO NICOTINE IN HUMAN LUNG EPITHELIAL CELLS. Nahid MOGHBEL, BoMi Ryu and Kathryn J. Steadman; The University of Queensland, St. Lucia, Australia

8:50 AM

109. 3D RECONSTRUCTED HUMAN AIRWAY MODELS: EFFECT OF ACCLIMATION CONDITIONS ON BIOMARKER AND INFLAMMATORY RESPONSE FOLLOWING TISSUE CHALLENGE. Holger BEHRING, Hans A. Raabe, Devin W. Sheehan, Elizabeth A. Sly and Rodger D. Curren; Institute for *In Vitro* Sciences, Gaithersburg, MD USA

9:10 AM

110. QUANTITATIVE BIOMONITORING OF URINE MUTAGENICITY: AN ALTERNATIVE TO CLASSICAL AMES TEST. Rafiqul ISLAM¹ and Clarinda Islam²; ¹Celerion Inc., Lincoln, NE USA and ²Somru BioScience Inc., Charlottetown, Canada

 WEDNESDAY MORNING, SEPTEMBER 23, 2015

9:30 AM *Not Presented*

104. EFFECT OF DIFFERENT NITROGEN APPLICATION ON FLUE-CURED TOBACCO HAZARD INDEX AND ENDOGENOUS HARMFUL COMPONENTS IN MAINSTREAM CIGARETTE SMOKE. GENG Zhao-liang, Zhang Jie, Ge Yong-hui, Xiang Zhang-min and Feng Yong-gang; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

9:30 AM

111. ANALYSIS ON IMMUNOTOXICITY INDUCED BY CIGARETTE SMOKE CONDENSATE BY A RAPID DETECTION AND EVALUATION METHOD BASED ON LUMINEX LIQUICHIP. KANG Yu¹, Zhao Junwei¹, Li Xiang¹, Yang Zhihua², Zhu Maoxiang², Liu Huimin¹ and Xie Fuwei¹; ¹Zhengzhou Tobacco Research Institute, Zhengzhou, China and ²Institute of Radiation Medicine, Beijing, China

 9:50 AM BREAK

10:20 AM *Not Presented*

105. DIFFERENCE OF THE NEUTRAL AROMA COMPOUNDS OF FLUE-CURED TOBACCO BEFORE AND AFTER CURING. ZHAO Huina, Lei Bo, Cai Kai, Wu Shengjiang, Pan Wenjie and Cai Bin; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

10:20 AM

112. PLASMA PERFORATION OF TIPPING PAPER: SELECTED BENEFITS FOR CIGARETTE CONSUMPTION. Michael LINDNER¹ and Renata Raunic' Vadanjel²; ¹Tannpapier GmbH, Traun, Austria and ²TDR d.o.o., Rovinj, Croatia

10:40 AM

106. PHOTOSYNTHETIC AND GROWTH CHARACTERISTICS OF FLUE-CURED TOBACCO SEEDLINGS IN WELL-CELLAR STYLE TRANSPLANTING. LIN Yechun, Chen Wei, Gao Weichang, Chen Yi, Ding Fuzhang, Li Hongxun, Liang Guilin and Pan Wenjie; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

10:40 AM

113. ESTIMATION OF HEAT GENERATION IN SMOLDERING PACKED BED OF TOBACCO. Yasunobu INOUE¹ and Masataro Suzuki²; ¹Japan Tobacco Inc., Yokohama, Japan and ²Nagaoka University of Technology, Nagaoka, Japan

11:00 AM *Not Presented*

107. ISOLATION AND FUNCTIONAL ANALYSIS OF TOBACCO GLUTAREDOXIN NBGRX1 IN RESPONSE TO DROUGHT STRESS. GUO Yushuang, Li Ruiyuan, Yu Jing, Zou Jie and Zhao Jiehong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

 ADJOURN

69th Tobacco Science Research Conference

MONDAY MORNING, SEPTEMBER 21, 2015

Symposium

8:45 AM WELCOME: Rob Stevens, ITG Brands, 69th TSRC Chair

8:55 AM SYMPOSIUM: “The Scientific Basis of Harm Reduction and the Risk Continuum”
Chair: Clarissa Tatum, Eastman Chemical Company

9:00 AM MONDAY

1. HISTORICAL, CURRENT, AND FUTURE PERSPECTIVES OF HARM REDUCTION IN THE TOBACCO INDUSTRY. Michael W. OGDEN and Summer N. Hanna; RAI Services Company, Winston-Salem, NC USA

Over sixty years ago, scientists began to establish an association between cigarette smoking and disease. Scientific inquiries, including mouse skin painting and chemical analysis of tobacco and smoke resulted in a call to action from both the public health community and the U.S. government to reduce smoke yields. Cigarette manufacturers responded with a variety of design modifications that included filtration and filter ventilation, porous cigarette papers and paper additives, reconstituted tobacco sheet, and expanded tobacco. Until 2001, these general reduction strategies were applauded by both the government and public health community through a variety of actions and publications.

In concert with general yield reduction efforts, cigarette manufacturers also explored novel cigarette design approaches as a means to potentially reduce harm. These approaches, which have generally not found much commercial success in the U.S. to date, include selective filtration, tobacco substitutes, altered tar-to-nicotine ratios, and heating rather than burning tobacco.

Meanwhile, epidemiology of smokeless tobacco use in the U.S. and Sweden unequivocally established that smokeless tobacco, when used exclusively, was a less risky form of tobacco consumption than cigarettes. That, along with introduction of nicotine replacement therapies, highlighted that a continuum of risk exists among different tobacco and nicotine products. Over the last 10 to 15 years, many public health advocates and consumers have recognized the need for a more diversified suite of switching options, and manufacturers have again responded with a broad array of products along the risk continuum in an effort to reduce the disease associated with smoking. Such examples in the U.S. include introduction of dissolvable tobacco products, Swedish-style snus, and more recently, electronic cigarettes.

With FDA oversight in the U.S. and the Framework Convention in much of the rest of the world, the modern regulatory landscape of tobacco products is more dynamic than ever. An exploration of historical, current, and future approaches to tobacco harm reduction will give perspective to both regulators and industry members alike.

9:30 AM MONDAY

2. A FRAMEWORK FOR THE ASSESSMENT OF REDUCED RISK TOBACCO AND NICOTINE PRODUCTS. Frazer Lowe, Ian M. Fearon, Oscar M. Camacho, Emmanuel Minet and James MURPHY; British American Tobacco (Investments) Limited, Southampton, UK

Cigarette smoking is a cause of many human diseases including heart disease, lung disease and cancer. The use of novel tobacco and nicotine products which yield lower levels of toxicants, such as tobacco heating products and electronic cigarettes, holds great potential in reducing the harms associated with smoking. However, this harm reduction potential has yet to be scientifically substantiated.

Currently, the FDA is the only national regulator to have provided a draft framework with which to assess novel tobacco and nicotine products for their harm reduction potential. In this presentation I will present an overview of how we believe such products could be assessed and describe how novel product assessment can be achieved with an integrated approach drawing on data from four key areas:

- Stewardship science;
- Toxicant exposure studies examining both machine-derived product yields and actual human exposure;
- Individual risk reduction using both pre-clinical *in vitro* models and clinical studies examining biomarkers of biological effect;
- Population risk reduction, including studies on user behaviour, risk perception, abuse liability and postmarket surveillance/population modelling.

Data will be presented to exemplify how components of this integrated approach have already been used to provide preliminary evidence for the reduced harm potential of novel products. This will focus on the use of *in vitro* models and cardiovascular disease Adverse Outcome Pathways (AOPs) to evaluate tobacco heating products and electronic cigarettes, and will also outline approaches for assessment of these products in human studies. During the conference, we will present further examples of the use of stewardship science and system dynamics population modelling in product assessment.

10:30 AM MONDAY

3. VERY LOW NICOTINE CIGARETTES AND LOW-TAR-TO-NICOTINE CIGARETTES AS POTENTIAL REDUCED EXPOSURE TOBACCO PRODUCTS. Michael R. MOYNIHAN; 22nd Century Group, Inc, Clarence, NY USA

Nicotine sustains regular cigarette smoking, but most of the harm is due to other smoke constituents. Smokeless tobacco and e-cigarettes provide lower exposures to many harmful substances, but the numbers of users of such products is dwarfed by the number that continue to smoke cigarettes. 22nd Century's focus is on development of products that may reduce exposure to harmful substances in adults who continue to smoke and facilitate transition to tobacco products even lower on the risk continuum. While cigarettes with very low nicotine content (VLN cigarettes) and cigarettes with a substantially lower tar-to-nicotine ratio (LTN cigarettes) lie much closer to conventional cigarettes on the risk

continuum, they may be acceptable to smokers who are not willing to switch to other products. In a recently completed independent trial, 840 smokers were provided with usual brand or one of six experimental cigarettes with different nicotine content for six weeks. Smokers provided with VLN cigarettes not only reduced their nicotine intake, but consumed fewer cigarettes per day than those provided with usual brand cigarettes, had lower scores on several measures of nicotine dependence, and reported more quit attempts during follow-up. For current smokers whose immediate objectives are to reduce nicotine exposure and smoke less, VLN cigarettes may be useful. The concept of providing a desired level of nicotine with a lower exposure to other smoke constituents is not new, but has proven to be challenging. A limiting factor is the nicotine content in tobacco varieties used in cigarettes. 22nd Century has developed novel tobacco varieties with leaf nicotine more than twice the concentration in conventional cigarette filler. LTN cigarettes produced from these tobaccos may reduce toxicant exposure.

11:00 AM MONDAY

4. PRODUCTS FOR TOBACCO HARM REDUCTION – IS THE LIGHT AT THE END OF THE TUNNEL A LED? Carl D. D’RUIZ; ITG Brands, Greensboro, NC USA

The relative merits of traditional prohibitionist tobacco control measures and tobacco harm reduction approaches as strategies to reduce the smoking-related burdens of morbidity and mortality has been debated for several decades now within the scientific and public health communities. The concept of a risk continuum for tobacco and nicotine products has emerged, in parallel with a sense that the most appropriate regulatory oversight should encourage users of nicotine-delivering products to migrate to less-harmful product categories. This approach is intended to foster innovation and advancement of disruptive nicotine delivery technologies to achieve immediate reductions in smoking-related morbidity and mortality. A sound base of scientific research on the relative exposures to users and non-users is rapidly accumulating, and seems already sufficient to assign provisional rankings of certain products on the continuum of risk between conventional cigarettes and medicinal nicotine products. While additional work is certainly needed to more fully characterize and quantify the both the risks and the benefits of emerging nicotine delivery products, the potential for harm reduction or benefit is indisputable. However, realization of the full potential of tobacco harm reduction to advance the public health will require a progressive regulatory apparatus, enlightened public health and academic research communities, and a similarly enlightened regulated community of tobacco and nicotine product manufacturers to work together in unprecedented ways for the betterment of society. A snapshot of the current regulatory environment provides some insight as to whether a more enlightened regulatory paradigm, inclusive of tobacco harm reduction products, is forthcoming.

1:00 PM POSTERS

5. DETERMINATION OF THE MOST SUITABLE RACK, AMOUNT OF TOBACCO AND CURING REGIME FOR BASMA TOBACCO IN SEMI BULK CURING. Reza MOHSENZADEH, Iranian Tobacco Company, Behshahr, Mazandaran, Iran

There are a number of various techniques for curing oriental tobacco, based on specific

variety and location. The time required to complete the curing process depends upon tobacco type, stalk position, body, ripeness and weather conditions. This study was done to determine the most suitable curing, type of rack and amount of tobacco per rack with 13 treatments and observation in Tirtash Tobacco Research and Education center in 2008-2009 in the 5 pick ups. Treatments were rack types two (Japanese rack and Iranian rack) and tobacco amount (5-7 and 7-9 kg in Japonica rack, 2/3 and full tobacco in card rack) and stringing tobacco then curing in collector (check), 3times of yellowing, Temperature increase 1 degree in 2 hours, Temperature increase 3 degree in 3 hours and 24 hour barn was off and then Temperature increase 1 degree in 2 hours. The second, third and fourth periods in tobacco curing are the same for all treatment. Temperature increased from 35 to 40, 41 to 45, and from 46 to 50, 1 degree per hour, respectively. Stop times were in all processes free. Average price of tobacco, sugar, nicotine, time of stringing and racking were recorded. Results showed that treatments card rack with full tobacco (8-10 kilogram per rack) with Average price of tobacco 1\$ and regimes Temperature increase 1 degree in 2 hours with Average price of tobacco 0.8\$ in comparison to others treatments exception stringing (1\$) were the most suitable. The card racks reduce number and expensive of labor amount 52 percent in comparison to stringing.

6. DETERMINATION OF THE MOST SUITABLE RACK TYPE AND TOBACCO AMOUNT IN COLLECTOR AND COMPARISON IT'S QUANTITY AND QUALITY CHARACTERISTICS WITH METHOD OF BASMA TOBACCO STRINGING. Reza MOHSENZADEH; Iranian Tobacco Company, Behshahr, Mazandaran, Iran

Harvesting, stringing and the preparation of leaves for curing and marketing are the most factors for tobacco production in most countries. Stringing of oriental tobacco leaves is very labor intensive in comparison to other tobacco. This study was done to determine the most suitable type of rack and amount of tobacco per rack with 9 treatments in collector at the Tirtash Tobacco Research and Education center in 2008-2009 with 3 replications in factorial test based on RCBD in 4 pick ups. Treatments were rack types two (Japanese rack and Iranian rack) and tobacco amount (2, 3, 4 and 5 kg in Japonica rack and 1/2, 1/3, 2/3 and full tobacco in card rack) and stringing tobacco (check). Average price of tobacco, sugar, nicotine percent time of stringing and racking were recorded. Variance analysis results showed that there were significant differences for sugar, nicotine percent and Average price of tobacco in Treatments and pick ups. Using of Japonica rack and card rack reduced time and number of labor ratio to stringing, 15-35 percent. Stringing, 1/2, 1/3 tobacco in card rack and 2 kg in Japonica rack treatments were with Average price of tobacco 0.87\$, 0.8\$, 0.77\$ and 0.76\$ rials the best suitable treatments ratio to others. But treatment of 1/2 tobacco in card rack was the best for reduce of number labor and net income.

7. COMPARISON OF THE CERULEAN E-CIGARETTE PRESSURE DROP TESTING INSTRUMENT TO THE CES 508 CIGARETTE PRESSURE DROP AND VENTILATION TESTER UNIT FOR ELECTRONIC CIGARETTES, CONVENTIONAL TOBACCO BURNING CIGARETTES, AND FILTER TIPS. Karen W. AVANTS¹, Steven A. Wilson² and T. Jeffrey Clark¹; ¹Liggett Group, LLC, Mebane, NC USA and ²Eastman Chemical Company, Kingsport, TN USA

The pressure drop or “resistance to draw” of cigarettes and cigarette-like products is a prominent experience for consumers. This poster is a comparison of two commercial

instruments with different approaches to measuring pressure drop. The instruments compared are the Cerulean EPD100 e-cigarette testing instrument and the CES 508 cigarette pressure drop and ventilation tester. The design of the two test units differs significantly as the Cerulean unit forces air out through the test article, whereas the CES 508 instrument draws air through the test article. The comparison was made using samples of commercially-available electronic cigarettes (e-cigs), conventional tobacco-burning cigarettes, and filter tips. Overall, both test units provided comparable results over a range up to 200-mm H₂O pressure drop. This poster presents the correlation and bias between the two different unit designs. The findings of this study demonstrate that conventional tobacco burning products and filter tips exhibit very low variability from unit to unit. The variation between units for electronic cigarettes is generally greater, presumably due to the hand-made nature of e-cigs compared to conventional machine-made cigarettes and filter tips.

8. TERMINATION SYSTEMS IN ROUTINE ANALYTICAL SMOKING: NON-CONTACT ALTERNATIVES TO COTTON. Ian TINDALL, James Vincent and Linda Crumpler; Cerulean, Milton Keynes, UK

When comparing the puff count results from WG10 it was observed that under ISO smoking conditions that infra-red termination systems consistently showed lower puff counts than cotton termination. However, under Health Canada Intense smoking this difference was eliminated. Subsequent video evidence showed that the cotton termination accurately represents when the burn front on the envelope paper reaches a butt mark.

Non-contact determination is necessary for rotary smoking machine and also has the merit that a physical butt mark is not required and so has some perceived benefits for users.

An alternate non-contact scheme has been devised and characterised that uses commercially available components and visible light. This has the merit of fast reaction times, no need for physical termination marking and the assurance of correct alignment for termination through a highly visible spot on the envelope.

The comparative puff counts for various brands and regimes between cotton and this new optical system is presented.

9. SYSTEMATIC ERRORS IN VENTILATION MEASUREMENT AND THEIR RESOLUTION. Ian TINDALL, James Vincent and Tim Mason; Cerulean, Milton Keynes, UK

Calibration of ventilation measurement equipment is an “ideal” process designed to remove differences between a transfer standard and the real world. However, when real measurements are made by instruments there is no practical method of making an inline flow measurement without disturbing the ventilation measurement as this introduces additional resistance into the flow path. In practice, calibration with a transfer standard is not sufficient to eliminate measurement errors and limits the accuracy of ventilation measurements.

The objective of this study was to investigate the impact of PD in the ventilation measurement path and the nonlinearity that this introduces. This was then related to flow modeling of the transfer standard which shows the cause of the observed sensitivity as a function of flow. A further objective is to develop schemes that minimise these errors.

It was found that inducing a small balancing PD in the reference path of approximately ImmWG, using a non-linear multipoint interpolation for calibration and constant referencing to three points on the interpolated curve significantly improves accuracy of measurement. Improvements in accuracy were shown across the range, the magnitude of the improvement being dependent upon the actual ventilation being measured. Typical improvements (absolute value) between the standard and new system would be 0.5% at 28% ventilation, 1% at 47% ventilation, 1.1% at 60% ventilation, 0.6% at 70% ventilation and 0.1% at 93% ventilation. Increasing the PD of the ventilation standard exacerbates the observed problem although in practice this should not happen with real samples.

The improved instrument design, which takes into account the flow modeling of the transfer standard and reference path PD, can give more accurate ventilation measurements.

10. MEASUREMENT OF E-CIGARETTE AEROSOL PARTICLE SIZE WITH A LOW FLOW CASCADE IMPACTOR. David B. KANE and Mark J. Rusyniak, Altria Client Services, Richmond, VA USA

Particle size is an important aerosol property related to dosimetry and aerosol dynamics. For e-cigarettes which produce condensation aerosols from propylene glycol and/or glycerin, measuring aerosol particle size can be particularly challenging due to the volatile and dynamic nature of these aerosols. In particular e-cigarette aerosol particle size measurements may be convoluted by evaporation due to high dilution ratios required for measurements made with conventional aerosol instrumentation and coagulation due to long residence times between sampling and measurement. To address these measurement issues, we have developed a particle size measurement system for e-cigarette aerosols using a low flow cascade impactor. This measurement system is not limited by the concentration of the particles and minimal dilution air is required for impactor operation, thereby minimizing the effects of evaporation. The impactor is interfaced with a sampling system that is capable of generating a puff on an e-cigarette and directly introducing the aerosol into the inlet flow of the impactor, thereby minimizing the time for coagulation.

With this system we have compared the median particle size of both cigarette smoke (0.4 microns) and electronic cigarette aerosols (0.6 - 0.8 microns). Measurements of the aerosol particle size produced by several commercially available e-cigarettes indicate that a majority of the products generate aerosols with median particle sizes within the sub-micron range. This measurement system has also been used to study the parameters that may affect the aerosol particle size. Particle size is found to be highly dependent on the puff flow rate, with higher puff flow rates reducing the aerosol particle size. Conversely particle size was found to be independent of the puff duration. An additional factor found to affect the particle size was the concentration of glycerin in the flavor formulation. The addition of 5% glycerin to a propylene glycol based flavor formulation reduces the median particle diameter from ~0.8 microns to ~0.6 microns.

11. EFFECT OF LABORATORY CONDITIONS ON E-CIGARETTE AEROSOL COLLECTION. Anthony BROWN, Karl Wagner, John Miller and Jason W. Flora; Altria Client Services, Richmond, VA USA

Smoking machines were first developed to generate smoke from tobacco cigarettes for the purpose of comparing cigarette tar and nicotine yields under consistent conditions. The International Organization for Standardization (ISO) specifies the atmosphere for the conditioning and testing of tobacco products in reference document ISO 3402:1999E. Conditioning is typically conducted for 48 hours and requires an atmosphere of $22\pm 1^{\circ}\text{C}$ and $60\pm 3\%$ relative humidity (RH). The testing atmosphere requires $22\pm 2^{\circ}\text{C}$ and $60\pm 5\%$ RH. No standardized environmental conditions or smoking regimes exist for e-cigarette aerosol collection. Therefore, the purpose of this work was to evaluate the effect of laboratory environmental conditions on the collection of e-cigarette aerosols using a consistent puffing regime. While temperature can typically be controlled in most laboratories, RH cannot. Therefore, RH was the primary focus of this investigation. Commercial e-cigarettes were puffed using a square wave profile for 4 seconds, 55 cc puff volume, and 30 second puff interval on a 20 port linear smoking machine. Twenty puffs were collected on conditioned Cambridge filter pads (CFP) and the aerosol mass (AM) collected was evaluated for total mass and concentration of nicotine, menthol, propylene glycol, glycerin, and water using gas chromatography with flame ionization and thermal conductivity detectors. Aerosol collection was conducted at $22\pm 2^{\circ}\text{C}$ with a %RH of 40, 60, and 80. Differences in analyte concentrations at the various RHs will be discussed.

12. METHOD FOR ALKALOID DETERMINATION IN TOBACCO THAT IS HIGHLY SELECTIVE, SENSITIVE AND SUITABLE FOR REGULATORY REPORTING. Anthony P. BROWN, Michael Morton, David Self, Jason W. Flora and Karl Wagner; Altria Client Services, Richmond, VA USA

Smokeless tobacco manufacturers have been required to annually report concentrations of nicotine in smokeless tobacco products (STP) sold in the United States to the Centers for Disease Control and Prevention (CDC) since 1999. The CDC protocol requires nicotine measurements conducted by gas chromatography with a flame ionization detector (GC-FID). Since the CDC protocol specifies a non-selective detector, new matrices and matrices with known interferences often require that calibration be performed by standards addition to accurately determine nicotine concentrations. The objective of the work was to validate and compare a more rapid, sensitive and selective method using GC with mass spectrometry detection (GC-MS) to the CDC's GC-FID protocol. Statistical analysis (*e.g.*, Schuirman's two one-sided tests (TOST) approach) determined that the two methods can be considered equivalent for the quantitation of nicotine in STPs and are thus both suitable for regulatory reporting. The GC-MS method had a broader calibration range (0.8-50 mg/g) compared to the CDC protocol (3.0-60 mg/g) and had no matrix interferences due to the selectivity of MS detection. The GC-MS method demonstrated a more than a tenfold increase in sensitivity. This method was validated for the quantitative analysis of nicotine as well as 3 additional alkaloids (nornicotine, anabasine, anatabine) in STPs, leaf tobaccos, cigarette filler, pipe tobacco and cigar filler.

13. DETERMINATION OF GAS-PHASE CARBONYLS IN E-CIGARETTE AEROSOL USING A SORBENT TUBE (EPA TO-11A) VS. AN IMPINGER COLLECTION. Celeste WILKINSON, James Wilkinson, John Miller and Jason W. Flora; Altria Client Services, Richmond, VA USA

The aerosols generated from e-cigarettes are primarily composed of fine particles of liquid and gas phases of the vaporized e-liquid. Low levels of thermal degradation products such as carbonyls (e.g., formaldehyde, acetaldehyde, acrolein, and crotonaldehyde) have been reported in e-cigarette aerosols. A rapid, selective and sensitive method specific to measuring carbonyls in e-cigarette aerosols using UPLC-MS has been developed. This method was optimized for aerosol collection using a 44mm Cambridge filter pad (CFP) followed by an impinger containing acidified 2,4-Dinitrophenylhydrazine (DNPH) to capture both liquid and gas phase carbonyls, respectively. While the use of CFPs and impingers are common for traditional cigarette smoke collection techniques, environmental air sampling techniques typically involve the use of sorbent tubes (e.g., DNPH impregnated silica) for the collection of gas phase carbonyls as described in the U.S. Environmental Protection Agency (USEPA) Compendium Method TO-11A. Therefore, this collection regime was evaluated as an alternative to the traditional impinger approach for gas phase collection. It was demonstrated that both methods are suitable for the collection of gas phase carbonyls in e-cigarette aerosols and they show equivalent trapping efficiencies. For 20 puff collections, it was observed that approximately 70% of the formaldehyde is trapped in the liquid phase on the CFP and approximately 30% is trapped in the gas phase by either the sorbent tube or the impinger. The sorbent tube collection had one major limitation. The tubes had inconsistent packing densities which could restrict air flow, thereby altering the puff volume. While there are no puff volume issues using the impinger method, sorbent tubes must be pre-selected based on packing density prior to aerosol collection.

14. PARTICLE BREAKTHROUGH COMPARISON OF COMMERCIAL CARBON FILTERS WITH CELFX™ CARBON/ACETATE FILTERS. R. M. ROBERTSON, J. N. Suthar and S. Basu; Celanese, Narrows, VA USA

Previous works have evaluated cigarette filter carbon particle release from various filter designs. The recently introduced CelFX™ Matrix Technology offers high loadings of carbon with low pressure drops and has not been studied for the particle release effect. This study will compare a filter with a CelFX™ segment against cigarette filter designs containing either a carbon-on-tow segment or a cavity segment. In all cases, a cellulose acetate segment is used as the mouth end. This study focuses on particle release from 0.125 micron to 5 micron. This particle range encompasses the respirable particles as listed by Organization for Economic Cooperation and Development (OCED) guidelines. Initial data shows CelFX™ carbon filters have significantly less particle release than a cavity filter for 0.125-5 micron size particle range. Commercial carbon-on-tow filters will also be evaluated.

15. CELFX™ CARBON TECHNOLOGY: PUFF-BY-PUFF PROFILE. R. M. ROBERTSON, J. N. Suthar and S. Basu; Celanese, Narrows, VA USA

CelFX™ carbon filter technology has been shown to reduce vapor phase smoke components by 40-90% in prior work¹. In order to expand the understanding of this filtration performance, this work will evaluate the puff-by-puff machine profile to assess the filter

performance on individual puffs. It is known that nicotine or tar delivery from the first puff is different than the last puff. This work will seek to assess the effect on that puff profile from presenting the carbon to the smoke in a much more efficient and higher loading format. Carbonyls and volatile compounds will be tracked and compared against a cellulose acetate filter control (Kentucky 3R4F cigarettes).

¹CelFX™ Matrix Technology Super Slim Filter comparison with commercial carbon filter, ST 31, Presented CORESTA Congress October 12-14, 2014, Quebec City Canada.

16. FDA COMPLIANT ANALYSIS OF NICOTINE PLUS NINE METABOLITES IN HUMAN URINE BY LC-MS/MS. Patrick MILLER, Ridha Nachi, G. Paul Brown, Veniamin Lapko, Christine Kafonek and Kirk Newland; Celerion, Lincoln, NE USA

With the U.S. Food and Drug Administration (FDA) having oversight for legal distribution and marketing of tobacco products, a rigorous approach to the analysis of tobacco-related compounds in biological samples for product assessment studies is prudent to ensure compliance with regulatory expectations. The measurement of total nicotine equivalents (*i.e.* nicotine plus its major metabolites) in urine is a direct and noninvasive method for assessment of human exposure to tobacco products. Selectivity for all of the analytical compounds in a method is paramount for accuracy and reproducibility. Although some methods may quantify nicotine and its metabolites from a single injection, the selectivity of such methods may not be sufficient for regulated bioanalysis (*i.e.* GLP-compliant).

A method for the determination of nicotine, cotinine, trans-3'-hydroxycotinine, and their N-, N-, and O-glucuronides (respectively) from 0.250 mL of urine has been validated with lower limits of quantification (LLOQs) of 10/10/10/10/20/50 ng/mL, respectively. Liquid-liquid and solid-phase extraction were combined with reversed-phase gradient chromatography and detection by multiple reaction monitoring (MRM) of ions on an AB Sciex API 4000™ triple quadrupole mass spectrometer for accurate and precise analysis with complete, FDA-compliant selectivity against trace urinary interferences. Development of a complementary method for analysis of nornicotine, norcotinine, nicotine oxide, and cotinine oxide, from 0.100 mL of urine with target LLOQs of 2/1/5/2 ng/mL, respectively, is in progress and nearing completion.

Concentrations of the analytes (or lack thereof) in smokers' and nonsmokers' urine, along with sample chromatography, are presented to demonstrate the accuracy, precision, sensitivity, and selectivity of the comprehensive methodology.

17. AN APPLICATION METHOD OF BACTERIOPHAGES AGAINST RALSTONIA SOLANACEARUM FOR PREVENTION TOBACCO BACTERIAL WILT. CAI Liu-ti, Lu Ning and Shi Junxion; Guizhou Duzhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

Tobacco bacterial wilt is one of the major diseases affecting flue-cured tobacco production in many countries and is caused by a complex pathogenic bacterial species, *Ralstonia solanacearum*. Preventing and controlling bacterial wilt is difficult because the pathogen contains many virulence factors, pathogenesis pathways and can exert many effects to kill plants. Traditional chemical control, rational crop rotation, resistant varieties and other

measures cannot effectively control the disease. The use of bacteriophages for bacterial control has a long history. Recently, a study on the application of phages to control the wilt diseases caused by *Ralstonia solanacearum* was reported. Here, we present an application method of bacteriophages against *Ralstonia solanacearum* for prevention of tobacco bacterial wilt. This method is very simple; the lytic bacteriophage solution was directly inoculated into the stem of tobacco with a sterile syringe needle, without pulling out the needle. Then the bacteriophage solution was covered with a drop of sterile mineral oil to prevent evaporation and pollution. After inoculation with bacteriophage ϕ PB2, it played a key role in protecting the tobacco K326 seedlings from bacterial wilt. Furthermore, plaque assay indicated that the bacteriophages were stable and survived more than 50 days at environmental temperature. Our results suggest that this method might be used as a potential new strategy for the control of bacterial wilt.

18. IDENTIFICATION OF NEW PHENYLPROPANOID GLYCOSIDES IN TOBACCO USING SOLID-PHASE EXTRACTION-GAS CHROMATOGRAPHY/MASS SPECTROMETRY WITH TRIFLUOROACETYLATION. CAI Kai, Zhao Huina, Xiang Zhangmin; Cai Bin, Pan Wenjie and Ledong Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

A novel method for the identification of new phenylpropanoid glycosides in tobacco was developed by gas chromatography-mass spectrometry (GC-MS) in electron ionization (EI) and negative chemical ionization (NCI). The glycosidic extract in tobacco was extracted with methanol, cleaned up with an Amberlite XAD-2 adsorption column. Glycosides were analyzed after enzymatic hydrolysis by GC-MS and directly after trifluoroacetylated (TFA) derivatization by GC-MS. In total four kinds of phenylpropanoid aglycones were identified by β -glucosidase hydrolysis method. Then, four kinds of phenylpropanoid glycosides were accurately identified by trifluoroacetylation. Among them, 2-Hydroxyphenylethanol β -D-Glucopyranoside, 4-Hydroxyphenylethanol β -D-Glucopyranoside and Homovanillyl alcohol β -D-Glucopyranoside were first identified in tobacco.

19. ANALYSIS OF AROMA COMPONENTS FROM MAINSTREAM CIGARETTE SMOKE USING LOW TEMPERATURE SOLVENT EXTRACTION FOLLOWED BY GC \times GC \times HR-TOFMS. XIANG Zhangmin, Geng Zhaoliang, Cai Kai, Zhou Shuping and Pan Wenjie; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

A method involving comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry was developed and applied to analysis of aroma components present in mainstream tobacco smoke trapped on self-designed equipment. The samples were prepared using low temperature solvent extraction under liquid nitrogen and analysed by comprehensive two-dimensional gas chromatography high-resolution time-of-flight mass spectrometry. Important experimental parameters, such as the type and volume of the extraction solvent and flow rate of smoking, were optimised to improve the analysis parameter. The results indicated that 180 mL of diethyl ether in the low temperature solvent extraction apparatus system with a 4 mL/min smoke flow rate were the optimal condition. Then, 112 aroma components were identified and quantified using a mass spectral library search. Finally, a comparison of the low temperature solvent extraction method and cambridge filter pad method indicated that more peak numbers, a higher peak volume and repeatability were obtained using the low temperature solvent extraction method.

20. APPLICATION OF LINEAR MODELS TO LINK CIGARETTE YIELDS DELIVERED UNDER ISO AND INTENSE REGIMES. Rémi JULIEN, Thomas Verron, Xavier Cahours and Stéphane Colard; SEITA - Imperial Tobacco Group, Fleury-Les-Aubrais, France.

Since it was first requested to measure and to report tar and nicotine in a limited number of countries, observation is made of an increasing trend for more testing and reporting requirements. From a single regime and two smoke yields, recent recommendations include utilising two smoking regimes and measuring up to 93 tobacco and smoke constituents.

Considering the increase in the number of data generated and the similarities between certain smoke constituent formations during combustion, it is worth exploring possible correlations between emissions. In that purpose, 90 smoke constituents have been analysed under ISO and intense regimes for 23 different brands as well as 36 tobacco constituents and 17 cigarette design parameters; in total 5359 data points were available for statistical evaluation.

A comprehensive search for the best subsets of up to 5 explanatory variables for predicting ISO smoking regimes in linear regression have been performed using an efficient branch-and-bound algorithm. Best models of all sizes have been selected using different classical criteria such as correlation coefficient, Akaike Criteria (AIC) or Bayesian Criteria (BIC).

It was then observed that many yields were predictable from a limited number of variables and consequently that a reduced number of tests could have been carried out without loss of information on product characteristics.

21. DIVERSITY AND DISTRIBUTION OF FUNGAL ENDOPHYTES IN *NICOTIANA TABACUM*. WANG MangSheng, Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

The diversity of culturable endophyte microfungi in root, stem, and leaf tissues originating from six flue-cured tobacco varieties (*Nicotiana tabacum*) in Guizhou province was surveyed. Our aims were to describe the distribution in different varieties and tissues of isolated endophyte species to assess their specificity and abundance. Microfungi were isolated from surface-sterilized tissues, and the strains were identified based on molecular characteristics of analyses of the ITS1 and 26S rDNA D1/D2 sequence. One hundred and fifty strains belonging to 17 different taxa, dominated by the asexual states of *Fusarium oxysporum* Schlecht., *Chaetomium globosum* and *Penicillium janthinellum*, were obtained from the 221 samples. *Gibellulopsis nigrescens* was found cited for the first time as an endophyte. The highest species richness and the Shannon index (H') index were found in Yunyan 85 ($S=9, H'=1.99$) and Guiyan No.1 ($S=9, H'=1.92$), followed by K326 ($S=7, H'=1.71$) and Dizhi101 ($S=7, H'=1.5$), and the lowest for Changbohuang ($S=6, H'=1.43$) and Honghuadajinyuan ($S=5, H'=1.36$). The highest species richness and the Shannon index (H') index in different parts of tobacco was roots ($S=10, H'=1.78$), then stems ($S=8, H'=1.75$) and the lowest was leaves ($S=7, H'=1.44$). Research of endophytic fungi in tobacco in this work not only enriched understanding of microbial resources, but also played an important role in revealing the host plant resistance.

22. THE DETERMINATION AND COMPARISON OF AMMONIA CONTENT IN TOBACCO PRODUCTS BY ION CHROMATOGRAPHY AND CONTINUOUS FLOW ANALYSIS. Andy STINSON¹, Sherry Gilliland¹, Amelia M Paolantonio², Thomas D. Lockhart² and I. Gene Gillman²; ¹Liggett Group LLC, Mebane, NC USA and ²Enthalpy Analytical Inc., Durham, NC USA

The FDA Center for Tobacco Products (CTP) has identified ammonia as a harmful or potentially harmful constituent (HPHC) in tobacco and tobacco products. However, there is currently not a single, generally accepted method for the determination of ammonia in tobacco products. For this study, three methods were used - ion chromatography by CORESTA Recommended Method (N°73), a modified version of CORESTA Recommended Method (N°73) using a C-16 column, and Continuous Flow Analysis (CFA). All these methods are routinely used for the quantification of ammonia in tobacco and tobacco products. We will present the ammonia concentration determined in 3R4F blend, CM7 blend and CRP3 smokeless tobacco by each method. The results for each method will be compared along with the advantages and disadvantages of each analytical technique.

23. THE DETERMINATION AND COMPARISON OF AMMONIA CONTENT IN MAINSTREAM TOBACCO SMOKE BY ION CHROMATOGRAPHY AND SPECTROPHOTOMETRIC METHODS. Andrew J. HUCKINS and Andy Stinson; Liggett Group LLC, Mebane, NC USA

The FDA Center for Tobacco Products (CTP) has identified ammonia as a harmful or potentially harmful constituent (HPHC) in tobacco and tobacco products. However, there is currently not a single generally accepted method for the determination of ammonia in mainstream tobacco smoke. Several analytical techniques are commonly used to measure ammonia, including Ion Chromatography (IC) with a conductivity detector, LC-MS/MS and spectrophotometric detection. For this study, two methods were used, ion chromatography and spectrophotometric detection. We will present the ammonia concentration determined in each method in the mainstream smoke from 3R4F, CM7 and 1R5F reference cigarettes under both ISO and Canadian Intense smoking regimes. The results for each method will be compared along with the advantages and disadvantages of each analytical technique.

24. NRF2 RESPONSE TO WHOLE SMOKE IN THREE DIMENSIONAL (3D) AIRWAY CULTURES. Wanda R. FIELDS, Brian M. Keyser and Betsy R. Bombick; R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

Lung diseases are frequently accompanied by molecular changes, including those associated with the Nrf2 signaling pathway. Cigarette smoke has been shown to activate this pathway in lung tissue. The goals of this study were to assess the effect of cigarette smoke on the Nrf2 promoter and on genes associated with oxidative stress, inflammation and metabolism in human 3D EpiAirway™ tissue models (MatTek, Inc.). Whole smoke (WS) exposures (8 – 64 minutes; 8 minutes/cigarette) with Kentucky Reference 3R4F cigarettes were conducted under ISO conditions using the VITROCELL VC1® smoke exposure system. Viability and tissue integrity were assessed with the lactase dehydrogenase and transepithelial electrical resistance (TEER) assays, respectively. Nrf2 promoter activation was determined by a luciferase assay, while gene expression changes were assessed via QRT/PCR at 6, 12, 18, or 24 hours post-exposure.

Dose-dependent decreases in viability and tissue integrity were observed. Cell viability was > 90% for exposures up to 32 minutes (4 cigarettes); while a maximum response of 38% viability and diminished TEER were observed for the 64 minute (8 cigarettes) treatment group. Therefore, luciferase and gene expression studies were conducted between 8 and 32 minutes. Time- and dose-related increases in Nrf2 promoter activation were observed with levels exceeding 200-fold at the 12 and 18 hours post-exposure time-points, and the Nrf2 promoter was also differentially regulated in WS as compared to gas vapor phase exposures. Statistically significant increases ($p < 0.05$) ranging from 2 to >100-fold were observed across the time-course for genes associated with oxidative stress, inflammation and metabolism.

Collectively, the data indicate that putative biomarkers of effect in the Nrf2 signaling pathway were responsive to cigarette smoke exposures in human 3D EpiAirway™ tissue models. These models may be useful in evaluating tobacco and aerosol exposures and may further understanding of the biological relevance of the responses.

25. ENGINEERING TOBACCO FOR RESISTANCE TO TOMATO SPOTTED WILT VIRUS. Muqiang GAO and David Zaitlin; KTRDC, University of Kentucky, Lexington, KY USA

Tomato Spotted Wilt Virus (TSWV) ranks among the top 10 most economically important plant viruses, accounting for >\$1 billion in annual crop losses. TSWV routinely causes extensive damage to crops in the southeastern US. Genetic resistance is known in tomato and pepper but not in commercial tobacco (*Nicotiana tabacum* L.). Therefore, we investigated the use of RNA interference (RNAi) to introduce TSWV resistance into tobacco and *Nicotiana benthamiana*. TSWV is a complex RNA virus with a tripartite genome consisting of small (S; 2.9Kb), medium (M; 4.8Kb), and large (L; 8.9Kb) single-stranded RNA molecules that encode five proteins (S1, S2, M1, M2, L1 genes). We made hairpin RNAi constructs for all five TSWV genes using the pHellsgate8 binary vector. Transgenic plants were obtained via Agrobacterium-mediated transformation. T0-generation plants were inoculated with TSWV to screen for virus resistance.

For flue-cured tobacco variety K326, seven S1 lines, six S2 lines, seven M1 lines, two M2 lines, and nine L1 resistant transgenic lines were obtained. Two of the M2 transgenic plants displayed only weak resistance to TSWV infection.

For the burley variety KY14, we obtained seven S1, three S2, 11 M1, and six L1 resistant transgenic lines. For *N. benthamiana*, five S1, four S2, seven M1, three M2, and two L1 resistant transgenic lines were obtained. Three of the M2 transgenic plants expressed weak resistance to TSWV. TSWV inoculation of T1-generation plants showed that virus resistance was segregating in all T1 progeny sets.

PCR assays showed that all resistant plants carry both the forward and reverse fragments of the RNAi constructs. Also, results of DAS-ELISA showed that the resistant plants were TSWV-free or contained low levels of the virus.

26. CONSIDERATIONS FOR AUTOMATION OF STANDARDIZED METHODS FOR TOBACCO/SMOKE INVESTIGATIONS. Julian A. COX and Justin Lu; Sirius Automation Inc, Buffalo Grove, IL USA

Much of tobacco & smoke research comprises repetitive implementations of standardized methodologies and/or the appropriation of well-defined technologies to novel requirements.

Additionally, regulatory compliance is now an undeniable feature of the industry and is recognized as being of huge import moving forwards.

Laboratory automation is therefore key to both challenges; If a task is completed by robotic means, both the actions and the data trail is viewed by regulators in a different (and usually more positive) light than by equivalent manual methods.

Topics discussed will include the challenges of different method types typified by examples of extraction/concentration vs 'smoke & report' investigations; How automation can be used to ease ratification of the requirements of 21CFR11 and similar rationales; Considerations of approaches to verification dynamics; and how to ensure 'quality data' with associated considerations for long term storage and accessibility.

27. INTEGRATE MEASURES BIO-CONTROL TOBACCO BACTERIAL WILT AND REMEDIATE TOBACCO MONO-CROPPING LAND, LIU Yanxia, Li Xiang, Cai Liuti and Shi Junxiong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Tobacco mono-cropping obstacle caused a lot of serious problem in China, leading to the burst of soil-borne diseases, such as tobacco bacterial wilt. In order to find an environment-friendly method to control tobacco bacterial wilt and remediate tobacco mono-cropping land, a four-year field experiment was conducted to evaluate the bio-control efficacy. Four treatments were designed: T1, chemical fertilizer application; T2, chemical fertilizer plus bioorganic fertilizer application; T3, deep plough before chemical fertilizer plus bioorganic fertilizer application; T4, deep plough and quick lime application before chemical fertilizer plus bioorganic fertilizer application. Biolog-ECO was provided to contrast rhizosphere soil bacterial functional diversity of four treatments. The paired-end sequencing of soil 16S rDNA revealed the bacterial community structure. The results obtained were listed as follows. The bio-control efficacy of tobacco bacterial wilt in the T4 treatment was 71.7%. In contrast to the T1 treatment, the counts of bacterial and actinomycetes increased while the populations of fungi and *Ralstonia solanacearum* decreased in rhizosphere soil of the T4 treatment. The bacterial functional diversity of the T2, T3 and T4 treatments significantly increased, compared with the T1 treatment. Besides, according to the soil abundance clustering map, the evenness index of the T4 treatment was higher than that in the T1 treatment, suggesting that there were less dominant microorganisms when the soil was treated by integrate measures. The phylum distribution varied under different treatments. In conclusion, the integrate measure, as deep plough and quick lime application before chemical fertilizer plus bioorganic fertilizer application, can efficiently control tobacco bacterial wilt and remediate tobacco mono-cropping land. It is a promising method for the long-term development of tobacco soil.

MONDAY AFTERNOON, SEPTEMBER 21, 2015

SESSION A – Methods Development

2:20 PM MONDAY

28. DETERMINATION OF NINE VOLATILE NITROSAMINES AND HYDROXY-NITROSAMINES IN CIGARETTE FILLER AND MAINSTREAM TOBACCO SMOKE USING ISOTOPE-DILUTION LC-MS/MS. Mehran SHARIFI, Peter Joza and William Rickert; Labstat International ULC, Kitchener, ON, Canada

Volatile nitrosamines (VNA) and N-nitrosodiethanolamine (NDELA) in tobacco and tobacco smoke have been traditionally quantified using multi-step procedures involving either chemoluminescence (GC-TEA) or more recently mass spectrometric (GC-MS) techniques. The high cost and complexity associated with these procedures prompted an investigation into alternative analytical approaches. As a result, a new liquid chromatography/tandem mass spectrometric method was developed utilizing LC-MS/MS for the analysis of nine nitrosamines and NDELA in cigarette filler and mainstream smoke.

VNA were collected by passing mainstream tobacco smoke through two traps, each containing 25 mL of an ammonium sulfamate /sulfuric acid buffer solution, followed by a treated Cambridge filter pad. Isotopically labeled nitrosamines, used as internal standards, were added to the trapping solution then used to extract the filter pad (30 minutes, wrist-action shaker). For tobacco filler, the internal standard mixture was added to the sample, extracted with 50 mL of water at 40°C for 16 hours, then acidified using ammonium sulphamate/sulphuric acid and saturated with ammonium sulphate. In both cases (smoke and filler) an aliquot of the extract was partitioned using a ChemElut® cartridge with ethyl formate containing 2% ethanol as eluent. Further clean-up was required for the hydroxyl-nitrosamines, consisting of a florisil SPE clean-up of the extract using methanol as eluent. The solvent was evaporated and the concentrated sample analyzed by LC-APCI+-MS/MS, with the analyzer operating under multiple reaction monitoring mode.

The method showed excellent recovery with values ranging from 98 to 111% for lab fortified matrix samples, with a good precision (ca. 10%). The method limits of quantification range between 1-10 ng/cig under ISO smoking regimen and 0.2-0.4 ng/g in cigarette filler.

2:40 PM MONDAY

29. A SINGLE STEP SOLID-PHASE EXTRACTION METHOD FOR GC/MS ANALYSIS OF AROMATIC AMINES IN MAINSTREAM CIGARETTE SMOKE. Chorng B. HUANG, Jason W. Flora and Karl Wagner; Altria Client Services, Richmond, VA USA

As stated in the Federal Register (Vol. 77, No. 64) Docket No. FDA-2012-N-0143, Aromatic Amines (AAs) are included in the “Established List of the Chemicals and Chemical Compounds Identified by FDA as Harmful and Potentially Harmful Constituents [HPHCs] in Tobacco Products and Tobacco Smoke.” To date, no standardized method for AA determination in mainstream cigarette smoke has been developed. The two previously reported techniques for AA determination require either 2 or 3 solid phase

cartridge extractions and the use of a solid phase extraction (SPE) manifold because of the complexity of the matrix and the trace amounts of AAs in cigarette smoke (ng per cigarette). These multistage techniques are time consuming and difficult to automate. The purpose of this work was to develop a simplified and automated extraction technique for 3 AAs (1-aminonaphthalene, 2-aminonaphthlene and 4-aminobiphenyl) in mainstream cigarette smoke without compromising analyte recovery. The method uses solid-phase mixed-mode cationic exchange cartridges (e.g., Waters Oasis® MCX or Phenomenex Strata™-X-C), is compatible with the RapidTrace® automation, eliminates multiple classes of interferences and samples can be analyzed by gas chromatography with mass spectrometry (GC/MS). Recovery of AAs was evaluated using labeled internal standards and ranged from 48.2 to 52.4% for 1-aminonaphthalene, 2-aminonaphthlene and 4-aminobiphenyl with the cigarette smoke matrix and ranged from 77.7 to 85.0% without the sample matrix. Recovery yields were consistent with the previously proposed methodologies.

3:00 PM MONDAY

30. APPLICATION OF NEAR INFRARED SPECTROSCOPY TO DETECT MOLD CONTAMINATION IN TOBACCO. YANG Lei^{1,3}, Hou Ying¹, Li Jing-Jing¹, Li Wei¹, Wang Jia-Jun², Yang Qian-Xu², Wang Bao-Xing² and Pan Xue-Jun³; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China, ²China Tobacco Yunnan Industrial Co., Ltd., Kunming, Yunnan, China and ³Kunming University of Science and Technology, Kunming, Yunnan, China

Mold infection is a significant postharvest issue for processors of tobacco, which can cause value reduction of product and direct product loss. However, mold is mostly undetectable at early stages by traditional sorting techniques. In this paper, Near-Infrared (NIR) spectroscopy technique used in detection of the percentage of mold infection in tobacco samples was studied. A good to bad (GBA) algorithm for feature selection with visual analysis grading Linear Discriminant Analysis (LDA) routines was applied, which achieved low classification error rates as 2.92% of total error, with a Wilk's λ 0.216 ($P < 0.001$). The optimal features corresponded to Abs [1066 nm], Abs [1130 nm], Abs [1832 nm], and Abs [1474 nm]. The accurate classification of 5.43% unmold and 3.06% mold (0.00% slight mold, 0.00% low mold, 5.00% medium mold and 7.14% high mold) error was achieved. According to the results, the sorting system developed based on multispectral NIR bands showed the potential for rapid detection and removal of mold contaminated tobacco as well as the reduction of the incidence of early mold contamination in tobacco lots.

KEYWORDS: Mold contaminated tobacco; Near-Infrared spectroscopy; Feature selection; GBA algorithm

3:50 PM MONDAY

31. CARBON MONOXIDE AND NITROGEN OXIDE DIFFUSION THROUGH CIGARETTE PAPER II. Joseph WANNA; SWM Intl., Alpharetta, GA USA

Cigarette paper plays a critical role in carbon monoxide (CO) diffusion during the smoking process. Introducing bands with low diffusion to comply with LIP regulations reduce CO

diffusion through these bands. Upon the request by SWM a custom made test that measures CO diffusion through the bands and paper during the smoking process was assembled by Arista Laboratories. The device can be used to measure CO diffusion through cigarette paper at various positions along the tobacco column during cigarette puffing in an ISO or Intense smoking regime. It was successfully demonstrated and results were reported at the 68th TSRC conference¹.

In this work ten experimental cigarettes with different band composition, diffusion, and base paper properties were selected. Band diffusion ranged from 0.05 cm/sec to 0.18 cm/sec and base diffusion from 1.1 cm/sec to 2.0 cm/sec. The base paper properties that were selected for this study had different porosity, filler, and citrate levels. Nitrogen oxide (NO) diffusion was also measured through the bands and base paper surfaces with a method that is already available at Arista laboratories. This paper will discuss the impact of band and base paper properties on CO and NO diffusion.

¹Wanna, J. "Carbon Monoxide (CO) Diffusion Through Cigarette Paper," 68th Tobacco Science Research Conference, Paper #58, September 2014

4:10 PM MONDAY

32. IGNITION PROPENSITY OF CIGARETTES ACCORDING TO ISO 12863 USING TWO DIFFERENT SUBSTRATES. Mario MAYR; delfortgroup / Papierfabrik Wattens GmbH & Co KG, Austria

There are two standards available to determine the ignition propensity of cigarettes: ASTM E.2187-09 and ISO 12863. One of their main differences is that ISO 12863 allows using other substrate materials as an alternative to Whatman No.2 filter paper as long as such substrates are equivalent. The target of this study was to determine if an alternative substrate LIPCan filter paper produced by Tervakoski of delfortgroup would be a proper candidate for a substrate. These filter papers comply with the requirements in section 7.3.2 in ISO 12863. Four different lower ignition propensity cigarettes with two different styles, king size and super slim, were used in this study. The cigarettes were tested according to ISO 12863 on Whatman No.2 and LIPCan filter paper. The results show that every cigarette brand passed the test according to ISO 12863 and ASTM E.2187-09 on Whatman No.2 and LIPCan filter paper.

In conclusion the results show that within the typical variation of the test according to ISO 12863 LIPCan filter paper is equivalent to Whatman No.2 as no statistical difference between the results obtained with two substrates could be found at statistical significance levels of 95% or 99%.

4:30 PM MONDAY

33. THE HEAT OF VAPORIZATION OF NICOTINE FROM TOBACCO. E. Kelley ST.CHARLES¹ and Serban Moldoveanu²; R. J. Reynolds Tobacco Co., ¹Lewisville, NC USA and ²Winston-Salem, NC USA

The enthalpy of vaporization (ΔH_{vap}) has been reported, or can be derived from published

data for pure nicotine, but no published value exists for nicotine in a tobacco matrix. We measured the nicotine headspace concentration for pure nicotine and for tobacco stored at 23, 30, and 40°C. This allowed for conversion to vapor pressure and for the estimation of ΔH_{vap} . Burley, flue-cured, oriental and cigarette blends were tested. Experiments were conducted with pure nicotine to allow comparison with previously published values of vapor pressure to validate the experimental technique. The ΔH_{vap} for pure nicotine was determined to be 56.6 kJ/mol with a standard error of 1.2 kJ/mol. For tobacco, the ΔH_{vap} ranged from 77 kJ/mol to 92 kJ/mol with no obvious trends due to tobacco origin, type, stalk position or tobacco nicotine concentration. The mean value for all tobacco types combined was 86.7 kJ/mol with a relative standard deviation of 6.5% indicating that this was an intrinsic property of the nicotine form in tobacco rather than the specific tobacco properties. This value was about 30 kJ/mol greater than that of pure nicotine and is similar to the energy needed to remove a proton from monoprotonated nicotine.

4:50 PM MONDAY

34. A QUANTITATIVE ANALYSIS OF TERTIARY AMINES IN TOBACCO LEAVES. Kei KOBAYASHI, Atsushi Nagai and Shinsuke Sato; Japan Tobacco Inc., Yokohama, Japan

The aroma and taste of cigarette smoke, and tobacco aroma, are affected by many components in tobacco leaves. Some of these components give a strong aroma, even in small amounts. Trimethylamine (TMA) and N-methylpyrrolidine (NMP) are classified as tertiary amines, and are known to be contained in tobacco leaves. TMA gives off a foul 'fish-like' odor, and has a low detection threshold. NMP also gives off a foul odor that smells like ammonia. There is a possibility that they affect the cigarette aroma/taste and the tobacco aroma. There are reports based on the qualitative analysis of TMA and/or NMP, but not many quantitative reports, because an easy-to-use and reliable quantitative analytical method has not yet been developed. Therefore, it is not clear if either component is an important element affecting the tobacco aroma.

In this study, we developed a quantitative analysis method to analyze these amines in tobacco leaves, and the amount of TMA and NMP in several types of tobacco leaves was quantified. In this method, the above-mentioned amines were derivatized into quaternary amines with tert-butyl bromoacetate, and analyzed by using tandem mass spectrometry coupled with liquid chromatography. As a result, this method achieved high sensitivity LOD/LOQ: TMA(0.39, 1.31 [ng/mL]), NMP(0.96, 3.21 [ng/mL]), high accuracy (Recovery: 80-110%), and an intra-day/inter-day error rate of under 3%. By using this analytical method, TMA and NMP in several types of tobacco leaves were measured. As a result, the diversity of the contents in different leaves was clarified. This presentation will report on the detail of an analytical method, the content of tertiary amines in tobacco leaves, and how additional factors, from cultivating to curing, affect that content.

5:10 PM MONDAY

35. QUICK MOISTURE MEASUREMENT IN THE LABORATORY WITH THE MICROWAVE RESONANCE METHOD. André TEWS; TEWS Elektronik GmbH & Co. KG, Hamburg, Germany

Not Presented

Moisture measurement is a basic quality check in the tobacco laboratory because water has a big influence on the properties of tobacco and on the properties of the finished cigarettes.

In practice, moisture measurements are carried out by several methods. There are basic differences between these methods -- some methods are direct measuring methods while others are indirect and have to be calibrated. Some methods are water selective, others determine the volatile parts of the tobacco. There is a big difference in time consumption between the different techniques.

The different methods of moisture measurement in the tobacco laboratory are discussed. One of the methods, the microwave resonance technique, is brought into focus and possibilities of the application in the tobacco laboratory are introduced and measuring examples are shown.

MONDAY AFTERNOON, SEPTEMBER 21, 2015

SESSION B – E-Cigarettes

2:20 PM MONDAY

36. SECONDHAND EMISSIONS IN AN ENVIRONMENTAL CHAMBER AFTER E-CIGARETTE VAPING. John W. CARAWAY, Paul R. Nelson, Tao Jin and Eckhardt Schmidt; R. J. Reynolds Tobacco Company, Winston-Salem, NC USA

Selected secondhand smoke (SHS) constituents were evaluated in a 28 m³ unventilated environmental chamber after e-cigarette vaping or combustible cigarette smoking. Subjects were enrolled into 3 e-cigarette groups (VUSE Original, VUSE Menthol, and a market sample) and 2 cigarette groups (leading non-menthol and leading menthol brands). Each group consisted of 11-12 subjects. Ten test sessions were conducted for each group (5 smoking/vaping and 5 non-smoking/non-vaping). Four subjects from a group were randomly selected to participate in each session. Sessions started with a 10-min background collection after subjects entered the chamber, followed by a 10-min ad libitum smoking/vaping period (or non-smoking/non-vaping). Subjects then exited the chamber, and a 120-min sample collection period was initiated. Real-time data collection was also performed throughout the entire test session. Airborne concentrations of 25 SHS constituents were determined using methods validated according to the principles of GLP. Analytes included formaldehyde, acetaldehyde, nicotine, benzene, toluene, glycerin, propylene glycol (PG), PM_{2.5}, carbon monoxide and ammonia.

By design, test conditions resulted in SHS concentrations much higher than expected in real-world smoking environments. Even under these conditions, most SHS constituent

levels after e-cigarette vaping were not statistically significantly different than after sessions without vaping. Generally, e-cigarette SHS levels were at least 95% lower than cigarettes. PG levels after vaping were higher, lower, or no different than after smoking, depending on the product comparison. Glycerin concentrations after vaping were higher than cigarette smoking. Although e-cigarette secondhand nicotine concentrations were higher than the non-vaping blank, they were 88-99% lower than cigarettes. The results demonstrate that consumption of the most commonly used cigarette-like e-cigarettes will have negligible impact on air quality in most indoor environments.

2:40 PM MONDAY

37. ARE CHEMICAL CONSTITUENTS EXHALED IN A ROOM WHERE E-VAPOR PRODUCTS ARE USED? Mohamadi SARKAR¹, Jianmin Liu¹, Qiwei Liang¹, Xuejun Peng¹, Michael Oldham¹, Ali Rostami¹, Karl Wagner¹, Gene Gillman² and Anne Marie Salapatek³; ¹Altria Client Services Inc., Richmond, VA USA, ²Enthalpy Analytical Inc., Durham, NC USA and ³Inflamax Research Inc., Toronto, Canada

A controlled clinical study was conducted to determine potential constituents in the atmosphere where e-vapor products and cigarettes were used. Levels of nicotine, propylene glycol (PG), glycerol, 15 carbonyl compounds (including formaldehyde), 12 volatile organic compounds, and 4 trace metals were measured using ISO or EPA methods. Exhaled breath, room air and surface samples were investigated. The products used were MarkTen® 2.5% Classic (M10), a Prototype GreenSmoke® 2.4% (GS), Ego-T Tank with subjects' own e-liquids (Tank) and subjects' own conventional cigarettes (CIG). Exhaled breath samples (EBS) were collected at baseline (sham use) and with test products from 37 subjects (23 males and 14 females). Room air measurements were made in a controlled exposure chamber (EC). Products (M10 and GS) were used under controlled conditions (10 puffs/person, once/30 minutes for 4 hours) and ad lib use (all four products). Baseline measurements (without product use) were made in the EC for a 4-hour period, and background measurements were conducted without people in the EC. Room air levels of nicotine, PG and glycerol, under both controlled and ad lib use, were several-fold below the current published limits for workplace exposure to airborne contaminants. Room air formaldehyde levels from M10, GS and Tank systems were similar to the background and baseline. Most of the other constituents measured were below the limit of quantification during M10, GS and Tank use. Significant levels of most constituents were observed during CIG use. Under the study conditions, for the e-vapor products tested, the few chemical constituents exhaled are several-fold below the permissible limits. The results from surface sample measurements suggest that third-hand exposure to nicotine is unlikely.

3:00 PM MONDAY

38. DETERMINATION OF EMISSION FACTORS FOR CIGARETTES AND ELECTRONIC CIGARETTES. Paul R. NELSON, John W. Caraway, Tao Jin and Eckhardt Schmidt; R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

An emission factor is the amount of a specific compound produced by a product relative to the amount of product used. For combustible or electronic cigarettes, units may typically be mass/cigarette, mass/use interval, or mass/ml e-liquid. They are a key input for indoor

air quality models to estimate potential contaminant concentrations in indoor spaces where a product is used.

Emission factors are typically determined from measurements made in a controlled environmental test chamber. Determining the peak concentration of analytes collected over time in a minimally ventilated chamber presents a challenge for accurately determining emission factors. If the emission factor is calculated from the average concentration, emissions will be underestimated due to some material escaping the chamber over the sampling period. Carbon monoxide (CO) has been used previously as a surrogate compound to determine a correction factor (peak to time-weighted average ratio) for sample loss. However, this correction cannot be made for electronic cigarettes because e-cigarettes do not yield CO. A simple method for predicting analyte peak concentrations will be described, where CO₂ decay rate is used to determine the chamber ventilation rate. The ventilation rate is then used to calculate a correction factor for relating peak concentration to time-weighted average concentration.

This method was utilized in the determination of emission factors for tobacco-burning and electronic cigarettes in a clinical study. Emission factors for the analytes that were present above background levels were determined from two leading brands of cigarettes and e-cigarettes. The emission factors can be applied in models to predict the impact of electronic cigarettes use on indoor air quality.

3:50 PM MONDAY

39. COMPUTATIONAL TOOL FOR ESTIMATING INDOOR AEROSOL/VAPOR CONCENTRATION. Ali A. ROSTAMI, Yezdi Pithawalla, Mohamadi Sarkar and Jianmin Liu, Altria Client Services Inc., Richmond, VA USA

E-cigarette use is a potential source of particulate matter and chemical exposure in indoor spaces such as cars, homes and offices. A fraction of the aerosol inhaled by the e-cigarette users is exhaled into the space. A computational model has been developed to predict changes in particle size and concentration of chemical constituents over time. The model is based on the thermodynamic vapor/liquid equilibrium for tracking phase partitioning and overall mass balance of each chemical constituent. The results from the first principle model have been validated using published experimental data for machine generated aerosols. The model prediction for the nicotine concentration in the room averaged over 12 runs is within 5% of the experimental value. Further validation will be carried out as data from human studies become available.

Modeling results indicate that the concentration of chemicals drops substantially as the room size and ventilation rate are increased. The results from the model also show that particle size drops rapidly due to evaporation of volatile compounds from the particles. A sensitivity analysis has shown that by increasing the frequency and volume of aerosol release into the room, the concentration of chemicals might reach an upper plateau at low ventilation rate. The model may be used to estimate the indoor level of various chemicals and aerosol concentrations at different release rates, room sizes, air ventilation rates, aerosol compositions, and room and ventilation air temperatures and humidity. It can also be

used to compare different devices and/or different liquid formulations in terms of room concentration of chemicals of interest.

4:10 PM MONDAY

40. TOXICANTS IN BIOFLUIDS ORIGINATED FROM STABLE ISOTOPES OF PROPYLENE GLYCOL IN ECIGARETTES. Raymond H. FARMEN, Kirk E. Newland, Ridha Nachi and Chris J. Kafonek; Celerion, Lincoln, NE USA

The power of using a stable isotope analog of propylene glycol (13C3-PG) in eCigarettes was demonstrated during the TSRC last year. From a clinical study we monitored the pharmacokinetics of propylene glycol (PG), 13C3-PG and nicotine in both vapers and non-vapers. The data demonstrated that the second-hand exposure of non-vapers to 13C3-PG was 1/5000 that of the vapers. We selected 13C3-PG instead of deuterated PG as our stable isotope analog because 13C3-PG might afford us the opportunity to monitor metabolites, degradants, or toxicants, such as 13C2-acetaldehyde and 13C1-formaldehyde in biofluids.

In a letter to the New England Journal of Medicine entitled “Hidden Formaldehyde in e-Cigarette Aerosols”, the authors reported the detection of formaldehyde hemiacetal in the aerosolized liquid from eCigarettes under high voltage conditions. Detection of compounds related to known toxicants in eCigarette vapor increases the importance of measuring toxicants from 13C3-PG in biofluids to determine exposure levels under typically pleasurable vaping conditions.

The measurement of acetaldehyde and formaldehyde in biofluids presents many bioanalytical challenges. First, their small atomic mass and highly polar nature make them very susceptible to interference and matrix effects using traditional LC-MS/MS. Second, aldehydes are chemically reactive with nucleophilic groups such as amines. And third, there are 19 aldehyde dehydrogenase (ALDH) isoforms in humans. While most ALDH isoforms are intracellular, some ALDH activity may be present in plasma which would adversely affect acetaldehyde and formaldehyde stability. Consequently, we chose to determine the exposure level of 13C2-acetaldehyde and 13C1-formaldehyde in plasma using their glutathione conjugates.

4:30 PM MONDAY

41. A NOVEL NICOTINE SUBLINGUAL TABLET WHICH MIMICS CIGARETTE SMOKING NICOTINE PHARMACOKINETICS. John MCCARTY¹, Frank J. Vocci² and Matt Torrington²; ¹IntraTab Labs Inc, Miami, FL USA and ²Friends Research Institute Inc., Baltimore, MD USA

Very little by way of new nicotine products have been introduced into the market, despite scientists' and clinicians' pleas specifically for a faster-acting product that resembles nicotine pharmacokinetics as produced by cigarette smoking. Pharmacokinetic (PK) data obtained from a novel nicotine sublingual tablet demonstrates that such a product has now been developed. Our novel nicotine tablet uses a patented drug delivery technology that enables very rapid, accurate and repeatable delivery of drugs transmucosally from a small tablet placed sublingually in the mouth. A pharmacokinetic study compared the

novel nicotine tablet to the same strength nicotine lozenge in an open-label, randomized, crossover design in 6 healthy, heavily-dependent smokers participating in both arms of the trial. The outcomes measured were time to maximum nicotine plasma concentration (T_{max}), maximum plasma concentration (C_{max}) and the area under the nicotine plasma curve (AUC) which was compared between the novel nicotine tablet and the lozenge. The novel nicotine sublingual tablet had rapid absorption into the plasma with a median T_{max} = 17 minutes as compared to 84 minutes for the nicotine lozenge. The mean C_{max} values between the treatment groups were not statistically different having a p-value of 0.56. The mean AUC₀₋₁₈₀ values were statistically different with a p-value 0.03 with the lozenge having greater exposure. This novel sublingual tablet promises to provide the smoker with a real unmet medical need for a safe, convenient, inexpensive nicotine replacement therapy that mimics nicotine pharmacokinetics obtained from cigarette smoking. This pilot PK study was funded by NIH through a Phase I STTR NIDA Grant (1R41DA033710-01A1).

4:50 PM MONDAY

42. ASSESSING THE HEALTH EFFECTS OF LAUNCHING E-CIGARETTES USING SYSTEM DYNAMICS: A UK CASE STUDY. Ian M. Fearon¹, Oscar M. CAMACHO² and Andrew Hill²; ¹British American Tobacco (Investments) Limited, Southampton, UK and ²Ventana Systems, Salisbury, UK

Introduction: The introduction of electronic cigarettes (e-cigarettes) is a potential approach to tobacco harm reduction. In Britain, around 2.1 million adults use e-cigarettes, one-third are ex-smokers and two-thirds are smokers. Here we describe the development of a population model to assess the impact of the introduction of e-cigarettes in the UK using all-cause mortality as an endpoint.

Methods: We used a system dynamics approach for model development. A status quo scenario with e cigarettes in the UK was compared against a counterfactual scenario where e-cigarettes had never existed. Data informing the model were obtained from public sources and bespoke studies.

Results: Data gaps and inconsistencies between different public data sources were significant, especially among age categories when most smoking experimentation and initiation occurs. Consequently, assumptions were introduced to inform the model such as: English and British data can be extrapolated to the rest of the UK; relapse rates are not age specific; and the relative risk between different smoking statuses is 1, up to age 35 years. Smoking prevalence appeared to model estimates in agreement with historic data.

Conclusions: E-cigarettes may be a tool for smoking harm reduction. The projected population from our model including only smokers, never smokers and ex-smokers closely matched predictions from public data. Inconsistent definitions and age breakdowns between data sources made it difficult to combine data. Inclusion of relevant measurements on nicotine products in nationwide studies could provide valuable insights into nicotine consumption patterns. Systems Dynamics could be a useful approach to assess the potential health effects of new nicotine products when epidemiological data are not available.

TUESDAY MORNING, SEPTEMBER 22, 2015

SESSION A – Methods Development

8:00 AM TUESDAY

43. DEAD VOLUME AND IMPINGER CAPTURE: WILL MACHINE DESIGN CHANGE PUFFING CONDITIONS? Ian TINDALL, Linda Crumpler and Akinwande Cole; Cerulean, Milton Keynes, UK

Minimising dead volume between cigarette and capture pad in routine analytical smoking machines has been shown to be critical in achieving total capture of total particulate matter especially when smoking intensively. Less attention has been paid to dead volume in smoking experiments where the volatile component of mainstream smoke is captured in liquid impingers after particulate matter has been filtered out.

The objective of this study is to determine the impact of dead volume on vapour condensation before capture and how the pressure drop of the system is altered by the dead volume and in turn how this changes the combustion conditions of the cigarette.

This was examined using two types of linear smoking machine (SM450 and SM450i with dead volumes of 19.73 and 11.26 ml respectively) and a puff capture device that could record the profile parameters as experienced by the test piece.

It was found that profile shape of puffs when impingers are added change from the “normal” smoking condition. Comparing no impinger with impingers in increasing dead volume it was found that peak flow is reduced by 13% and 16% respectively, asymmetry increased for the largest dead volume from 1:1 symmetry to 5:4 asymmetry and that the peak maximum occurred at 1s, 1.25s and 1.35s respectively.

The consequences for analytical measurement are discussed in the context of “peak clipping” and combustion temperature, notably that the elongation of peaks due to the elasticity of the drawn smoke present particular problems with some machine designs that utilise a single engine. Reducing the dead volume between smoking article and impinger system from traditional systems has a positive impact on the puff shape and peak velocity.

8:20 AM TUESDAY

44. DIRECT EXTRACTION OF CAMBRIDGE FILTER PAD HOLDERS. Thomas SCHMIDT¹, Nils Rose¹ and Beata Kowalski²; ¹Borgwaldt KC GmbH, Hamburg, Germany and ²BKC Hamburg, Germany

Quantification of cigarette smoke constituents, using a smoking machine designed in accordance to ISO 3308 is the basis for research and development within the tobacco industry.

The loss by the incomplete removal of TPM and therewith the precipitated smoke

constituents from a classical Cambridge Filter Pad (CFP) holder is often discussed. To minimize this loss of analyte quantities and the impact of aging processes and contamination possibilities of the collected TPM, a new filter pad holder has been developed, as an integral unit. It allows the complete flushing and direct extraction without opening the holder and separate handling of the filter pad.

Earlier published studies have already shown that direct extraction techniques do have an impact on the deliveries and repeatability rates of smoke constituents. The new application simply has the advantage that the filter pad holder can be used directly for mechanical extraction and rinsed out without any further instrumentation.

Correspondingly, first results showing differences in nicotine and water deliveries, especially for the determination of higher molecular and instable substances. It could be of further interest to operate with an enclosed system.

Last but not least, the system as it is designed gives advantages related to occupational health and safety requirements as the operator does not handle open solvents anymore.

Beside the technical description, the paper will share some first analytical data showing different amounts in nicotine and water deliveries and give an outlook into further automated handling.

8:40 AM TUESDAY

45. ABBREVIATED METHOD FOR TNCO ANALYSIS - MODIFIED ISO SMOKING.
Rana TAYYARAH; ITG Brands, Greensboro, NC USA

ISO standards for analysis of mainstream smoke for 'tar', nicotine, and carbon monoxide (TNCO) have been in routine use for characterization of cigarettes in analytical testing laboratories for many years. While these methods are consistent and reliable, strict adherence to these methodologies requires defined time constraints. This presentation focuses on a proposed approach to generate and report TNCO results with a more rapid process with minimal impact on variability of reported values. Approaches to streamline sample collection, logging, and reporting data will be presented. Technical aspects of the ISO methodology for which modifications would yield efficiency improvements were explored. Results from evaluation of these variables will be presented. Results from the new method will be presented along with potential advantages and disadvantages to streamlining the TNCO method.

9:00 AM TUESDAY

46. QUALIFICATION OF THE JB2090 SMOKING MACHINE FOR E-CIGARETTE TESTING ACCORDING TO CORESTA AND TOBACCO CIGARETTE TESTING ACCORDING TO ISO 3308. Dritan XHILLARI, Daniel Bachman, Henry DeFord and Rudolph Jaeger; CH Technologies, Westwood, NJ USA

The testing of tobacco burning cigarettes is governed by the testing methods set forth in ISO 3308 and the Canadian Intense Regime (CIR). These both call for a two second

“puff” that is taken once (FTC) or twice (CIR) per minute in the amounts of 35 and 55 ml respectively. Electronic cigarettes or e-cigarette vapor generation methods are likely to be covered by guidelines defined by CORESTA. This calls for a 3 second puff of 55 ml volume taken twice per minute.

While vapor and aerosol toxicology studies need to be conducted, the availability of equipment suitable for the reliable, precise and accurate generation of e-cigarette vaporization aerosols has yet to be addressed in the industry.

The Jaeger-Baumgartner Cigarette Smoking Machine, model 2090, was tested to determine if it had the combination of reliable, accurate and precise characteristics that would allow it to be used for such determinations.

In the following presentation we report that the JB2090 smoking machine satisfactorily meets all the requirements set by the CORESTA recommended method for e-cigarette testing, including puff duration, puff volume and carousel rotation time, with either built in piston pumps or external continuously drawing pumps such as peristaltic and FMI pumps. Also, we demonstrate that the JB 2090 meets with high accuracy the ISO 3308 and CIR puffing requirements.

9:20 AM TUESDAY

47. INFLUENCE OF MEASUREMENT UNCERTAINTY ON RECOMMENDED REGULATION FOR CIGARETTE SMOKE CONTENTS. Li Zhonghao, Hu QingYuan, Tang GangLing, Yang Fei, Pang YongQiang, Fan ZiYan, Zhang HongFei, Liu ShanShan, Bian Zhaoyang, Jiang Xingyi, Zhang Wei, Chen Huan and Chen ZaiGen; China National Tobacco Quality Supervision & Test Center, Zhengzhou, Henan, China

According to the WHO recommended regulation for 9 priority toxicants in cigarette smoke, the collaborative study on specific analysis methods was achieved in 2014. The results of precision under reproducibility condition showed that the Reproducibility Relative Standard Deviation (RSDR) for tar, nicotine, CO in mainstream smoke were clearly lower than other Harmful chemicals. Comparing with the analysis precision results under two smoking regimens conditions, no significant difference of RSDR for Harmful chemicals was found between ISO and HCI smoking regimes. Besides, the RSDR and overall mean () of Harmful chemicals in mainstream smoke accorded well with the Horwitz curve under ISO regime, but the RSDR did not have strong dependences on under HCI regime. Accord to ISO/TS 21748:2004 , the measuring uncertainty for 9 high priority toxicants level in μg per mg nicotine in cigarette smoke based on the reproducibility results were calculated. The results demonstrated that: Tar, nicotine and CO under ISO smoking regime were the most robust characterization in the tobacco mainstream smoke, and also good characters for product ranking. According to the requirements for assessment and reporting of compliance with specification, because measurement variability among 20 typical commercial samples was very little under HCI regime, the measuring uncertainty had an extremely great influence on judgment of compliance with ceiling of priority toxicant recommended by WHO TobReg.

10:10 AM TUESDAY

48. METHOD DEVELOPMENT AND VALIDATION OF SELECTED METALS IN E-LIQUID SAMPLES. Jeremy BROWN; Global Laboratory Services, Inc., Wilson, NC USA

Over the past several years, the use of electronic cigarettes and devices has increased. Analysis of Harmful and Potentially Harmful Constituents is at a forefront in both the conventional and electronic cigarette industries. The analysis of various metals in E-liquids pose multiple challenges when analyzing by ICP-MS. A method for analyzing As, Cd, Cr, Ni, Pb, Zn, Co, Cu, and Sn in E-liquids using ICP-MS was developed and validated. Linearity ($r^2 > 0.999$), accuracy ($\pm 20\%$), precision (RSD $< 7\%$) and specificity (RSD $< 12\%$), and intermediate precision (RPD $\pm 15\%$), limit of quantitation and detection were demonstrated. Potential sources of interferences are discussed for As, Cd, and Sn.

10:30 AM TUESDAY

49. ON-LINE REAL-TIME ANALYSIS OF E-CIGARETTE VAPOURS AND HEAT-NOT-BURN TOBACCO PRODUCTS BY PHOTO-IONIZATION MASS SPECTROMETRY. Ralf ZIMMERMANN¹, Sven Ehlert², Thorsten Streibel¹, Jan Heide¹, Jan Wolter¹ and Andreas Walte²; ¹University of Rostock, Germany and ²Photonion GmbH, Schwerin, Germany

Photo ionization time-of-flight mass spectrometry (PI-TOFMS) is an established technology for on-line, puff-resolved characterization of organic compounds in smoke of conventional cigarettes. Many toxicants, such as butadiene, acetaldehyde, phenol or polycyclic aromatic hydrocarbons (PAH) can be detected in real-time with single puff-resolution in cigarette smoke. A PI-TOFMS system, based on a special VUV-light source, integrated with a smoking machine, is commercially available (LM2x-Photo-Tof, Borgwaldt KC, Hamburg/Germany) and suited for many industrial routine and research tasks. The high-end laser-PI-TOFMS technology (Photonion GmbH/Germany) allows a parallel use of different PI technologies (resonance enhanced multi photon ionization, REMPI and single photon ionization, SPI) in conjunction with conventional electron ionization (EI). This instrument was applied for highly sensitive on-line detection of smoke constituents and vaporization/thermal-break-down products from e-cigarettes (eCig) and heat-not-burn tobacco smoking devices (HnB). Different HnB- (electrical tobacco heating, charcoal-tip and a tobacco-extract pod system) and eCig-systems were investigated. While the composition of the eCig-aerosols is rather simple, the HnB aerosol is quite complex. However, most toxicants are dramatically reduced in HnB-aerosol if compared to cigarette smoke. The puff-resolved release of nicotine, aerosolizing compounds (glycerol and propylene glycol), flavor compounds (e.g. vanillin or menthol) and toxicants (benzene, butadiene etc.) of different HnB- and eCig-devices have been investigated using different smoking regimes (e.g. ISO, HCl). Large differences in puff release profiles of the relevant compounds are obtained for the different devices. A suppression of organic/aromatic tar formation in HnB-devices is very well visible. The smoking regime also has a strong influence on the release dynamics of the compounds. The results from the HnB- and eCig-devices are finally compared to findings obtained from standard reference cigarettes.

10:50 AM TUESDAY

50. DETERMINATION OF ALLYL ALCOHOL IN ELECTRONIC CIGARETTE (E-CIG) AEROSOL AND LIQUIDS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY. Xinyu LIU, Peter Joza and Bill Rickert; Labstat International ULC, Kitchener, ON, Canada

Allyl alcohol is a potential contaminant or degradation product of the glycerol commonly used in electronic cigarette (e-cig) solutions. The aim of this work was to develop a fast and simple procedure for the determination of allyl alcohol in electronic cigarette (e-cig) aerosol and e-liquids.

Aerosol collection was achieved using a collection pad followed in series by a cryogenic impinger containing methanol (-70°C). The sample preparation consisted of adding internal standard (allyl alcohol-d5) to the collection pad and extracting the pad with the trapping solution using a wrist shaker. The filtrate was then analyzed by GC-MS using a ZB-wax (60 m x 0.25 mm x 0.25 µm df) column using selected ion mode (SIM) using ions (m/z) 57 and 58 as the quantifier and qualifier respectively. The e-liquid was analyzed using the same instrumental parameters, simplifying the sample preparation procedure by adding internal standard to 500mg of sample, then performing a simple dilution with methanol and filtration.

Validation experiments demonstrated good sensitivity, specificity and reproducibility. Calibrations from 15 to 1900 ng/mL exhibited good linearity ($R^2 > 0.9999$) and precision (RSD < 15%) resulting in an LOD and LOQ for e-liquids of approximately 340 and 1100 ng/g respectively. These limits were equivalent to 70 and 220 ng per collection for e-cig aerosol. Total allyl alcohol deliveries was found at levels of 1510 ng/collection (n=5) in one of the e-cig aerosols generated using an 80/3/30/ "square wave" profile puffing regimen (volume/duration/frequency) with a 100 puff collection. For the e-liquids, one test sample was identified to contain 3733 ng/g (n=5) of allyl alcohol.

11:10 AM TUESDAY

51. INVESTIGATE PARTICLE SIZE DISTRIBUTIONS OF ELECTRONIC CIGARETTE AEROSOLS USING A MULTI-ANGLE LIGHT SCATTERING INSTRUMENT. Chen SONG¹, Don Holve² and Steve L. Alderman¹; ¹R. J. Reynolds Tobacco Company, Winston Salem, NC USA and ²EnviroMetrix Instruments LLC, Berkeley, CA USA

Particle size distribution measurement of electronic cigarette aerosols represents a great experimental challenge due to the volatile nature of the particulate compositions and the high number density (up to 1010 per cubic centimeter). Efforts have been made to measure particle size distribution of e-cigarette aerosols in recent studies using techniques such as electrical mobility, cascade impactor and spectral light transmission method. In this study, we demonstrate the capability of a newly developed multi-angle light scattering instrument to accurately measure particle size distribution and mass concentration of e-cigarette aerosols in real time without sample dilution. The instrument, developed by EnviroMetrix Instruments, uses laser light scattering collected at three angles (10, 40 and 90 degree) to calculate the total particle size and number concentration, based on MIE theory. Once the size and distribution width are determined by a proprietary algorithm, the particle number,

mass, and surface area are computed from these parameters over a wide dynamic range. It is capable of measuring particles with sizes range from 50 to 1000 nm and mass concentrations range from 0.1 to 100000 mg/m³ at up to 50 Hz data rates. Several different e-cigarettes were evaluated using the instrument under a constant flow, square wave puff profile (55 mL puff volume and 3 second puff duration) and approximately 80% mass closures, relative to filter pad measurement, were obtained. The average mass mean diameters of the tested e-cigarette aerosols were observed to fall between 350 and 450 nm, and these values are comparable to particles found in tobacco burn-down cigarette aerosols. The real-time mass concentration and mass mean diameter measurements provide valuable information for product developments and evaluating the behavior of e-cigarette aerosols in the respiratory tract.

11:30 AM TUESDAY

52. NEW HIGHLY SENSITIVE AND SELECTIVE METHOD FOR CARBONYL DETERMINATION IN E-CIGARETTE AEROSOLS. Jason W. FLORA, Celeste Wilkinson, James Wilkinson and John Miller; Altria Client Services, Richmond, VA USA

Low levels of thermal degradation products such as carbonyls (formaldehyde, acetaldehyde, acrolein, and crotonaldehyde) have been reported in e-cigarette aerosols. The collection techniques and analytical methodologies used for the quantification of carbonyls in e-cigarette aerosols have been adapted from methods developed for tobacco cigarettes. These methodologies typically use HPLC-UV and are often not sensitive enough to detect the low levels of carbonyls found in e-cigarette aerosols (*e.g.*, LOQ ~0.3 ug/puff). These methods are also subject to interference from e-cigarette flavor systems resulting in a potential for false positive identifications or incorrect quantification of carbonyls. Therefore, the objective of this work was to develop and validate a rapid, selective and sensitive method optimized for the analysis of carbonyls in e-cigarette aerosols using UPLC-MS. For this work, E-cigarette aerosols were trapped in sequential 20-puff collections following a puff regimen of 4 second duration, 55 mL volume, 30 second interval and square wave profile. The collection apparatus involved a 5-port linear smoking machine fitted with a 44mm Cambridge filter pad followed by an impinge containing acidified 2,4-Dinitrophenylhydrazine. This optimized method showed high trapping efficiency, an LOQ of 0.016 ug/puff and an instrument run-time of only 4 minutes. Six leading commercially available e-cigarettes were evaluated (five devices each) to confirm that the method was fit-for-purpose. All commercial products tested contained formaldehyde and in most cases, the levels were well below those observed in conventional tobacco cigarettes (less than 3 ug/puff). However, for some commercial products, levels above tobacco cigarettes were detected with the highest at 14.1 ug/puff.

TUESDAY MORNING, SEPTEMBER 22, 2015

SESSION B – Regulations & Quality

8:00 AM TUESDAY

53. INFLUENCE OF CIGARETTE FILTER VENTILATION ON SMOKERS' MOUTH LEVEL EXPOSURE TO TAR AND NICOTINE: A RETROSPECTIVE META-ANALYSIS OF 11 STUDIES IN 9 COUNTRIES. Jan M. FEARON¹, Sheri A. Bowman², John W. Caraway², Peter Chen², Paul R. Nelson², Madeleine Ashley¹, Christopher J. Shepperd¹ and Graham Errington¹; ¹British American Tobacco (Investments) Limited, Southampton, UK and ²R. J. Reynolds Tobacco Company, Winston-Salem, NC USA

Cigarette filter ventilation allows air to be drawn into the mainstream smoke which, when the ventilation is unblocked, dilutes the smoke. When cigarettes are machine-smoked using the Health Canada Intense method, ventilation holes are blocked and this gives rise to higher smoke yields than those produced under ISO conditions. However, in normal use, few smokers block all ventilation holes; therefore, it is beneficial to study the effect of filter ventilation on human smoke exposure.

To investigate the effect of filter ventilation on exposure, British American Tobacco and R. J. Reynolds Tobacco collated and reviewed data from 11 studies across 9 countries. These studies were performed between 2005 and 2013 and contained data on mouth level exposure (MLE) from 1,690 products with filter ventilation between 0% and 87%. Mouth level exposure of ~6,400 subjects to tar and nicotine was estimated using the part-filter analysis method from spent filter tips.

For each of the countries studied, MLE to tar and nicotine consistently decreased as filter ventilation increased. Across the countries, per cigarette MLE to tar and nicotine decreased 57% and 54%, respectively, as filter ventilation increased from 0% to 87%. Daily MLE to tar and nicotine decreased 61% and 58%, respectively, across the same range of filter ventilation.

Cigarette filter ventilation was associated with a reduction in MLE to tar and nicotine when examined under subjects' natural smoking behaviour. Therefore, these data do not support the view that smokers fully compensate for cigarette ventilation but instead suggest that smokers of ventilated cigarettes are exposed to lower amounts of nicotine and toxicants.

8:20 AM TUESDAY

54. HARMFUL AND POTENTIALLY HARMFUL CONSTITUENTS (HPHC) LEVELS IN TOBACCOS AND MAINSTREAM SMOKE FROM CIGARILLOS AND FILTERED CIGARS. John H. LAUTERBACH; Lauterbach & Associates, LLC, Macon, GA USA

Until recently, there had been little in the literature on cigarillos and filtered cigars since the 1976 article by Schmeltz *et al.* (Beiträge zur Tabakforschung) and the 2011 article by Rickert *et al.* (Regulatory Toxicology & Pharmacology). The 2014 article in New England Journal of Medicine by Pankow *et al.* that included quantitative analyses of flavors in filtered cigar

products was followed in 2015 by the article by Caruso *et al.*, on physical properties of filtered cigars (Nicotine & Tobacco Research) and by Pappas *et al.* (Journal of Analytical Toxicology) on toxic metals in the tobacco fillers. However, none of these articles included data on the US FDA Harmful and Potentially Harmful Constituents (HPHC) in mainstream smoke (MSS), and there were no reports of HPHC in the tobacco fillers other than toxic metals. Consequently, this study was undertaken to provide the missing information. Four cigar products (200 cigars each) were obtained at retail in the Atlanta, GA, area in early 2015. One of the products (A) was a traditional European cigarillo; and the other three (B, C, and D) were 100-mm filtered cigars. None of the products had a characterizing flavor. The products were sent to a commercial laboratory for routine tobacco and MSS determinations, including HPHC (3R4F used as embedded control). In general, the results for tobacco and smoke analytes were higher for the cigar products than they were for the 3R4F except for blend nicotine and mainstream smoke nicotine. Interpretations of the results will be presented based on available data and product design.

8:40 AM TUESDAY

55. RELATIONSHIPS BETWEEN HPHCS IN CIGARETTE SMOKE. Beatrice TEILLET, Thomas Verron, Xavier Cahours and Stéphane Colard; SEITA - Imperial Tobacco Group, Fleury-Les-Aubrais, France

Manufacturers are increasingly being asked by regulatory authorities to report information on their products. For example, the FDA has published a draft list of 93 harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke and published draft guidance on the reporting of an abbreviated list of 20 HPHCs. The objective of this reporting is presumably either to gain a better understanding of the products, to be able to discriminate them, or to impose limits on selected constituents.

In this context, the added value of such extended list was assessed in terms of information and knowledge delivered compared to the FDA's abbreviated list of 18 HPHCs in smoke or the 9 priority constituents highlighted by the WHO.

Using an analytical dataset generated from 22 commercial cigarette brands sold on the US market, we observed that, among the 93 HPHCs, 30 to 40% of the constituents were systematically non-quantifiable or not detectable for each smoking regime suggested by the FDA (ISO and Intense smoking regimes).

For the remaining 60 to 70%, the strong correlations observed between constituents tend to prove that the extended list provides a limited added value compare to the shorter list for both smoking regimes. The reliability of the relationships varied from strong to weak, depending on particular constituents. The ISO machine-smoking data show more distinctly the influence of some cigarette design features, and in particular those related to filtration or gas phase diffusion.

It can be concluded that the decision of requiring the best and most useful information is very certainly not simply requiring more redundant information making even less discriminant the comparison of product.

9:00 AM TUESDAY

56. ROUTINE AND DETAILED ANALYSES OF SOME TRADITIONAL PIPE TOBACCOS ON THE US MARKET. John H. LAUTERBACH¹ and Mark Ryan²; ¹Lauterbach & Associates, LLC, Macon, GA USA and ²Daughters & Ryan, Inc., Kenly, NC USA

Traditional pipe tobacco that is blended, cased, cut, and flavored for use in pipes represents a very small percentage of the US tobacco market. However, despite its very small appeal (probably 1% or less of all tobacco users), pipe tobacco has been included in the US FDA's deeming regulations along with cigars and e-cigarettes. Product regulation requires an excellent knowledge of product composition. However, there are few details on composition of contemporary pipe tobaccos. Consequently, the purpose of this presentation is to provide routine and detailed (GC-MS scans techniques) analytical data blends/casings/flavorings on contemporary pipe tobaccos along with comparisons to data on the same brand-styles that can be found in older literature. Data will also be provided on components of blends through various stages of tobacco processing.

9:20 AM TUESDAY

57. U.S. PHARMACOPEIA DISSOLUTION TECHNIQUE FOR THE DETERMINATION OF NICOTINE AND FLAVOR RELEASE FROM SMOKELESS TOBACCO PRODUCTS. John MILLER, Helen Miller, Richard Schibetta, Anthony Brown, Tim Danielson, Karl Wagner and Jason W. Flora; Altria Client Services, Richmond, VA USA

Smoking machines were first developed to generate smoke from cigarettes for the purpose of comparing cigarette tar and nicotine yields under consistent conditions. Smoking machines with standardized puffing protocols have been used to measure the components in cigarette smoke for decades. There are no standardized methods for measuring the release of components in smokeless tobacco. The objective of this work was to develop a standardized dissolution technique for smokeless tobacco products using a SOTAX CE7 Smart USP-4 flow-through dissolution apparatus. The flow-through dissolution apparatus was configured for off-line collection of USP artificial saliva (no enzymes) with a pH of 6.8. The flow rate was 4 mL/minute, temperature was held at 37°C and fractions were collected (*e.g.*, every 4 minutes) for 60 minute. The dissolution fractions were analyzed by GC/MS for flavor and nicotine release from pouch moist smokeless tobacco (MST) and snus products. Rates of release showed a quadratic distribution and a faster release of nicotine and flavors for pouch MST compared to snus. This technique demonstrated excellent reproducibility and can be applied to measure a variety of constituents that are released from smokeless tobacco for comparative and regulatory reporting purposes.

10:10 AM TUESDAY

58. STABILITY OF REFERENCE CIGARETTES IN DIFFERENT STORAGE CONDITIONS. Huihua JI, Ying Wu and Neil Fannin; University of Kentucky, Lexington, KY USA

Kentucky Tobacco Research & Development Center reference cigarettes are widely used in tobacco research laboratories as tobacco reference products in various areas including

analytical method development and modified risk tobacco product development. Reference cigarettes provide stable physical and chemical characteristics for these investigations. Storage conditions are critical for maintaining reference cigarette quality within its effective period. Some experimental data from the literature indicate our current storage conditions are adequate to store our reference cigarettes. However, there is no integrated data to support this conclusion. The objective of this study was to analyze filler tobacco contents and smoke from 3R4F cigarettes after 1, 2, 3, 6, 9 and 12 months of storage at -20°C, 4°C and room temperature to determine stability of selected constituents. The sampled cigarettes were conditioned for 48hr at 22°C and 60% relative humidity prior to filler analysis and smoking. Filler analysis included oven volatiles, individual alkaloids and TSNAs. Smoke analysis included measurement of individual alkaloids and TSNAs, CO and TPM under ISO smoking regime. Five replications of 20 cigarettes in a single smoking machine run (five cigarettes per port) were assayed. There were no significant changes in the oven volatiles, nicotine and NNN in the filler from the three storage conditions. Also, there were no significant changes in puff/cigarette, CO, TPM, alkaloids and TSNAs in the smoke of cigarettes stored in these conditions. Our experimental data demonstrated 3R4F cigarettes were relatively stable after one year storage under these storage conditions.

10:30 AM TUESDAY

59. CENTER FOR TOBACCO REFERENCE PRODUCTS UPDATE ON 1R6F PRODUCTION AND PROFICIENCY TESTING PROGRAM. Orlando CHAMBERS, Socrates Canete and Huihua Ji; Center for Tobacco Reference Products, University of Kentucky, Lexington, KY USA

The Center for Tobacco Reference Products (CTRP) at the University of Kentucky has completed production of 50 million 1R6F reference cigarettes. The final specifications and characterization status of the 1R6F will be presented along with an update on additional reference products that will be provided by the CTRP. Significant changes are being made to the CTRP in terms of ordering reference products and services provided. The status of the proficiency testing program and schedule of future proficiency testing will be presented. The CTRP is funded through a cooperative agreement with the Food and Drug Administration's Center for Tobacco Products.

10:50 AM TUESDAY

60. WAS THERE A TEMPORAL INCREASE IN CIGARETTE NICOTINE YIELD-TO-CONTENT RATIOS IN PRODUCTS REPORTED TO THE MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH? Michael J. MORTON, David A. Self, Raquel M. Olegario and Scott Appleton; Altria Client Services, Richmond, VA USA

Since 1997, Philip Morris USA (PM USA) and the other major US cigarette manufacturers have been required to provide the Commonwealth of Massachusetts with smoke nicotine yields and nicotine in filler for high volume brand families. In 2014, researchers from the Massachusetts Department of Public Health (MDPH) published a paper concluding, among other things, that “average [nicotine] yield-to-content ratios of recent years were markedly higher than those of earlier years.”

We explored the PM USA data submitted to MDPH between 1997 and 2013 examining the finding that the nicotine yield-to-content ratio had increased. For the years 1998-2008, the filler nicotine of PM USA products was tested using CORESTA Recommended Method No. 35 (CRM35). From 2009 to the present, the filler nicotine for the PM USA products was measured by the CDC test method.

It is well known that CRM35 is sensitive to secondary alkaloids in addition to nicotine, and has been found to result in higher values than nicotine-specific methods similar to the CDC method. Testing on 3R4F cigarette filler showed 13% lower results for the CDC method than was measured for 3R4F at the time of manufacture when tested with CRM35. A lower measured value for filler nicotine makes the denominator in the nicotine-to-content ratio smaller, and therefore makes the ratio larger.

We conclude from our investigation that changing the test method from CRM35 to the CDC method explains most, if not all, the observed increase in average nicotine yield-to-content ratios for PM USA cigarettes.

This investigation illustrates the difficulties in making inferences of product time trends, particularly when analytical methodology changes during the period under investigation.

11:10 AM TUESDAY

61. CAN US FDA SUBSTANTIALLY EQUIVALENT PREDICATES BE DEVELOPED WITHOUT KNOWLEDGE OF AND A SAMPLE OF THE PREDICATE PRODUCT? John H. LAUTERBACH; Lauterbach & Associates, LLC, Macon, GA USA

The passage in 2009 of the Family Smoking Prevention and Tobacco Act (the Act) has created unexpected problems for many companies in the small business tobacco manufacturing industry. Section 905(j) of the Act dealing with the substantially equivalent (SE) requirement, whereby the predicate product had to be on the market on February 15, 2007, and the FDA guidance on what constitutes SE product has been less than clear. If a company did not market a product on February 15, 2007, or was not even in business at that time, then what do they do for a predicate product? Since SE difficulties will increase as other tobacco products fall under FDA jurisdiction, we have investigated several approaches for recreating predicate products. These will be presented for filter tubes, conventional cigarettes and filtered cigars, and unwrapped tobacco products such as pipe tobacco, roll-your-own tobacco, and chewing tobacco. These approaches involve a combination of analytical and physical testing and reports in the tobacco literature.

11:30 AM TUESDAY

62. A COMPARISON OF STATISTICAL AND MATHEMATICAL METHODS IN SUPPORT OF EQUIVALANCE TESTING. Rana TAYYARAH; ITG Brands, Greensboro, NC USA

In many study designs, data evaluation involves comparison of results for which there is an expectation of differences between data sets. In these cases, evaluations include use of statistical tests such as Student's t test and analysis of variance. For data evaluations,

if the hypothesis is that the data sets are equivalent, equivalence testing techniques may be applicable. In equivalence testing, the null hypothesis is established such that the statistical test is for similarity. Typical applications of equivalence testing include process scale-up, control charting, methods transfer between laboratories, and product design and maintenance. In order to support studies for which the results were hypothesized to be equivalent, statistical and mathematical techniques were compared and contrasted using a range of exemplary data sets. These techniques included methods such as repeatability & reproducibility, critical difference, confidence interval analysis, and two one-sided t-test (TOST). The results of these comparisons along with advantages and disadvantages of the techniques will be discussed.

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

1:15 PM POSTERS

63. EFFECT OF ELECTRICITY OFF AMOUNT ON THE CURING DIFFERENT STAGES ON QUALITY AND YIELD OF FLUE-CURED TOBACCO. Reza MOHSENZADEH; Iranian Tobacco Company, Behshahr, Mazandaran, Iran

Rate and extent of biochemical reactions are controlled by leaf temperature and moisture content, while air velocity provides uniformity of curing conditions and facilitates the removal of moisture from the curing system. This study was conducted to determine the effect of time of turn off on quality characteristics of flue curing tobacco in Tirtash research and education center in 2008–2009. In this study leaves were cured in 4 pick and 12 treatments and observation. Treatments were: 3 times turn off (2, 4 and 6 hours) in temperatures 42, 48 and 54 centigrade. Two treatments were times turn off in 6 hours at temperatures 36 and 68 centigrade and control treatment. Processes others for all treatments were uniform and based on form. The mean of price tobacco, sugar and nicotine were tested. Results showed that all treatments were reduced in mean of price tobacco and quality in comparison to control. The most reduction were in 42 and 48 temperatures and time of 6 hour. Percent of sugar was in control treatment 13 and the lowest and the highest of sugar were in 3 pick up in 48 and 36 treatments and time of 6 hour. Treatments were not differences in percent of nicotine.

64. STUDYING RESEARCH AND EXTENSION TOPPING HEIGHT (LEAF NUMBER) AND TIMING ON QUALITATIVE AND QUANTITATIVE YIELD IN FLUE-CURED TOBACCO. Reza MOHSENZADEH and M. R. Seraji; Iranian Tobacco Company, Behshahr, Mazandaran, Iran

Tobacco topping on a proper stage and at the right time gives the opportunity for the maximum potential and develop leaves and tips, and subsequently obtaining a good yield and quality. This study was carried out to reduce production costs including harvesting, curing, sorting and handling according to maintaining quality and quantity of tobacco based on new methods of topping and sucker control. This study was carried out in Golestan province in (north of Iran) 2010. Treatments for farmers who did not perform topping were including: 1) without topping and sucker control 2) Topping at bottom, stage and harvest 22 leaves and for farmers that do perform topping were: 1) Topping based on common regional approach 2) Topping at bottom, stage and harvest 22 leaves. Treatments

were compared with t test and paired observations. The results showed that there were significant difference among treatments with no topping and topping on button stage for average length and width of leaves, green and dry leaf yield and income. Also there were significant differences between treatments based on common methods with topping on button stage for green and dry yield and gross income. According to results the best time for topping is suggested button stage with 22 leaves.

65. COMPARISON OF INITIAL AND NEAR END-OF-LIFE ELECTRONIC CIGARETTE AEROSOL YIELDS. J. A. BODNAR¹, S. L. Alderman², S. K. Pike² and R. J. Potts¹; ¹R. J. Reynolds Tobacco Company, Winston-Salem, NC USA and ²RJR Vapor Company, Winston-Salem, NC USA

The typical major components of electronic cigarette e-liquids include glycerin and/or propylene glycol, nicotine, and flavoring ingredients. It was hypothesized that the temperature of the heating element could be high enough to decompose some components of the e-liquid. Since the effect of cartridge depletion on aerosol composition is not known, it was of interest to compare the initial puffs with those from a semi-depleted cartridge using freshly charged batteries for both aerosol collections.

Thirteen electronic “cigalike” rechargeable cigarettes from the 2014 U.S. marketplace were puffed using a 55/30/3 square wave machine regimen. The aerosol yield of the initial 20 puffs was compared to yields of 20 puffs taken from a semi-depleted cartridge. Because each electronic cigarette brand potentially has a different end of life puff number, for comparison across brands, puffs 101-120 were chosen for the semi-depleted cartridge analysis. Cigarette reportable HPHC analytes and other analytes of interest were examined.

Increases were seen in carbonyl yields (formaldehyde, acetaldehyde, acrolein), metals (nickel, lead) and volatiles (benzene, propylene oxide) from the semi-depleted cartridge in some electronic cigarette aerosol compared to the initial analyses. Decreases in yield of some electronic cigarettes were noted for styrene, ethylene glycol and diethylene glycol from the semi-depleted cartridge compared to the initial analyses. Yields of many toxicants were below the limit of quantitation in the electronic cigarette aerosols, and all toxicants quantifiable were substantially lower when compared to the 3R4F reference cigarette yields.

66. STUDY OF THE DECOMPOSITION OF TOBACCO/CITRATE/SBA-15 MIXTURES IN NITROGEN AND AIR ATMOSPHERES. A. MARCILLA, M. I. Beltrán, A. Gomez-Siurana, I. Martinez and D. Berenguer; Alicante University, Alicante, Spain

Combustion is a complex physical-chemical process. The products of combustion strongly depend on the temperature and oxygen availability in the different zones of the cigarette. Potassium citrate is widely used as a cigarette ingredient, acting as a moisturizing and surface-active agent for flavor application and having the ability of reducing the amount of carbon monoxide and tar. SBA-15 has been studied as a catalyst for reducing the amounts of nicotine, tar and carbon monoxide.

In the present work, the effect of SBA-15 zeolite catalyst on the qualitative composition of gases produced during the pyrolysis of tobacco and tobacco-citrate mixtures was studied by TGA/FTIR, under N₂ and air atmospheres.

Three processes are observed in the thermal decomposition of potassium citrate in both atmospheres; the first two occur at around 230 and 290°C and the third one occurs at 510°C in N₂ and at 430°C in air. The addition of SBA-15 produces different and noticeable changes depending on the atmosphere used. Tobacco, tobacco-citrate and tobacco-citrate-SBA-15 samples present almost the same weight loss processes, though their intensity decreases when adding the citrate and the catalyst in both atmospheres. The presence of SBA-15 produces a separation of the last decomposition process into two processes in air. The evolution with the temperature of the more significant IR bands of the gases produced shows significant reduction, in both atmospheres, mainly when adding potassium citrate and SBA-15 to tobacco (except in N₂ atmosphere for the R-O-H and R-O-R', and carbonyl bands).

Acknowledgments: Financial support for this investigation has been provided by the Spanish "Secretaría de Estado de Investigación" del Ministerio de Economía y Competitividad (MAT2011-24991) and by the Generalitat Valenciana (PROMETEO/2012/015).

67. STUDY OF THE EFFECT OF DIFFERENT CATALYSTS IN THE DECOMPOSITION OF NICOTINE. A. MARCILLA, M. I. Beltrán, A. Gomez-Siurana, I. Martinez and D. Berenguer; Alicante University, Alicante, Spain

Nicotine is the principal alkaloid component of tobacco, occurring throughout the plant (*Nicotiana tabacum*), and is the major inducer of tobacco dependence. It has not been classified as a carcinogen. Numerous studies concerning the thermal pyrolysis of nicotine indicate that nicotine is stable in inert atmosphere up to temperatures in excess of 600°C. In an oxidizing atmosphere (*i.e.*, in air) nicotine begins to decompose around 300°C. Some of the pyrolysis and/or oxidation products of nicotine include myosmine, nicotyrine, nornicotine, and a variety of pyrrolidine ring-compounds, where most of them are considered carcinogenic. Zeolites and molecular sieves have been employed as additives in cigarettes in order to remove such dangerous products, but their effect on nicotine is not well known.

In this work, three catalysts with different physicochemical properties, and different concentrations, were employed: one without acidity (SBA-15), one with a low acidity (NaMCM-41) and another with high acidity (HMCM-41). When using SBA-15, similar results in air and N₂ atmospheres were observed. A shift of the nicotine evolution peak at higher temperatures was observed when the SBA-15 concentration increased, probably because nicotine is adsorbed within its porous structure. IR spectra showed mainly nicotine in the gas evolved at all temperatures in N₂ and also the presence of CO₂ bands in air. The other two materials present a distinct behaviour, and a premature decomposition of the nicotine was observed at very low temperatures, both in air and N₂ atmospheres. Two steps of desorption of nicotine are observed in both atmospheres. The IR spectra in air experiments show the presence of relatively intense bands not corresponding to nicotine. On another hand, a clear evolution of CO₂ was observed in air at high temperatures as a consequence of the residue evolution. Consequently, it can be concluded that nicotine reacts in the presence of both MCM-41 type catalysts.

Acknowledgments: Financial support for this investigation has been provided by the Spanish "Secretaría de Estado de Investigación" del Ministerio de Economía y Competitividad (MAT2011-24991) and by the Generalitat Valenciana (PROMETEO/2012/015).

CHEMICAL FINGERPRINTING OF TOBACCO AND RELATED PRODUCTS BY

TD–GC–TOF MS. Laura McGregor¹, Bob GREEN¹, Caroline Widdowson¹, Kevin Collins¹, Chris Hall² and Pete Grosshans²; ¹Markes International, Llantrisant, South Wales, UK and ²Markes International Inc., Cincinnati, OH USA

The hazardous constituents of cigarette smoke have attracted considerable media attention, especially with increasing regulation around the world limiting or banning smoking in public places – and even in private cars if children are present.

Furthermore, the recent surge in tobacco-replacement devices, such as e-cigarettes, is driving the development of fast and efficient quality control procedures. E-cigarette solutions may contain potentially harmful chemicals, including nitrosamines and polycyclic aromatic hydrocarbons (PAHs). The presence of such chemicals naturally gives rise to some concern, and confident chemical fingerprinting is required for both research and development and regulatory purposes.

Although e-cigarettes emit less particulate matter than regular tobacco cigarettes (since no combustion takes place), they still produce a wide range of compounds at trace levels. Organic constituents of tobacco smoke have historically been analysed by gas chromatography coupled with quadrupole mass spectrometry (GC–MS). However, quadrupoles are mass filters, with a high percentage of ions being wasted, which limits sensitivity. Moreover, in selected ion monitoring (SIM) mode, only target compounds can be monitored, meaning that full characterisation of the sample is not possible in a single run and retrospective searching of data is limited.

The use of time-of-flight mass spectrometry (TOF MS) overcomes this issue by providing highly sensitive detection whilst acquiring full-range mass spectra, to allow both target and unknown identification in a single, rapid analysis.

This presentation explores the use of a multi-functional thermal desorption (TD)–GC–TOF MS system to capture and identify whole e-cigarette emissions using a single, highly-automated platform.

69. EFFECT OF PUFF DURATION AND PUFF VOLUME ONE-CIGARETTE AEROSOL COLLECTION. John MILLER and Jason W. Flora; Altria Client Services, Richmond, VA USA

Smoking machines were first developed to generate smoke from tobacco cigarettes for the purpose of comparing cigarette tar and nicotine yields under consistent conditions. Smoking machines have been used worldwide to verify cigarette designs and ensure regulatory compliance for decades. Two standardized machine smoking protocols frequently used for regulatory reporting are the International Organization of Standardization (ISO) smoking regime and the Health Canada Intense (HCI) smoking regime. Both protocols call for specific puff volumes (35 and 55 cc), puff profile (bell shaped), puff durations (2 seconds), interval between puffs (60 and 30 seconds) and percent ventilation blocking (0 and 100%). There are no such standardized methods for collecting e-vapor product aerosol. In 2013, a Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Task Force (TF) was formed to address this and other relevant topics regarding e-cigarette testing and

has made commendable progress. The purpose of this work was to evaluate the effect of puff volume (within the constraints of standard linear smoking machines) and duration (2 to 5 seconds) on e-cigarette aerosol mass (AM) collection. This information would be useful to both the CORESTA TF as well as relevant regulatory bodies. It was observed that puff volume has little effect on AM while puff duration plays a key role in the amount of AM collected. Larger puff volumes do appear to create some evaporative loss particularly with longer puff durations. For single device designs, the puff duration has a linear increase on AM from 2 to 5 seconds.

70. UPLC-MS/MS FOR HIGH-THROUGHPUT ANALYSIS OF AROMATIC AMINES IN CIGARETTE SMOKE. Xiaohong JIN, Chornng B. Huang, Karen Avery, Karl Wagner and Jason W. Flora; Altria Client Services, Richmond, VA USA

Aromatic Amines (AAs) are included in the “Established List of the Chemicals and Chemical Compounds Identified by FDA as Harmful and Potentially Harmful Constituents [HPHCs] in Tobacco Products and Tobacco Smoke” (Federal Register (Vol. 77, No. 64) Docket No. FDA-2012-N-0143). To date, no standardized method for AA determination in mainstream cigarette smoke has been developed. Previously reported techniques for the quantitative analysis of trace amounts of AAs in cigarette smoke (ng per cigarette) include gas chromatography with mass spectrometry (GC-MS) involving multistep solid phase extractions (SPE) or high performance liquid chromatography with multistage mass spectrometry (HPLC-MS/MS) with liquid-liquid extraction. The purpose of this work was to evaluate a higher throughput approach using ultra-performance liquid chromatography (UPLC) with MS/MS with a single step SPE clean-up. This method demonstrated applicability to all AAs on the FDA HPHC list and was validated for the 3 AAs on the current abbreviated HPHC list. All requirements for method validation were met such as linearity, accuracy, precision, limits of detection (LOD), and limits of quantitation (LOQ). For example, the linearity was demonstrated with a coefficient of determination of greater than 0.990 for the calibration ranges of 1.5 to 150 ng/cigarette for 1-aminonaphthalene, 0.75 to 75 ng/cigarette for 2-aminonaphthalene, and 0.6 to 30 ng/cigarette for 4-aminobiphenyl under the ISO smoking regime.

71. DETERMINATION OF DIACETYL IN E-VAPOR PRODUCTS USING GAS CHROMATOGRAPHY AND MASS SPECTROMETRY. Chornng B. HUANG, Karl Wagner and Jason W. Flora; Altria Client Services, Richmond, VA USA

While diacetyl is approved for food use, the United States National Institute for Occupational Safety and Health (US NIOSH) has suggested it may be associated with respiratory disease when heated and inhaled. NIOSH has defined safety limits for occupational exposure to diacetyl. Farsalinos and coworkers recently (2015) investigated 159 sweet-flavored e-vapor refill formulations where they observed that 110 contained measurable amounts of diacetyl with many exceeding NIOSH limits. Farsalinos and coworkers used a modified method developed for the analysis of carbonyls in mainstream cigarette smoke to quantify diacetyl in e-vapor formulations. This method was validated for e-vapor formulations and involved derivatization using 2,4-dinitrophenylhydrazine (DNPH) followed by high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector. The purpose of this work was to develop a more sensitive and selective method for the analysis of diacetyl using gas chromatography and mass spectrometry (GC/MS). This method does not require

derivatization, uses a labeled internal standard, and the MS is operated in the selected ion monitoring (SIM) mode to maximize selectivity and sensitivity. All requirements for method validation were met such as linearity, accuracy, precision, limits of detection (LOD), and limits of quantitation (LOQ). For example, the coefficient of determination is greater than 0.990, the calibration ranged from 0.1 µg/g to 16.2 µg/g of e-cigarette formulation and the recovery ranged from 90 to 110%. The method is suitable for potential regulatory reporting and the quality control purposes that may be needed in this product category.

72. QUANTITATIVE SCREENING OF POTENTIAL CONTAMINANTS IN E-CIGARETTE FORMULATIONS: ETHYLENE GLYCOL AND DIETHYLENE GLYCOL. Niti H. SHAH, Karl Wagner and Jason Flora; Altria Client Services, Richmond, VA USA

The US Food and Drug Administration (FDA) evaluated two commercial e-cigarettes and a nicotine replacement therapy inhaler in 2009 (DPATR-FY-09-23). Ethylene glycol (EG) and diethylene glycol (DEG) were included in this evaluation as potential impurities in e-cigarette formulations. DEG was found in one e-cigarette cartridge in the study (quantities were not included). The U.S. Pharmacopeia (USP) discusses permissible levels of EG and DEG in polyethylene glycol and glycerin (< 0.1%), the major components of most e-vapor product formulations. The USP only provides non-selective methods for the analysis of these potential contaminants in USP grade propylene glycol and glycerin. These methods are subject to potential interferences caused by flavor systems found in e-cigarette formulations. Therefore, the purpose of this work was to develop and validate a sensitive and selective method specifically for the quantitative screening of e-vapor formulations for EG and DEG. The method developed uses gas chromatography with mass spectrometry (GC/MS). All requirements for method validation were met such as linearity, accuracy, precision, limits of detection (LOD), and limits of quantitation (LOQ). The linearity was demonstrated with a coefficient of determination of >0.995 for the calibration range of 10 to 800 µg/g of e-cigarette formulation.

73. A VERSATILE METHOD FOR THE ANALYSIS OF TSNAS IN TOBACCO PRODUCTS AND CIGARETTE SMOKE BY LC-MS-MS. Jeff ZHU¹, Nancy Qian¹, Shalina Jones¹ and Serban Moldoveanu²; ¹Eurofins Lancaster Laboratories, Winston-Salem, NC USA and ²R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

A versatile method for the analysis of TSNAs has been applied to different tobacco sample matrices such as tobacco filler, smokeless and raw tobacco materials, as well as cigarette smoke. The method used an HPLC separation on a Phenomenex Gemini C18 column with 3 micron particle size, and gradient mobile phase with aqueous ammonium acetate buffer/acetonitrile and acetic acid/acetonitrile as mobile phases A and B. The detection was performed by MS/MS with multiple reaction monitoring (MRM) in positive mode using specific transitions precursor ion to product ion, specific for each TSNA compound. The instrumentation used for the analysis was a 1290 Infinity from Agilent Technologies and API 4000 from AB Sciex. The method provides good selectivity with no interference from the sample matrix. For this reason, after the sample extraction, no clean up procedure was necessary regardless of the sample type. The method has a wide calibration range (1-600 ng/mL for NNN, NAT and NNK, 0.25-150 ng/mL for NAB) that allows the analysis to be performed on all tobacco and smoke samples without modification. The method is rapid,

highly reliable, and shows excellent repeatability and robustness. The procedure has been applied successfully in the laboratory for a number of years and on a variety of samples.

74. METHYLATION PROFILES IN CHRONIC SMOKERS AND MOIST SNUFF CONSUMERS. G. L. PRASAD and Michael F. Borgerding; R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

Alterations in gene methylation and other epigenetic changes regulate normal development as well as drive disease progression. Chronic cigarette smoking results in hypermethylation or hypomethylation which could contribute to smoking-related diseases. For example, methylation status of genes involved in xenobiotic metabolism and growth regulation is altered in smokers (SMK). Yet there is limited information on the global methylation changes in smokers. Further, it is unclear whether consumers of non-combustible tobacco, such as moist snuff, also exhibit perturbations in their methylome. Here, we present global methylation changes in the buccal cells collected from smokers and moist snuff consumers (MSC) in a biomarker discovery study.

Generally healthy adult male study subjects were recruited into SMK, MSC and Non-Tobacco Consumers (NTC) cohorts (40 subjects/cohort). The subjects fasted overnight from food and tobacco, and buccal cells were collected. Global methylation profiling was performed on the Illumina 450K methylation array using the buccal cell DNA. Approximately 1250 gene loci were found to be differentially methylated in tobacco consumers (SMK and MSC) relative to NTC. Overall, the SMK cohort exhibited the largest qualitative and quantitative changes relative to MSC. Hierarchical clustering of the top 20 significant gene loci suggested that MSC and NTC co-cluster. Approximately half of the total number of gene loci, classified as Combustible Tobacco-Related Signatures/changes, and a third of the changes, termed Tobacco Related Signatures/changes, were commonly detected in the tobacco consumers. Consistent with other published work, AHRR and F2RL3 were hypomethylated in smokers. Initial bioinformatic analyses indicated that SMK, not MSC, exhibit an activated AHR pathway and perturbations in vitamin metabolism. In summary, we describe global gene methylation changes in buccal cells of long-term tobacco consumers.

75. IMPACTS OF TOTAL PARTICULATE MATTER FROM CIGARETTE SMOKE ON EARLY DEVELOPMENT OF ZEBRAFISH (*DANIO RERIO*). G. L. PRASAD¹, Andrey Massarsky², Nishad Jayasundara², Richard T. DiGiulio², Jordan Bailey³ and Ed Levin³; ¹R. J. Reynolds Tobacco Co., Winston-Salem, NC USA, ²Duke University, Durham, NC USA and ³Duke University Medical Center, Durham, NC USA

Cigarette smoke has been associated with a number of pathologies; however mechanisms leading to developmental effects are yet to be fully understood. This study examined the effects of Total Particulate Matter (TPM) from 3R4F reference cigarettes on the early development of zebrafish (*Danio rerio*). Zebrafish embryos were exposed to two concentrations of TPM, corresponding to 0.4 and 1.4 ug/mL equi-nicotine units. The exposures (single and acute) began at 2 h post fertilization (hpf) and lasted until 96 hpf. Several physiological parameters were assessed during or after the exposure. We show for the first time in a zebrafish model that TPM increased mortality, delayed hatching, and increased the incidence of pericardial edema and other deformities (assessed by microscopic observations). TPM exposure also increased the incidence of cranial hemorrhage (microscopic observations

and o-Dianisidine staining) and disrupted the proper angiogenesis of the major vessels in the brain (alkaline phosphatase staining). Moreover, TPM exposure reduced the larval body length, and decreased the heart rate. Several oxidative stress parameters were also affected, including glutathione levels and activities of several antioxidant enzymes, suggesting that oxidative stress contributes to the toxicity of TPM. TPM-exposed zebrafish also differed behaviorally: at 24 hpf the embryos had a higher frequency of spontaneous contractions (microscopic observations) and at 144 hpf the larvae displayed hyperactivity, which was assessed by monitoring the swimming using Danio Vision. It is important to note that the effects reported for TPM are not attributable to nicotine, since embryos treated with nicotine alone did not differ from the control embryos. This study demonstrates that TPM disrupts early development in zebrafish.

76. EFFECT OF MATURITY ON THE ENZYMES AND CHEMICAL COMPOSITIONS RELATED TO CARBON-NITROGEN METABOLISM IN FLUE-CURED TOBACCO DURING CURING STAGE. WEI Kesu, Wu Sheng-jiang, Pan Wen-jie, Li De-lun, Jiang Jun, Li Guo-bin and Xie Yi-shu; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

The current study was carried out to investigate the effects of maturity (immature, mature, over-mature) on the key enzymes and main chemical substances related to carbon-nitrogen metabolism of flue-cured tobacco leaves during the curing process by using K326 cultivar as material, to dissect the transformation rule of carbon-nitrogen metabolism in flue-cured tobacco leaves and provide a reference to improve the availability of leaves. The results showed as follows: higher starch reduced in immature tobacco leaves than the two other maturities. The total sugar and reducing sugar content showed two peak values during curing process, at 48h and 96h, respectively. The protein content decreased along with curing time, showing immature > mature > over-mature. Nicotine and total nitrogen content were also show higher in immature tobacco leaves. It was found that the amylase(AMS) and starch phosphorylase(SPS) activities in mature tobacco leaves showed higher activity level and stayed longer period than the other maturities, which would be help the starch degradation in tobacco leaves. For another, the nitrate reductase (NR) and glutamine synthetase(GS) showed higher activities in immature tobacco leaves than the other two mature leaves. The AMS activity showed significant correlate with carbohydrate, but not correlate with nitrogen compositions. However, the SPS, NR, GS activities were also not correlate with ht carbon and nitrogen compositions at the same time. The ratio of NR and SPS (NR/SPS) was positive significant correlated with starch and protein during curing process ($p < 0.01$), while negative significant correlated with total sugar and reducing sugar ($p < 0.01$). So it was suggested that the ratio of NR/SPS might be the indicator of carbon-nitrogen metabolism in flue-cured tobacco leaves during curing process.

77. ACTIVITIES OF AZOXYSTROBIN AND DIFENOCONAZOLE AGAINST ALTERNARIA ALTERNATA AND THEIR EFFICACY FOR THE CONTROL OF TOBACCO BROWN SPOT. WANG Hancheng, Wang Maosheng, Chen Xinjiang, Wang Jin and Shang Shenghua; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

Tobacco brown spot caused by *Alternaria alternata* is a devastating disease of tobacco worldwide. In this study, we report on the effects of the stobilurin fungicide azoxystrobin and the sterol inhibitor difenoconazole on mycelial growth, spore germination, and

control of brown spot caused by *A. alternata*. Mycelial growth *in vitro* was most sensitive to difenoconazole (EC₅₀ and EC₉₀ values of 0.30 and 10.67 mg L⁻¹, respectively) and least sensitive to azoxystrobin (EC₅₀ and EC₉₀ values of 25.83 and more than 100 mg L⁻¹, respectively). Germination was most sensitive to azoxystrobin - the EC₅₀ and EC₉₀ values with and without SHAM were 0.054 and 0.32 mg L⁻¹, and 0.0038 and 0.12 mg L⁻¹, respectively. Germination was least sensitive to difenoconazole, with EC₅₀ and EC₉₀ values of 26.58 and more than 100 mg L⁻¹, respectively. Azoxystrobin and the compound of azoxystrobin plus difenoconazole provided excellent control of tobacco brown spot in the field. Three sprays of azoxystrobin at doses of 93.75, 187.50 and 281.25 g a.i./ha presented disease efficacy of 87.48, 89.67 and 87.18%, respectively; and of azoxystrobin plus difenoconazole at doses of 146.25, 219.38 and 292.50 g a.i./ha showed efficacy of 89.23, 86.14 and 88.71%, respectively; while 120 g a.i./ha difenoconazole presented 55.14% disease control effect. No phytotoxic symptoms were observed by any chemical in the field, and these compounds potentially could be used for brown spot control in tobacco.

78. INTEGRATE MEASURES BIO-CONTROL TOBACCO BACTERIAL WILT AND REMEDIATE TOBACCO MONO-CROPPING LAND. LIU Yanxia, Li Xiang, Cai Liuti and Shi Junxiang; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Tobacco mono-cropping obstacle caused a lot of serious problem in China, leading to the burst of soil-borne diseases, such as tobacco bacterial wilt. In order to find an environment-friendly method to control tobacco bacterial wilt and remediate tobacco mono-cropping land, a four-year field experiment was conducted to evaluate the bio-control efficacy. Four treatments were designed: T1, chemical fertilizer application; T2, chemical fertilizer plus bioorganic fertilizer application; T3, deep plough before chemical fertilizer plus bioorganic fertilizer application; T4, deep plough and quick lime application before chemical fertilizer plus bioorganic fertilizer application. Biolog-ECO was provided to contrast rhizosphere soil bacterial functional diversity of four treatments. The paired-end sequencing of soil 16S rDNA revealed the bacterial community structure. The results obtained were listed as follows. The bio-control efficacy of tobacco bacterial wilt in the T4 treatment was 71.7%. In contrast to the T1 treatment, the counts of bacterial and actinomycetes increased while the populations of fungi and *Ralstonia solanacearum* decreased in rhizosphere soil of the T4 treatment. The bacterial functional diversity of the T2, T3 and T4 treatments significantly increased, compared with the T1 treatment. Besides, according to the soil abundance clustering map, the evenness index of the T4 treatment was higher than that in the T1 treatment, suggesting that there were less dominant microorganisms when the soil was treated by integrate measures. The phylum distribution varied under different treatments. In conclusion, the integrate measure, as deep plough and quick lime application before chemical fertilizer plus bioorganic fertilizer application, can efficiently control tobacco bacterial wilt and remediate tobacco mono-cropping land. It is a promising method for the long-term development of tobacco soil.

79. DEVELOPMENT OF USER-FRIENDLY MARKER FOR NIC2 IN TOBACCO. Qiulin QIN, Dandan Li, Robert Miller, Anne Jack and Shengming Yang; University of Kentucky, Lexington, KY USA

Nicotine is synthesized in the tobacco root under the control of two independent genes, Nic1 and Nic2. Tobacco breeding for low levels of nicotine has been hampered by the lack

of user-friendly markers for both genes. Gene cloning of Nic2 revealed that this locus comprises clustered ethylene response factors (ERFs); while in the nic2 mutants, these ERF genes are deleted altogether. As a result, the gene-specific markers for Nic2 can only be dominant, which is inefficient for breeding selection. Near-isogenic lines (NILs) of Burley 21 with high (HA), high intermediate (HI), low intermediate (LI) and low (LA) nicotine levels were generated 50 years ago. Based on the transcriptome analysis of these four NILs of Burley 21, several SNPs closely linked to Nic2 were identified. We have successfully converted one SNP to a co-dominant dCAPS marker which is user-friendly for the breeding purpose. Linkage analysis showed that this marker was co-segregating with the Nic2 locus. Therefore, the user-friendly marker we developed provides convenient and efficient tool for low-nicotine breeding in tobacco.

80. IMPACT OF DIFFERENT PARAMETERS ON THE COLLECTION AND GENERATION OF E-CIGARETTE AEROSOL FOR NICOTINE DELIVERY ANALYSES. David K. COOK, Aaren N. Routh, Ryan C. Mills and Daniel G. Morgan; ITG Brands, Greensboro, NC USA

With increasing interest in electronic cigarette testing, different e-cigarette aerosol collection approaches were investigated. The use of a machine designed specifically for e-cigarette aerosol generation and collection (Cerulean E-cigarette Testing Instrument 8, CETI8) was evaluated and compared to a standard smoking machine (Cerulean Linear Smoke Machine 450, SM450). Data will be presented that compares the aerosol generation and collection by both machines and highlights the novel features of the CETI8 for e-cigarette testing. Data for aerosol collected matter (ACM) and nicotine delivery will be presented using both aerosol collection systems. In addition, data illustrating the impact of collecting the e-cigarette aerosol in a conditioned environment versus a non-conditioned environment will be discussed. Lastly, results will be presented showing that the CETI8 is functionally equivalent to the SM450 for collection of e-cigarette aerosol, while providing additional e-cigarette specific capabilities for testing e-cigarettes.

81. FORMATION OF NNK FROM PSEUDOXYNICOTINE (PON). Ying WU, Huihua Ji, Neil Fannin and Lowell Bush; University of Kentucky, Lexington, KY USA

Oxidation of nicotine may result in three nitrosamines being formed - 4-(N-methyl-N-nitrosamino)-4-(3-pyridyl)-1-butanal (NNA), nitrosonornicotine (NNN), and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK generally is considered to be formed from the nitrosation of pseudo-oxynicotine (PON), an oxidation product of nicotine. PON was found in green tobacco but accumulation of NNK was not detected. The objective of this research was to determine the nitrosation rate of PON to NNK in different conditions. Kinetics of PON nitrosation to NNK was determined at pH levels of 3.0, 5.5, 7.5 at 20°C and 37°C. Measurement of PON and NNK were done by UPLC/MS/MS protocols. Reactions were done in citric acid (0.16M) and disodium phosphate (0.08M) buffers at the three different pH levels. Very little NNK was formed in the two higher pH levels in 24 h. At pH 3 and 20°C with an initial PON of 6.3 nmole ml⁻¹ and 180 µmole ml⁻¹ NO₂ a very rapid formation of NNK was measured. Increased NO₂ from 25 µmole ml⁻¹ to 250 µmole ml⁻¹ increased NNK about 5X with initial PON of 6.3 nmole ml⁻¹ in 30 min at 20°C. When PON was increased from 0.63 to 25.2 nmole ml⁻¹ NNK accumulation was linear over 30 min at 20°C with maximum NNK accumulation

of 3.56 nmole ml⁻¹. The reaction was more rapid at 37°C with about 2X the NNK formed within 60 min. These results indicate that the generation of NNK is pH dependent and temperature can affect the reaction rate from PON to NNK. These conditions will greatly influence NNK formation and accumulation in the green leaf and during curing.

82. ASSESSMENT OF TWO HIGH THROUGHPUT *IN VITRO* METHODS FOR THE QUANTIFICATION OF CIGARETTE SMOKE INDUCED MICRONUCLEI. Bethany COOPER¹, Manoj Misra¹ and Robert Leverette²; ¹ITG Brands, Greensboro, NC USA and ²R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

Micronuclei (MN) formation, *in vitro* and *in vivo*, is a DNA damage endpoint utilized to determine the genotoxicity potential of individual compounds or complex mixtures. Historically, MN data collection relied on a manual scoring process; however, advances in technology have led to automated detection and scoring methods such as image-based high content screening (HCS) and high throughput flow cytometry (HTFC). The *in vitro* MN assay has been applied to cigarette smoke condensates (CSC), utilizing an HCS approach (Cellomics ArrayScan VTi, Micronucleus Bioapplication V4); however, this particular approach is limited by protracted analysis time (≥ 90 minutes / 96-well plate). A new HTFC instrument and method (Intellicyt iQue HTFC; *In Vitro* MicroFlow, Litron Labs) have been introduced and adopted, with substantially decreased plate analysis times (10 – 12 minutes / 96-well plate). A side-by-side comparison between the HCS and HTFC methods was conducted using positive control compounds Mitomycin C (MMC) and Vinblastine (VB) and CSC from 3R4F and 1R5F reference cigarettes. Chinese hamster ovary (CHO-K1) cells in 96-well plates were exposed to increasing concentrations of MMC, VB and CSC for up to 24 hours prior to HCS or HTFC analysis. For CSC, cell survival (EC₅₀) differed slightly between methods; however, CSC induced MN levels (1.5 – 2.5 fold increase above control) were similar in both methods, at doses with $\geq 45 \pm 5\%$ cell survival. The Coefficient of Variation (CV) for MN induction was considerably lower for HTFC (~20%) versus HCS (~50%), the result of more overall events per well captured by HTFC versus HCS. Immediate HTFC advantages were speed and reproducibility. Method protocols, instruments and advantages and disadvantages will be presented in further detail.

83. *IN VITRO* TOXICITY SCREENING OF BLU ELECTRONIC CIGARETTE LIQUIDS AND IMPLICATIONS FOR HUMAN EXPOSURE. Manoj MISRA¹, R. D. Leverette², B. T Cooper¹ and M. B. Bennett¹; ¹ITG Brands, Greensboro, NC USA and ²R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

The popularity and sales of electronic cigarettes (e-cigs) have increased significantly with thousands of available flavors, many with limited knowledge regarding their toxicity. An initial *in vitro* toxicological screening was conducted for several commercial blu e-cigarette flavors (e-liquids); Classic Tobacco, Cherry, Cherry Crush, Vanilla, Tobacco Gold, Rich Tobacco, and NRG. For comparative purposes, tobacco burning Kentucky reference cigarettes (3R4F & 1R5F) were included. The standard CORESTA *in vitro* battery of established assays was used to examine the mutagenicity (Ames), cytotoxicity (Neutral Red Uptake; NRU), genotoxicity (Micronucleus; MN) and inflammation (IL-8 release). Dose-dependent effects in all assays were observed with tobacco burning cigarettes at doses 20-40 fold lower than e-liquids. EC₅₀ values for 3R4F and 1R5F cigarettes were 0.17 and 0.22 mg/mL, respectively for cytotoxicity, and for inflammation 0.15 mg/mL for both.

Mutagenicity specific activity values for 3R4F and 1R5F were 1261-1391 and 523-593 revertants/mg, respectively. For all four assays, the EC50 or NOAEL values for e-liquids could not be calculated due to the absence of response. A proposed approach to assess the potential human exposure via extrapolation of *in vitro* results was performed by comparing the experimental exposure surface area to that of human lung. The observed severe *in vitro* toxicity of tobacco cigarettes occurred at approximately a 1000-fold higher dose than expected for human exposure (18 mg Tar/cig; 20 cig/day). No toxicity was observed experimentally for e-liquids at doses 4000-fold higher than expected for human exposure levels (5.0 mL e-liquid consumption/day). Limitations, complexities and other factors involved in extrapolating our current experimental data to realistic human exposure will be discussed in further detail.

84. THE PREPARATION OF ADDITIVES TO REDUCE PHENOL AND ITS APPLICATION IN CIGARETTE. XIONG Shan-Shan¹, Wu Jing-Qiang¹, Xu Jian-Rong², Shen Jing-Xuan¹, Xiao Wei-Yi¹, Yao Zhen-Yu¹ and Xu Lan-lan¹; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China and ²China Tobacco Fujian Industrial Co., Ltd., Xiamen, China

Cellulose diacetate and glycerol triacetate are recognized as materials for selective adsorption of phenol in the tobacco industry. 1% CA-TC solution in this article was prepared, where TC is the solvent, CA is the solute, and the solution was applied on the central line of the cigarette. Compared with the blank central line, the results show that, 1% CA-TC central line selectively reduced the rate of cigarette mainstream smoke phenol by 10%; there is no statistically significant difference ($P > 0.05$) through toxicological evaluation (Ames, micronucleus cells and neutral red blood cell experiments); compared with the control sample, 1% CA-TC center line has no negative impact on the sensory quality of cigarettes.

85. DISCUSSIONS ON HARM REDUCTION MECHANISM OF BIOMASS STEM GRANULE MATERIAL IN CIGARETTES. ZI Wenhua, Long Minghai, Yang Lei, Shen Yan and Li Biao; Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China

The development of biomass stem granules has provided a viable new application for the utilization of waste tobacco stems. Its effect on the deliveries of harmful components in cigarette mainstream smoke was studied in this paper. The harm reduction mechanism was discussed thoroughly via pyrolysis kinetics of stem granule and its impact on the cigarette combustion temperature. The results showed that the application of biomass stem granules in cigarette blends could significantly reduce the deliveries of harmful components in mainstream smoke and the hazard evaluation index (H), such as CO, HCN, NNK, NH₃, B[α]P, phenol, crotonaldehyde, etc. The H value was decreased from 8.36 to 5.14 with its mix proportion increased to 8% in cigarettes, and the decreasing degree reached 38.53%. The mechanism of harm reduction was related to the pyrolysis activation energy of different cigarette materials and the effect on cigarettes combustion temperature. Although the pyrolysis process of biomass stem granule, cut tobacco blend, and reconstituted tobacco sheet is similar, its activation energy was lower than cut tobacco blend, which was 25.114 kJ/mol, 28.756kJ/mol, 20.551 kJ/mol under air atmosphere at heating rate of 10°/min, respectively. The peak maximum temperature, peak average temperature, suction average temperature and smoldering average temperature of cigarette samples decreased

significantly with the increased mix proportion of stem granule within the experimental range. Meanwhile, the H values also decreased obviously with the combustion temperature reduction of cigarettes, which further indicated that the harm reduction mechanism of biomass stem granule could be explained via the pyrolysis activation energy of cigarette materials and the effects on cigarettes combustion temperature, provided a theoretical basis for the development of low-hazard cigarette products.

86. STUDY ON INFLUENCE OF PROCESSING STRENGTH ON FLUE-CURED TOBACCO LEAF QUALITY IN THE THRESHING AND REDRYING PROCESS. LONG Minghai¹, Hua Yikun², Wang Xianguo², Lin Nan¹ and Zi Wenhua¹; ¹Yunnan Reascend Tobacco Technology (Group) Co. Ltd., Kunming, Yunnan, China and ²Hongyun Honghe (Group) Co., Ltd., Kunming, Yunnan, China

To improve the technological level of threshing and redrying and quality of products, the influences of processing strengths (steam moistening, steam mixing water moistening, slow redrying with low-temperature, high-temperature redrying) on the qualities of flue-cured tobacco leaves were studied in this paper. The results showed that the variation coefficients (Cv) of moisture and appearance colors fluctuations for redried lamina of steam moistening and slow redrying with low-temperature were 3.24%, 9.76% (L*), 7.82% (a*) and 8.39% (b*), respectively. The uniformities were increased by 42.65%, 16.44%, 46.73%, 3.12% compared to steam mix water moistening and high-temperature redrying, respectively. Compared to flue-cured tobacco leaves, the colorants contents were decreased by 8.13% and 18.29% via steam moistening and steam mix water moistening, respectively, while the polyphenol contents were 5.05% and 8.99%. Similarly, the colorant contents were decreased by 2.12% and 5.94% via slow redrying with low-temperature and high-temperature redrying, while the polyphenol contents were 15.63% and 19.46% compared with moistening tobacco leaves, respectively. However, the total contents of flavor precursors in tobacco leaves processed via steam moistening and slow redrying with low-temperature were higher than those by steam mix water moistening and high-temperature redrying, improved the flavor richness, aroma quantity and aftertaste of redried lamina. Therefore, the steam moistening and slow redrying with low-temperature were advantageous to improving the qualities of redried lamina in the threshing and redrying process, providing a theoretical basis for the characteristic processing techniques of the threshing and redrying.

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

SESSION A - Agronomy

2:20 PM TUESDAY

87. NICOTINE ANALYSIS IN SEVERAL NON-TOBACCO PLANT MATERIALS. Serban C. MOLDOVEANU, Scott A. Wayne and Darlene M. Lawson; R. J. Reynolds Tobacco Co., Winston-Salem, NC USA

Nicotine is a natural compound in plants from Solanaceae family. The plants from Nicotiana genus contain large levels of nicotine, and low levels are in plants from Solanum genus including potato, tomato, eggplant, and from Capsicum genus, which are used as food. In addition, contamination with nicotine was reported, for example, for mushrooms.

Because the levels of nicotine in these materials are in the range of parts per billion, the measurements are difficult and the results are very different from study to study. The present study evaluated the level of nicotine in a number of plants (fruits, leaves, tubers) from Solanaceae family (not including *Nicotiana* genus) and from several other vegetables commonly used as food. The analysis consisted of the treatment of plant material with an aqueous solution 5% NaOH at 70°C for 30 min, followed by extraction with TBME containing d3-nicotine as an internal standard. The TBME organic layer was analyzed on a 7890B/7000C GC/MS/MS system from Agilent with a 30m x 0.25, 0.25 um film CAM column (from J&W). The MS/MS system worked in MRM positive ionization mode monitoring the transition 162 – 84 for nicotine and 165 – 87 for d3-nicotine. Particular attention was given to the preservation of intact levels of nicotine in the plant material. The plant material was analyzed as is, without drying and with minimal exposure to contaminations. Separately, the moisture of the plant material was measured in order to report the nicotine level on a dry-basis. Levels of nicotine around 180 ng/g per dry material were obtained for tomatoes and eggplant (fruit) and lower levels were obtained for green pepper. Materials from other plant families showed traces of nicotine probably from contaminations.

2:40 PM TUESDAY

88. THE DEMETHYLASE MUTANTS – PANACEA OR NEW PROBLEMS? Anne JACK, Huihua Ji, Neil Fannin, Colin Fisher and Angela Schoengendorfer; University of Kentucky, Lexington, KY USA

The demethylase mutants contain knockout versions of three genes controlling nicotine to nornicotine conversion (CYP82E4, CYP82E5v2 and CYP82E10). These triple mutants have many advantages; lower conversion, lower NNN, cheaper and easier identification, elimination of expensive seed screening. However, there are several potential disadvantages. As a result of the lower conversion, nicotine levels in the mutants can be too high; three triple mutant lines failed the RQT (Regional Quality Test) on nicotine in 2013. The enantiomeric ratio is different in ultra-low converter lines, with a higher ratio of the more toxic S isomer of both nornicotine and NNN; this could result in higher absolute levels of S-NNN. We have developed a method to measure absolute amounts of R and S-NNN; previously, it was only possible to measure the ratio. It is also possible that the mutations could have an adverse effect on plant growth. In this study, we compared single (e4), double (e4e5) and triple (e4e5e10) mutants with the RNAi transgenic and equivalent wild types. Nicotine was not significantly higher in the triple mutants and transgenic than in the LC equivalent, nor was the absolute amount of S-NNN, despite the higher ratio of S-NNN. There were some consistent differences in growth parameters. The triple mutants generally had longer, narrower leaves and were taller with fewer leaves. The triple mutant and transgenic were later flowering with longer internodes in one variety but not in the other. In general, we conclude that the advantages of these triple mutants outweigh the potential disadvantages; they have a marked potential for harm reduction because the S-NNN, as well as the total amount of NNN, is greatly reduced in the triple mutants.

3:00 PM TUESDAY

89. EVALUATION OF ANDROGENIC AND GYNOGENIC DOUBLED HAPLOID LINES FOR USE AS PARENTAL LINES FOR HYBRID BURLEY TOBACCO VARIETIES. R.D. MILLER and Ezequiel Deoliveira; University Of Kentucky, Lexington, KY USA

Ten androgenic doubled haploid (ADH) lines, developed via anther culture followed by mid-vein culture to double the chromosome number, and ten gynogenic DH (GDH) lines, developed by an interspecific cross with *Nicotiana africana* followed by mid-vein culture, were derived from the inbred cultivar TN 90LC. Eight ADH and 4 GDH lines were also derived from the inbred line GR 149. For the TN 90LC family, each of the 10 ADH and 10 GDH lines were randomly paired with TN 90LC to form a triplet. The 10 triplets were evaluated for agronomic traits at three locations utilizing a randomized complete block with three replications. The GR 149 ADH and GDH lines were evaluated in a similar fashion. For the TN 90LC family, on average the ADH lines yielded 3.9% less and the GDH lines yielded 1.9% more than the inbred TN 90LC. For GR 149, the average yields of the ADH lines, GDH lines, and GR149 source were 3357, 3343, and 3351 Kg/ha, respectively. Each of the ADH and GDH TN 90 lines was crossed with TKS 2002LC, the female parental line of the hybrid variety KT 204LC. Similarly, each of the GR149 ADH and GDH lines was crossed with ms TN 90LC, the female parental line of the hybrid variety TN 97LC. The hybrid varieties were then evaluated in the same manner described for the parental lines. For both the KT 204LC and TN 97LC families, no yield differences were observed in hybrids having either ADH or GDH lines as the male parent in comparison to hybrids having the original inbred line as the male parent.

3:20 PM TUESDAY

90. EFFECT OF ARTIFICIAL ORDERING ON TSNAS DURING SHORT TERM STORAGE. Colin FISHER¹, Anne Jack¹, Lowell Bush² and Bob Pearce²; ¹KTRDC and ²Plant and Soil Sciences, University of Kentucky, Lexington, KY USA

Production guidelines caution burley tobacco growers against using water sprays to bring tobacco into case and storing high-moisture tobacco because of concerns that these practices can lead to the accumulation of TSNAs. A series of experiments over three years was done to test whether this is indeed the case. Low and a high converter burley tobacco lines were brought into order using several methods: naturally, in a conditioning chamber, with steam and with a mist or spray of water from a hosepipe. Samples for moisture content, alkaloids, TSNAs and nitrate and nitrite nitrogen were taken at takedown, before bulking and after 14 days in the bulk. The method of ordering did not cause an increase of TSNAs in any of the tests. After 14 days in a bulk, there was no increase in TSNA accumulation as the moisture content of the tobacco increased. The method of application however, did make a difference to moisture content of the tobacco, more because of the difficulty of distributing the water evenly if a coarse spray or even a mist were used. Takedown and stripping in Kentucky is typically done between November and February when the average low temperatures are just below freezing and the average high temperatures are 40 to 45°F (4 to 7°C). In warmer production areas, especially those with higher humidity, there could still be some effect of ordering on TSNA accumulation, especially at higher moisture contents.

4:10 PM TUESDAY

91. TRANSCRIPTOME-ANALYSIS ENABLED THE DEVELOPMENT OF CO-DOMINANT MARKERS FOR NIC1 AND NIC2 IN TOBACCO. Shengming YANG, Qiulin Qin, Dandan Li, Robert Miller and Anne Jack; University of Kentucky, Lexington, KY USA

With the impending FDA regulation, it is expected that tobacco plants with low alkaloid levels will be more and more favored in future tobacco breeding. Nicotine is the predominant alkaloid in most commercial varieties of tobacco, and it is synthesized in the tobacco root under the control of two independent genes, Nic1 and Nic2. Genetic studies revealed that *nic1* and *nic2* mutations are semi-dominant and act synergistically, with effects of *nic1* being 2.4 times stronger than those of *nic2*. Even though the *nic* mutants have long been available, breeding selection is difficult. Without efficient molecular markers the introgression of *nic1* and *nic2* into the commercial varieties has been seriously hampered. Based on the RNA-seq analysis of four near-isogenic lines with various nicotine levels, co-dominant markers closely linked to Nic1 and Nic2 genes were developed. Our results will greatly benefit the tobacco breeders and industry to generate tobacco varieties with low level of alkaloids.

4:30 PM TUESDAY

92. METABONOMICS STUDY OF TWO NICOTIANA GENOTYPES UNDER CADMIUM STRESS. ZHANG Yanling, Guo Yuanyuan, Zhou Huina and Zhai Niu; Zhengzhou Tobacco Research Institute, Zhengzhou, Henan, China

A metabonomics method based on Ultra high performance liquid chromatography-quadrupole-time of flight mass spectrometry (UHPLC-Q-TOF/MS) was applied to study the metabolite changes in different tobacco genotypes under cadmium stress. Mass Hunter was used to extract the mass spectrometry information, MPP (Mass Profiler Professional) was used to screening and processing data. 13 differential metabolites including Homocarnosine, Hydroxylysine, Pantothenic Acid and 5'-Methylthioadenosine were identified by using accurate mass, ms/ms date and mass spectrometry database. Metabolic pathway analysis showed that under cadmium stress, 13, 6, 6 kinds of different metabolites were found in the roots, stems, leaves of K326 and *N. rustica*. Metabolites changes of K326 and *N. rustica* roots could increase the biosynthesis of lignin and jasmonic acid, suggested that both of the two nicotiana genotypes could reduce cadmium toxicity by root lignification and increase the amount of jasmonic acid. Serine content reduced, glycine, threonine, alanine content increased of K326 and *N. rustica* roots. In the stems, leaves of K326 and *N. rustica* the secondary metabolites had the same trend and glycine, threonine, alanine content all increased. Arginine, cysteine, methionine, tryptophan content reduced and proline content increased in the roots of K326. Lysine, arginine content increased, proline content decreased and cysteine, methionine, tryptophan had a little change in the root of *N. rustica*. Serine, arginine content in the stems and leaves of K326 reduced, glycine, threonine, proline content increased, *N. rustica*, on the other hand. Cadmium stress made GSH synthesis precursor cysteine content reduce in the roots of K326, *N. rustica* changed little and had contrary trend in the stems and leaves, K326 upgrades and *N. rustica* downgrades. Consistent with the trend of root cadmium chelating related gene expression.

4:50 PM TUESDAY

93. TRANSCRIPTION FACTOR NTPIF1 INTERACTED WITH NTRAP2.2 TO NEGATIVELY REGULATE EXPRESSION OF NTPSY. LEI Bo, Ding Fuzhang, Zhao Huina, Cai Kai, Pan Wenjie and Cai Bin; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Carotenoids are important secondary metabolites in flue-cured tobacco. Phytoene synthase (PSY) is the first specific enzyme in the carotenoid biosynthesis pathways, because of its crucial regulatory role of PSY in the carotenoid pathway, the PSY promoter region was analyzed in more detail in other plants. Transcription factor AtRIFs and AtRAP2.2 regulate expression of AtPSY with bind with G-box element and ATCTA-motif in its promoter in Arabidopsis. In this work, Phytochrome-interacting Factor 1 (NtPIF1), RELATED TO APETALA2.2/Ethylene-responsive element-binding protein (NtRAP2.2) and the promoters of two NtPSY gene family members were cloned in *Nicotiana tabacum*, and G-box element and ATCTA-motif in the promoters were found to be bind by proteins NtPIF1 and NtRAP2.2 by EMSA, separately. The interaction between NtPIF1 and NtRAP2.2 were identified through yeast-two-hybrid, GST-pull down and Co-Immunoprecipitation (Co-IP). Agrobacterium-mediated transient expression results showed that expression of NtPSY could be negatively regulated by NtPIF1 and NtRAP2.2, and further reduced by simultaneously expression of NtPIF1 and NtRAP2.2. These results preliminary uncovered the upstream regulatory network of carotenoid pathway and laid the theoretical foundation for transcription regulation of carotenoid pathway genes in flue-cured tobacco.

TUESDAY AFTERNOON, SEPTEMBER 22, 2015

SESSION B – E-Cigarettes

2:20 PM TUESDAY

94. EFFECT OF POWER LEVEL ON THE YIELD OF TOTAL AEROSOL MASS AND FORMATION OF ALDEHYDES IN E-CIGARETTE AEROSOLS. I. Gene GILLMAN, Emil W. Stewart and Amelia R. Paolantonio; Enthalpy Analytical Inc, Durham, NC USA

The study objective was to determine the effect of power applied to the atomizer of refillable tank-based e-cigarette (EC) devices. Five different devices were evaluated, each at four power levels. Aerosol samples were collected using a 55 mL puff with a four second duration. Aerosol results will be reported for each puff block as mass/puff and normalized for the power applied to the coil in mass/watt. The range of aerosol produced on a per puff basis ranged from 2.5 to 34.4 mg, and, normalized for power applied to the coil, ranged from 0.38 to 1.38 mg/watt. Aerosol samples were also analyzed for the production of formaldehyde, acetaldehyde, and acrolein at each power level. The amount of formaldehyde, acetaldehyde, and acrolein produced per puff ranged from 0.04 to 74.4 µg, 0.05 to 56.1 µg, and <0.02 to 12.9 µg, respectively. The amount of formaldehyde, acetaldehyde, and acrolein produced per mg of aerosol produced was from 0.009 to 9.49 µg, 0.004 to 7.16 µg, and <0.002 to 1.65 µg, respectively. These results were used to estimate daily exposure to formaldehyde, acetaldehyde, and acrolein from EC aerosols, and were compared to estimated daily

exposure from both consumption of cigarettes and to workplace exposure limits. Two of the devices produced aerosol that exceeded aldehyde workplace exposure limits and the estimated exposure due to combustible cigarettes usage. The other three devices produced aldehyde amounts below both the estimated exposure due to combustible cigarettes usage and a workplace exposure limit.

2:40 PM TUESDAY

95. INFLUENCE OF RELATIVE HUMIDITY CONDITIONING ON AEROSOL AND LIQUID CHEMISTRIES IN ELECTRONIC CIGARETTES. Candice K. CUNNINGHAM¹, Steven L. Alderman² and Doug Brown²; ¹R. J. Reynolds Tobacco Company, Winston Salem, NC USA and ²R.J. Reynolds Vapor Company, Winston Salem, NC USA

With the growing market of electronic cigarettes, there is also a growing need to consider standardized methods for analyzing these products. As with standardized methods for combustible products (e.g. ISO, Health Canada), any method for electronic cigarettes may require specification of conditions in which these products are analyzed. This work investigated varying relative humidity (RH) conditions and the effect RH had on the nicotine, water, propylene glycol (PG), and glycerin content in generic formulations of electronic cigarette liquids (e-liquids) where the initial nicotine was 2% and the PG:Glycerin ratio was 50:50 for all formulations while the water content ranged from 0 to 15%. Aerosol from these formulations was also produced under different relative humidity conditions, and analyzed for nicotine, water, PG, and glycerin. Additionally, gravimetrically determined total particulate matter mass yields were compared to device weight loss measurements. Conditions investigated were 40% RH and 60% RH at 22 +/- 3°C for both e-liquid and aerosol, and additionally 24% RH at 33 +/- 1°C for e-liquids.

3:00 PM TUESDAY

96. **Withdrawn**

3:20 PM TUESDAY

97. **NON-TARGETED ANALYSIS OF EMISSIONS FROM TOBACCO HEATING PRODUCTS.** Kelly REES, Justin Frosina, Michal Brokl, Christopher Rawlinson and Chris Wright; British American Tobacco, Southampton, UK

The analysis of emissions from tobacco heating products presents a number of challenges. These include: ensuring that testing conditions are relevant to the mechanism of operation and to known or expected consumer use; design of representative sampling conditions; and ensuring that the testing, measurement or analytical method is fit for purpose.

As well as constituents that are known to be present in the tobacco consumable, a number of additional constituents may be found in the aerosol that are associated with the heating device or due to thermal degradation. In order to inform product understanding and to assure due diligence, testing methods are required to screen the chemical constituents of tobacco heating product emissions.

Further to the presentation by Rawlinson *et al* at the 68th TSRC in 2014, this presentation will demonstrate the evolution of the method from separate particulate and vapour phase trapping using Cambridge Filter Pad collection and sorptive trapping (respectively) to sorptive trapping alone, and the benefits this provides. In addition, developments in the gas chromatographic analysis and data processing will be highlighted.

4:10 PM TUESDAY

98. **EVALUATION OF BIOMARKERS OF SMOKE EXPOSURE IN ADULT SMOKERS FOLLOWING DUAL USE OF CIGARETTES WITH ELECTRONIC CIGARETTES.** Carl D. D'RUIZ; ITG Brands, Greensboro, NC USA

This study evaluated changes in various urine and blood biomarkers of smoke exposure following a switch from usual brand combustible cigarettes to dual use of e-cigarettes and combustible cigarettes over a 5-day period. Healthy adult smokers were randomized into groups that either partially substituted their usual brand cigarette with 1 of 3 commercial blu™ e-cigarettes ('dual use', n= 45, 15 per product) or discontinued all tobacco product usage for five days ('nicotine cessation', n=15). Following randomization, dual use subjects were allowed to use assigned combustible and e-cigarette products ad libitum, but could smoke no more than 50% of the number of cigarettes per day reported during screening. Urinary (NNAL, Nicotine equivalents, 3-HPMA, HMPMA, CEMA, 1-OHP, NNN, MHBMA, S-PMA, and 8-iso-PGF2 Type III) and blood (COHb, Nicotine, Cotinine, and trans-3'-hydroxycotinine) biomarkers were assessed at baseline and following randomized product use. With the exception of the nicotine equivalents, dual users exhibited reduced urinary biomarker levels that were approximately proportional to the reduction in cigarettes smoked, with significant, positive linear relationships observed between percent change in excretion and the percent change in cigarettes smoked. COHb also tended to be reduced compared to baseline. As expected, the cessation group experienced significant reductions in the biomarkers compared to baseline, and values were significantly lower at Day 5 compared to dual use. These results suggest that smokers who reduce their cigarette consumption through partial substitution with e-cigarettes may experience reduced

exposure to several of the harmful smoke constituents found in combustible cigarettes while replacing nicotine from combustible cigarettes with e-cigarettes.

4:30 PM TUESDAY

99. REDUCTIONS IN BIOMARKERS OF SMOKE EXPOSURE IN FOLLOWING COMPLETE SUBSTITUTION OF CIGARETTES WITH ELECTRONIC CIGARETTES.
Carl D. D'RUIZ; ITG Brands, Greensboro, NC USA

This study compared the short-term changes in selected urinary and blood biomarkers of smoke exposure in subjects that switched from combustible cigarettes to e-cigarettes versus a nicotine product cessation group to assess whether the use of e-cigarettes can significantly reduce exposure to harmful smoke constituents found in combustible cigarettes. Following screening, clinically-confined healthy adult smokers were allowed to smoke their usual brand of cigarettes for 1 day (baseline) prior to being randomized to ad libitum use of 1 of 3 commercial blu™ e-cigarettes ('exclusive use' group, n=45, 15 per product) or to discontinuation of all tobacco product usage ('cessation' group, n=15). Urinary (NNAL, Nicotine equivalents, 3-HPMA, HMPMA, CEMA, 1-OHP, NNN, MHBMA, and S-PMA) and blood (COHb, Nicotine, Cotinine and trans-3'-hydroxycotinine) biomarkers of smoke exposure were assessed at baseline and following randomized product use. With the exception of nicotine equivalents, after 5 days there were no significant differences in urinary excretion of the biomarkers of exposure and COHb concentration in the blood between the exclusive use groups and the cessation group, with all groups experiencing significant reductions compared to baseline. Predictably, nicotine equivalents in urine and concentrations of nicotine and its metabolites in the blood were higher in the exclusive e-cigarette groups compared to the cessation group, but were lower after 5 days of exclusive use of the e-cigarette compared to baseline. These study findings are consistent with an expectation of significantly reduced exposures to harmful smoke constituents in smokers who completely replace their cigarettes with e-cigarettes, and further with an expectation of potentially reduced risks for diseases believed to be caused by those exposures.

4:50 PM TUESDAY

100. AN ONLINE SURVEY OF 5,000 VAPERS' PERCEPTIONS AND EXPERIENCES OF USING ELECTRONIC CIGARETTES AS AN AID TO SMOKING CESSATION.
Chris RUSSELL, Neil McKeganey and Tiffany Hamilton-Barclay; Centre for Drug Misuse Research, Glasgow, Scotland UK

Millions of smokers are now choosing, and succeeding, to quit or reduce tobacco smoking with the assistance of electronic cigarettes and vapourisers. However, the success of e-cigarettes in reducing tobacco-related harm is being limited because many smokers who trial e-cigarettes do not persist with them long-term, for reasons that are neither well understood nor well predicted. Instead, many smokers use e-cigarettes only experimentally or as a short-term alternative to cigarettes before resuming cigarette smoking. Understanding the psychosocial mechanisms and product features that attract smokers to trial e-cigarettes as an alternative to smoking, and then convert e-cigarette experimenters into regular users, is critical for mitigating e-cigarette users' odds of relapsing to smoking.

In this presentation, we will describe the findings from a currently live, multi-country, online survey of 5,000 e-cigarette users who identified the perceptions and experiences that attracted them to begin and then continue using e-cigarettes as an aid to smoking cessation. This survey is collecting post-market surveillance data on a multitude of factors that are specified by the U.S. Food and Drug Administration as 'priority data needs', including users' perceptions of the health risks and benefits associated with using e-cigarettes as a partial or complete substitute for conventional cigarettes.

The obtained data will identify perceptions and experiences of using e-cigarettes that rationalize smokers' decisions to initiate and continue e-cigarette use as a long-term alternative to smoking, and to desist from e-cigarette use and resume smoking. These data can guide regulatory bodies, health practitioners, and e-cigarette manufacturers to provide information, regulation, and products that more effectively persuade and assist smokers to use e-cigarettes as a means to reduce their daily smoking and smoking urges.

WEDNESDAY MORNING, SEPTEMBER 23, 2015

SESSION A –Agronomy

8:30 AM WEDNESDAY

101. QUANTITATIVE PROTEOMICS ANALYSIS THE TOBACCO TRICHOMES UNDER CADMIUM STRESS. FU Qiang, Lin Shifeng, Zou Jie, Yu Jing, Zhang Xiaolian and Ren Xueliang; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Nicotiana tabacum (tobacco) is known to both accumulate and tolerate high levels of heavy metals from polluted soils, its trichomes appeared to be the excretion of toxic metals in the form of inorganic particles To gain a comprehensive understanding of the effect of cadmium(Cd) treatment on *N. tabacum* leaf trichomes, control and Cd-treated trichome proteins were prepared in triplicate to be explored using a proteomics approach with high throughput isobaric tag for relative and absolute quantification (iTRAQ) technique using liquid chromatography tandem mass spectrometry (LC MS/MS). The 3901 protein groups were identified in the trichome protein samples, of which quantitative information was detected in the 3871 proteins. And the number of differentially expressed proteins was 164 between the control and Cd-treated *N. tabacum* leaf trichomes (ratio>1.2, p<0.05). The proteins up-regulated by Cd-treatment were associated with sulfur metabolism, defense/stress responses, and metabolic “housekeeping.” Down-regulated proteins were mostly associated with protein synthesis/processing or carbohydrate metabolism. Proteins for stress response as well as for primary metabolism scored highly, indicating that the trichome is a biologically active and stress-responsive tissue. Our results revealed that the trichome-specific proteomics approach was a powerful tool to investigate the defensive functions of trichomes against both abiotic and biotic stress. Trichomes are shown to be an enriched source of useful genes for molecular breeding towards Cd-tolerant plants.

8:50 AM WEDNESDAY

102. ISOLATION AND IDENTIFICATION OF TOBACCO SPECIFIC PLANT GROWTH-PROMOTING RHIZOBACTERIA AND ITS FIELD APPLICATION. LI Xiang, Liu Yanxia and Shi Junxiong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Plant growth promoting rhizosphere microorganisms (PGPR) could not only reduce the dose of pesticide and fertilizer, but also promote plant growth. Isolating efficient tobacco PGPR and making plant-growth-promoting bioorganic fertilizer have great effect on improving tobacco yield and quality.

In this investigation, three efficient plant growth promoting bacteria were screened from tobacco rhizosphere and organic fertilizer. Seedling and pot experiments were conducted to prove the plant growth promoting effect. It was studied that how the plant-growth-promoting bioorganic fertilizer had effect on soil rhizosphere microbe by high-throughput sequencing rhizosphere microbial genome, which investigated the plant-growth-promoting mechanism of bioorganic fertilizer affecting soil microorganism.

Strain LX4, LX5 and LX7 had significant growth promotion effect on tobacco among all the screened PGPR strains. These three strains were identified as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, respectively. The PGPR bioorganic fertilizer consisted of three strains could significantly ($P \leq 0.05$) promote tobacco growth in seedling period and after transplantation. The plant-growth promoting effect of T6 (MBOF) treatment was significantly higher than other treatments, with yield increasing by 14.21% and 12.35%, compared to T1 and T2 treatments. The agronomic trait of the T6 treatment was higher than other treatments in both resettling growth and topping stages, but not significantly ($P \leq 0.05$) higher than T4. The dry weight of root, root diameter, area and volume of T6 treatment increased by 17.96%, 12.64%, 82.35% and 168.00%, compared with T1. Besides, the activities of tobacco leaves anti-enzyme of the T6 treatment were significantly higher than other treatments. Soil microbial diversity revealed differently among different treatments. The diversity of Nitrospira in T6 was much higher than that in T1.

The PGPR bioorganic fertilizer consisted of three PGPRs not only increased tobacco yield and quality, but also efficiently improve soil microbial diversity, thus possessing a promising application prospect.

9:10 AM WEDNESDAY

103. THE SOLID-STATE-FERMENTATION OF THE DISCARDED TOBACCO LEAVES TO MAKE ORGANIC FERTILIZER AND ITS EFFECT ON TOBACCO GROWTH. LI Xiang, Liu Yanxia and Shi Junhong, Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

In China, there are more than 1.5 million tons of discarded tobacco leaves produced every year. In order to make better use of the discarded tobacco leaves, they were solid-state-fermented to produce organic fertilizer. Effective decomposing strains were screened, identified and re-inoculated to the discarded tobacco leaves to make organic fertilizer. Field experiment was conducted to evaluate the effect of the organic fertilizer on tobacco growth after its application. Three treatments were designed: T1, chemical fertilizers only (NPK); T2, chemical fertilizers plus unfermented tobacco leaves (NPK-N); T3, chemical fertilizers plus solid-state-fermented tobacco leaves (NPK-O). The results obtained are listed as follows. Two strains out of six selected strains, identified as *Bacillus amyloliquefaciens* and *Bacillus subtilis*, were screened by decomposing cellulose ability, nicotine tolerance and antagonistic experiment. The organic fertilizer contained 11.5 g nitrogen kg⁻¹, 301.6 g organic matter kg⁻¹, 2.7 g total phosphorous kg⁻¹ and 21.3 g potassium (K₂O) kg⁻¹. The C/N of organic fertilizer (15.9) was significantly high, compared with that of unfermented tobacco leaves (9.79). The NPK-O treatment gave significant increases of 10.1% compared with NPK-N. The dry root weight, root surface area and root volume of the NPK-O treatment were 15.7%, 70.6% and 52.1% higher than that of NPK, respectively. The peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and polyphenoloxidase (PPO) of the NPK-O soil significantly increased, compared with NPK. In conclusion, it has a wide application prospect that making the discarded tobacco leaves into organic fertilizer.

9:30 AM WEDNESDAY

104. EFFECT OF DIFFERENT NITROGEN APPLICATION ON FLUE-CURED TOBACCO HAZARD INDEX AND ENDOGENOUS HARMFUL COMPONENTS IN MAINSTREAM CIGARETTE SMOKE. GENG Zhao-liang, Zhang Jie, Ge Yong-hui, Xiang Zhang-min and Feng Yong-fang, Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

Nitrogen has the reputation of “biological element”, that is usually in the form of protein, nucleic acid, chlorophyll, nicotine and others in tobacco. In the field test with flue-cured tobacco K326, the effect of different nitrogen levels (45.0 75.0 and 105.0kg/hm²) on contents of main chemical constituents in flue-cured tobacco, endogenous harmful components in cigarette mainstream smoke and hazard index (H) was studied. Results showed that, nicotine, total nitrogen, protein and potassium contents in both upper tobacco leaves (B2F) and middle tobacco leaves (C3F) were increased with the increasing of nitrogen application, while sugar and reducing sugar content decreased. For endogenous harmful components in mainstream cigarette smoke, NNK, B[a]P, NH₃ and HCN contents were increased with the increasing of nitrogen application, while no significant relation was found for CO, crotonaldehyde, and phenol. And also, as nitrogen application increased, hazard index of both upper and middle tobacco leaves also increased, which along with the increasing gradient of 45.0, 75.0 and 105.0kg/hm² of nitrogen application, hazard index of upper leaves rose 9.4% and 5.4% on average, while that of middle leaves respectively, by an increase of 11.1% and 5.6%. Combining with our previous correlation study between main chemical constituents in tobacco and endogenous harmful components in mainstream cigarette smoke, it suggested that properly decreased nitrogen fertilizer use may reduce cigarette hazard index. The study aimed to provide the scientific basis for production of tobacco leaf with high quality and low harm.

10:20 AM WEDNESDAY

105. DIFFERENCE OF THE NEUTRAL AROMA COMPOUNDS OF FLUE-CURED TOBACCO BEFORE AND AFTER CURING. ZHAO Huina, Lei Bo, Cai Kai, Wu Shengjiang, Pan Wenjie and Cai Bin, Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Not Presented

In order to study the effect of the flue-curing process on neutral aroma compounds of tobacco, HS-SPME-GC×GC-TOFMS and the half leaf method were used to analyze types, contents and proportion of the neutral aroma compounds in two flue-cured tobacco cultivars (K326 and Nanjiang 3) before and after curing. The results showed that there were significant changes in types, contents, and proportion of the neutral aroma compounds. Many types of aroma compounds were degraded or transformed during the curing process - 19 aroma compounds uniquely found in tobacco leaves before curing and 36 extra aroma compounds found in tobacco leaves after curing. By the end of curing, the total amount of aroma compounds was significantly higher than before curing, and the total amount of aroma compounds (except neophytadiene) in K326 was significantly higher than that of Nanjiang 3, which transformed more fully. The proportion of phenylalanine degradation products and carotenoid degradation products in the total amount of neutral aroma compounds increased significantly. Conversely, the proportion of cembratriendiol

compounds significantly decreased after curing. In conclusion, 36 types of extra aroma compounds (mainly maillard reaction production and carotenoid degradation production) were generated during curing, but 19 types of aromatic components (mainly aldehydes, alcohols and esters) were lost.

10:40 AM WEDNESDAY

106. PHOTOSYNTHETIC AND GROWTH CHARACTERISTICS OF FLUE-CURED TOBACCO SEEDLINGS IN WELL-CELLAR STYLE TRANSPLANTING. LIN Yechun, Chen Wei, Gao Weichang, Chen Yi, Ding Fuzhang, Li Hongxun, Liang Guilin and Pan Wenjie; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

The well-cellar style transplanting (WCST), widespread in China, was an original transplanting method of flue-cured tobacco, and had obvious advantages when compared to conventional transplanting (CT). WCST accelerated the growth of tobacco seedlings; however, it is necessary to study the characteristics of photosynthetic physiology of tobacco seedlings in WCST. Combined with the field and pot experiments, the study examined photosynthetic active radiation (PAR) level, plant height, leaf length, leaf width and chlorophyll content, and the leaf photosynthesis and maximal photochemical efficiency (Fv/Fm) of photosynthetic system II (PSII) were analyzed in WCST, compared with CT. The results showed that PAR levels in WCST were decreased (i.e, such that PAR levels were 72.60% and 52.85% at 4 cm and 8 cm in WCST respectively, compared with the CT). Plant height, leaf length and leaf width of tobacco seedlings were improved, but the leaf chlorophyll contents were reduced in WCST. In WCST, the maximum net photosynthetic rates (Pmax) and the light saturation points (LSP) were decreased and the light compensation points (LCP) and dark respiration rates (Rd) were reduced by 20.43% and 17.78% respectively. The apparent quantum yield (AQY) was improved by 4.44%, compared to CT. PSII maximal photochemical efficiency (Fv/Fm) of tobacco seedlings was reduced when transplanted in WCST or CT, but the Fv/Fm of transplanted seedlings was significantly ($P < 0.05$) higher by 5% to 9% in WCST than in CT. The growth of tobacco seedlings transplanted by WCST was accelerated in WCST. The self-adaptation and the enhanced light use efficiency in a weak light environment may provide a physiological basis to promote tobacco growth.

11:00 AM WEDNESDAY

107. ISOLATION AND FUNCTIONAL ANALYSIS OF TOBACCO GLUTAREDOXIN NBGRX1 IN RESPONSE TO DROUGHT STRESS. GUO Yushuang, Li Ruiyuan, Yu Jing, Zou Jie and Zhao Jiehong; Guizhou Academy of Tobacco Science, Guiyang City, Guizhou, China

Glutaredoxins (Grxs) are ubiquitous small heat-stable disulfide oxidoreductases that play a crucial role in plant responses to oxidative stress. Recently, studies have extended our knowledge on the physiological and molecular functions of Grxs in plants. Drought is a major factor that limits plant growth, development, and geographical distribution and adversely affects crop production. The objective of this study was to explore the new Grxs in tobacco and study their function in plant responses to drought stress. A cDNA fragment named NbGRX1 was isolated using homologue cloning and in silico cloning methods. Sequence analysis shows that the DNA contains 1021 nucleotides which encode

a protein of 298 amino acids. Quantitative real-time RT-PCR (qRT-PCR) analysis revealed that NbGRX1 is expressed ubiquitously in the tobaccoplant, including leaf, root, stem and flower, and can be induced by low temperature, drought and salt stresses. To investigate the subcellular localization of NbGRX1 protein in plant cells, the NbGRX1 protein was fused to the C-terminus of GFP and the construct was transiently expressed in *N. benthamiana* leaf epidermal cells then monitored by capturing GFP fluorescence. Our results suggested that NbGRX1 protein was localized in both nucleus and cytoplasm. A NtGRX1 gene silencing vector was constructed based on the geminivirus satellite and then introduced into *Agrobacterium tumefaciens* strain EHA105. Virus-induced gene silencing of NbGRX1 in tobacco led to increased sensitivity to drought stress with plants easily yellowing and wilting. The coding sequence of NbGRX1 was then introduced into *Arabidopsis* under the control of the cauliflower mosaic virus (CaMV) 35S promoter. Independent homozygous lines with a single copy of NbGRX1 were allowed to grow for 3 generations for further studies. We found that over-expression of NbGRX1 in *Arabidopsis* plants enhanced the tolerance to drought in soil-grown conditions. Our research demonstrated that the NbGRX1 was essential for plant response to drought stress.

SESSION B – Toxicology & Materials

8:30 AM

WEDNESDAY

108. *IN VITRO* CYTOTOXICITY OF ABORIGINAL AUSTRALIAN SMOKELESS TOBACCO PRODUCT 'PITURI' COMPARED TO NICOTINE IN HUMAN LUNG EPITHELIAL CELLS. Nahid MOGHBEL, BoMi Ryu and Kathryn J. Steadman; The University of Queensland, St. Lucia, Australia

Aboriginal people of central Australia use a range of *Nicotiana* species to make a smokeless tobacco product that they call 'pituri'. Pituri is prepared by mixing dried leaves of wild tobacco plants with ash, usually from burned *Acacia* twigs, and macerated into a 'quid' that is chewed or stored in their mouth for prolonged absorption of nicotine. Although the main biological and physiological outcomes of tobacco consumption is attributed to its major alkaloid nicotine, smokeless tobacco contains a large spectrum of other biologically active compounds that might also contribute to effects on health. Therefore, the objective of this study was to compare cell toxicity of pituri extract with pure nicotine at the same concentration using an *in vitro* assay.

Aqueous extract of pituri was quantified for nicotine using HPLC. A range of concentrations of the extract and corresponding concentrations of nicotine standard was used against human lung epithelium cells (A549) within different treatment times along with appropriate negative and positive cellular control and replication. The one-step MTT assay (MTS) was used for assaying cell viability.

Results show almost two fold lower viability of cells treated with pituri extract than the cells treated with pure nicotine standard of the same concentration. *Nicotiana* species used for preparing pituri contain nicotine along with other alkaloids such as nornicotine, anatabine, and anabasine, which can give rise to carcinogenic tobacco-specific N-nitrosamines on drying. Higher cytotoxic effects of pituri extract could be due to the presence of these nitrosamines and other compounds such as benzo[a]pyrene.

8:50 AM WEDNESDAY

109. 3D RECONSTRUCTED HUMAN AIRWAY MODELS: EFFECT OF ACCLIMATION CONDITIONS ON BIOMARKER AND INFLAMMATORY RESPONSE FOLLOWING TISSUE CHALLENGE. Holger BEHRING, Hans A. Raabe, Devin W. Sheehan, Elizabeth A. Sly and Rodger D. Curren; Institute for *In Vitro* Sciences, Gaithersburg, MD USA

Human reconstructed, 3-dimensional (3D) airway tissues present researchers with organotypic models that consist of heterogeneous cell types that more accurately reflect the pulmonary airway *in vivo*. These *in vitro* models are now increasingly applied to assess inhalation exposures, including those from tobacco-based products such as whole cigarette smoke and E-cigarette vapors. We conducted an exploratory study to assess the impact of acclimation conditions on air-liquid interface (ALI) tissue biomarker and cytokine responses following apical challenge. MatTek EpiAirway™ tissues were received and acclimated using hydrocortisone (HC) free or HC containing (1 µg/mL) medium, for 24 or 48 hr prior to apical challenge with two reference chemicals known to elicit an inflammatory response. Following the designated acclimation period, groups (comprised of triplicate ALI tissues) were treated with either 15 µg/mL Poly I:C or 5 µg/mL lipopolysaccharide (LPS) for 24 hr. After measuring TEER, an apical rinse, the tissue lysate, and medium from each ALI insert were collected. Total protein, LDH, IL-6, IL-8, and IP-10 were assayed from each sample. Results of a comparison between 24 hr and 48 hr acclimation times indicated that a 48 hr acclimation could result in a greater baseline TEER value and 6-fold greater average biomarker increase (over negative control) following challenge. The 48 hr HC containing acclimation medium group typically had the greatest average cytokine response and this was found in the apical rinse where markers were up to ~5-fold higher than in either tissue lysate or medium samples. The marker with greatest increase over control was IP-10 (160-fold in tissue; 139-fold in apical rinse, and 25-fold in medium) following Poly I:C challenge in the 48hr HC inclusive group. No consistent correlation between tissue protein content and biomarker expression level was found. As tobacco products are increasingly evaluated using the 3D airway models, researchers should consider experimental approaches and marker sampling options that will best demonstrate relevant changes in the tissue following challenge. An optimized acclimation protocol may deliver a more robust response and the marker sampling location may reflect relevant events that should be considered when using this useful model of the human airway.

9:10 AM WEDNESDAY

110. QUANTITATIVE BIOMONITORING OF URINE MUTAGENICITY: AN ALTERNATIVE TO CLASSICAL AMES TEST. Rafiqul ISLAM¹ and Clarinda Islam²; ¹Celerion Inc., Lincoln, NE USA and ²Somru BioScience Inc., Charlottetown, Canada

For more than 30 years the Ames test has been a widely used in-vitro method for the biomonitoring of urine mutagenicity due to exposure to tobacco products. Briefly, the Ames' test detects point mutations and frame shifts based on the reversion of inactivating mutations in the biosynthesis operon of a given amino acid by using *Salmonella* or *E. coli*. While it is currently the test of choice for the detection of cancer-causing potential due to tobacco exposure, it has a number of limitations. The classic Ames test provides binary readout (*i.e.* growth vs. no growth), suffers from poor sensitivity, requires high sample volume, and not easily scalable.

This presentation will describe a highly sensitive reversion mutation assay that provides quantitative readout for mutagenesis. The assay is based on the functional fluorescence-antibiotic resistance fusion construct that act as a dual reporter. The dual reporter is cloned into a multi-copy plasmid. A host cell is transformed with this plasmid. The mutations at the reversion site allow read-through of the fusion protein producing both an antibiotic resistance protein and a fluorescent protein. In the presence of an antibiotic, the level of fluorescence emitted by the fluorescence protein is proportional to the number of mutation events at the reversion site.

The assay is highly specific, very sensitive and requires very low sample volume. It provides a quantitative and unambiguous fluorometric endpoint which is amenable to statistical analysis. The assay is scalable and provides a major cost savings opportunity.

9:30 AM WEDNESDAY

111. ANALYSIS ON IMMUNOTOXICITY INDUCED BY CIGARETTE SMOKE CONDENSATE BY A RAPID DETECTION AND EVALUATION METHOD BASED ON LUMINEX LIQUICHIP. KANG Yu¹, Zhao Junwei¹, Li Xiang¹, Yang Zhihua², Zhu Maoxiang², Liu Huimin¹ and Xie Fuwei¹; ¹Zhengzhou Tobacco Research Institute, Zhengzhou, China and ²Institute of Radiation Medicine, Beijing, China

Cigarette smoke consisted of thousands compounds while many of them are of immunotoxicity. In order to investigate the immunotoxicity of mainstream cigarette smoke, a rapid detection and evaluation method based on Luminex liquichip was developed for immunotoxicity induced by cigarette smoke. On the basis of this method, EL-4 cells and Ana-1 cells were selected and exposed to the condensates of 3R4F mainstream smoke with a series of concentration. Twenty cytokines related to immunity were collected and analyzed by Luminex liquichip. Results showed that, when EL-4 cells were exposed to 0 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 60 µg/mL and 80 µg/mL condensates, IP-10 expression level increased as the condensate concentration increased, whereas the expression levels of GM-CSF and VEGF decreased; when Ana-1 cells were exposed to 0 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL condensates, all three expression levels of MIP-1 α , MCP-1 and TNF- α increased as the condensate concentration increased. The expression levels of other 17 cytokines in each experiment did not show significant changes. The results revealed that the condensate of 3R4F mainstream smoke is of certain immunotoxicity. When EL-4 cells and Ana-1 cells were exposed to the condensates, the cytokine expressions may indicate to certain immunotoxicities by dose-response relationships. Thus, the rapid detection and evaluation method based on Luminex liquichip to detect immunotoxicity induced by cigarette smoke is efficient and feasible.

10:20 AM WEDNESDAY

112. PLASMA PERFORATION OF TIPPING PAPER: SELECTED BENEFITS FOR CIGARETTE CONSUMPTION. Michael LINDNER¹ and Renata Raunic' Vadanjel²; ¹Tannpapier GmbH, Traun, Austria and ²TDR d.o.o., Rovinj, Croatia

Plasma Perforation represents an advanced technology for the generation of pre-perforated Tipping Paper. During the perforation process within an inert gas environment, a so-called

low-temperature plasma triggers local micro-evaporation events on the Tipping Paper surface provoking the formation of small perforation holes with a high hole density. The stabilities of hole parameters, air permeabilities and cigarette properties like the degree of filter ventilation and open draw resistance are significantly higher with Plasma Perforation than with the standard techniques of electrostatic and laser perforation. Cigarettes made with plasma perforated Tipping Paper are more efficient in ventilation rates and smoke yields reduction than conventionally perforated cigarettes due to a more homogeneous air flow through the ventilation zone and larger diffusive contributions to the dilution process. The first target of the present study is to demonstrate the capability of Plasma Perforation to optimize significantly the carbon monoxide / tar and nicotine / tar ratios of specifically designed cigarette samples. In this context, quantitatively determined diffusion capacities of the respective Tipping Paper qualities are related to smoke analysis results for scientific confirmation. The second part of this contribution reveals the effect of plasma perforated Tipping Paper on the sensory properties of cigarette smoke. This is realized by carrying out a survey with a professional smoker panel. The findings confirm that Plasma Perforation is a smart way to improve the compliance with regulatory targets requested by the tobacco industry and to enhance the physiological perception of the natural taste of cigarettes.

10:40 AM WEDNESDAY

113. ESTIMATION OF HEAT GENERATION IN SMOLDERING PACKED BED OF TOBACCO. Yasunobu INOUE¹ and Masataro Suzuki²; ¹Japan Tobacco Inc., Yokohama, Japan and ²Nagaoka University of Technology, Nagaoka, Japan

There are few reports on estimation of heat generation from tobacco during smoldering. This study focuses on the heat generation, using a smoldering packed bed of tobacco cut. The smoldering is assumed to consist of three reactions: the pyrolysis of unburned tobacco (generating “char” and “pyrolysis-gas”), the surface combustion of the char (generating “exhaust-gas” and “residue”), and the gas phase combustion of the pyrolysis-gas (also generating “exhaust-gas”). The amount of heat generated from each of these reactions is evaluated from balance of enthalpies between reactants and generated products. The total heat release rate is calculated by oxygen consumption in the smoldering.

The bed is a pile of tobacco cut in a thermally-insulated vertical tube. Air is supplied from the bottom of the tube against the smoldering direction, which is vertically downward to prevent the exhaust gas from condensing, at a flow rate of 30 mm/s. Solid materials, unburned tobacco, char, and residue, are collected from the corresponding regions in the tube. The pyrolysis-gas is defined as a gas that is generated from unburned leaves heated to 400 °C in air. The concentrations of oxygen, carbon monoxide and methane of the exhaust- and pyrolysis- gas after removing particulates are measured by gas chromatography. Enthalpies of the solid and particulates are measured by bomb calorimeter. The enthalpy of pyrolysis-gas is assumed to be the summation of enthalpies of those gas components and particulates. The heats release rate from the pyrolysis, the gas phase and the surface combustion are estimated at 1.7, 6.6 and 14.0 J/s, respectively. The total heat release rate from the smoldering is 22.3 J/s.

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