PROGRAM BOOKLET AND ABSTRACTS

Volume 64

64th Tobacco Science Research Conference

October 3-6, 2010
Hilton Head Island, South Carolina USA

Hosts:
Cerulean
&
Global Laboratory Services

TOWN OF HILTON HEAD ISLAND

One Town Center Court, Hilton Head Island, S.C. 29928
(843) 341-4600 Fax (843) 842-7728
www.hiltonheadislandsc.gov

October 3 – 6, 2010

64th TSRC: "Tobacco Research in the Era of Biotechnology and Genomics"

Dear TSRC Delegates:

Hilton Head Island is honored to have been chosen as the location for your Conference. We welcome the approximately 300 scientists from over a dozen countries who will be in attendance representing all disciplines of the tobacco industry: academia, government agencies and health organizations.

Hilton Head Island is widely known as a major convention area, and a world renowned destination location. You will find fine dining, entertainment, golf, tennis, and of course many miles of beautiful beaches to provide you with a delightful visit. The Island is also a unique and diverse community because of its rich, historical heritage and the contributions of the Gullah Culture. The preservation of this culture remains a priority for all of us. For more information about our wonderful Island's history, I recommend a stop at the Coastal Discovery Museum.

We are sure that during your stay you will fall in love with our beautiful seascapes and wonderful weather. We know that all this and more, coupled with a rich experience at your meeting, will bring you back to the island many times.

Again, welcome and best wishes for the success of your conference.

Sincerely,

TOWN OF HILTON HEAD ISLAND

Thomas D. Peeples

Thomas D. Peeples
Mayor

GENERAL PROGRAM

Sunday, October 3, 2010

2:00 pm – 6:00 pm	Registration	Ballroom Lobby
2:00 pm – 6:00 pm	Speaker Ready Room	East Hall A
6:30 pm – 9:30 pm	Welcome Reception	Brasshead Deck - Lower Level
	Hosted by – Cerulean & Global Laboratory Services	

Monday, October 4, 2010

7:30 am – 8:45 am	Session Chairs Breakfast	Carolina Room
7:30 am – 8:45 am	ISO TAG Breakfast	Palmetto Room
7:30 am – 5:00 pm	Registration	Ballroom Lobby
7:30 am – 5:00 pm	Speaker Ready Room	East Hall A
8:30 am – 9:00 am	Morning Coffee	Ballroom Lobby
9:00 am – 11:45 am	Symposium	West Hall
	"Tobacco Research in the Era of Biotechnology and Genomics"	
	Chairman, Balazs Siminszky, Philip Morris International	
10:15 am – 10:45 am	Coffee Break	Ballroom Lobby
11:45 am – 1:00 pm	Lunch	East Hall
1:00 pm – 2:20 pm	Poster Session	East Hall D
2:20 pm – 5:30 pm	Session A	West Hall J
	Session B	West Hall G
3:20pm – 3:50 pm	Coffee Break	Ballroom Lobby
4:45 pm – 6:00 pm	TSRC Analytical Methods Meeting	Sabal Palm Room

Tuesday, October 5, 2010

7:00 am – 8:15 am	Policy Committee Breakfast	Carolina Room
7:30 am – 5:00 pm	Registration	Ballroom Lobby
7:30 am – 5:00 pm	Speaker Ready Room	East Hall A
8:00 am – 8:30 am	Morning Coffee	Ballroom Lobby
8:30 am – 11:40 am	Session A	West Hall J
	Session B	West Hall G
9:50 am – 10:20 am	Coffee Break	Ballroom Lobby
11:40 am – 1:30 pm	Lunch	East Hall
12:00 pm – 1:30 pm	Tobacco Science Council Meeting	Carolina Room
1:30 pm – 4:20 pm	Session A	West Hall J
	Session B	West Hall G
2:50 pm – 3:20 pm	Coffee Break	Ballroom Lobby
4:30 pm – 5:30 pm	TSRC Business Meeting	Sabal Palm Room
6:30 pm – 7:15 pm	Social Hour	Ballroom Lobby
7:30 pm – 9:30 pm	Award Banquet	Ballroom

Wednesday, October 6, 2010

8:00 am – 8:30 am	Morning Coffee	Ballroom Lobby
8:00 am – 11:40 am	Speaker Ready Room	East Hall A
8:30 am – 11:40 am	Combined Session	West Hall J
9:50 am – 10:20 am	Coffee Break	Ballroom Lobby
11:40 am	Adjourn	

LIFETIME ACHIEVEMENT AWARD

William Kerr Collins, Sr.

Collins was born on a flue-cured tobacco farm near Henderson, NC and graduated in a post-high school program from Randolph Macon Academy, Front Royal, Virginia. He received B.S. and M.S. degrees from North Carolina State University (NCSU) and worked four years in the Tobacco Variety Evaluation Program after serving two years as an officer in the U. S. Army. His M. S. thesis formed the basis for the Minimum Standards Variety Release Program which has been in place since 1963. He received a Ph.D. in Crop Breeding from Iowa State University in the Corn Breeding Program. He worked three years as an agronomist for R. J. Reynolds Tobacco Company and became a faculty member at NCSU in 1966 for the remainder of his professional career with current employment. He was a Tobacco Extension Specialist for 20 years and taught a tobacco production course to more than 1000 students during this period.

He was a leader in training MS and PhD students to be professionals to work as extension agents, University professionals, and in tobacco-related agribusiness. He played a significant role in involving public agency representatives to work with leaf buyers and companies developing tobacco sucker control agents. Collins was an advocate for growing flue-cured tobacco for the mechanization era when the labor required for producing one acre of tobacco in 1965 was 650 hours to the current 50 hours. During this period yields increased from about 1500 pounds per acre to 3000 pounds per acre. Collins was a pioneer in the use of TV in educational programs supplemented by radio and about 500 popular press articles.

Collins was a pioneer in the use of replicated on-farm tests to develop and bring about the adoption of tobacco sucker control chemicals (particularly alcohols), herbicides, fertilizers, and management practices as published in Tobacco Science. He was an expert witness in about 15 liability court cases involving tobacco pesticides and fertilizers.

He was associate head and/or Acting Head of the Crop Science Department in the College of Agriculture and Life Sciences for 8 years. In 1994 he became the Coordinator of Tobacco Programs in the NC Agricultural Research Service for 13 years when he regularly attended TSRC meetings and was a consistent supporter of the Tobacco Science publication and Tobacco Literature Service at NCSU.

He was the director or co-director of eight agricultural two-year leadership development programs, consisting of 50 days of training that helped develop many leaders in the tobacco industry, particularly the agricultural sector. He had a strong interest in young farmers and in 1978 helped establish a 4-day Short Course for Young Tobacco Farmers at NC State University with about 1200 attendees during the last 30 years.

He visited the tobacco production areas of 44 countries and understands the implications of international agricultural competition. In 1997 he became general manager of Turner Family Tobacco Farms near Warrenton, NC.

Collins is the co-author of a book, Principles of Flue-Cured Tobacco Production, published in five languages.

Membership in Professional Societies – NC Soil Science Society, President, 1970; Pesticide Association of NC; American Society of Agronomy and Crop Science Society of America; Plant Food Association of NC; and Crop Protection Association of NC.

Membership in Honorary Academic Societies at NC State University – Alpha Zeta as sophomore; President, Blue Key and Senior Class, Golden Chain; Sigma Xi, Gamma Sigma Delta (President, 1979); President, NC Alpha Zeta Alumni Association, 1977 and 1999.

Honors and Awards Received – Most Outstanding Senior Award in Agronomy, NCSU, 1954; Gamma Sigma Delta Recognition for Meritorious Service Award, 1975; Philip Morris Professor (one of three named Extension Specialists on campus), 1978; Outstanding Extension Service Award, NCSU, 1978-79); Superior Leadership Award, NC Agricultural Extension Service, 1979; Agronomy Club Outstanding Instructor Award in 1978; Agronomic Extension Award from the American Society of Agronomy, 1981.

He is married to the former Ann Kittrell Turner and has three children: Julie, Lori, and Kerr; and has three grandchildren: Alexa, Will and Robert.

64th TOBACCO SCIENCE RESEARCH CONFERENCE

Monday Morning, October 4, 2010
Combined Session

9:00 **WELCOME:** Linda Crumpler, Cerulean &
Amy Walker, Global Laboratory Services, 64th TSRC Chairs

9:10 **SYMPOSIUM: "Tobacco Research in the Era of Biotechnology and Genomics"** Chair: Balazs Siminszky

9:15 **1. GENETICS-BASED MODIFICATION OF TOBACCO CHEMISTRY IN A REGULATORY ENVIRONMENT.** Ramsey S. LEWIS and Ralph E. Dewey; Crop Science Department, N.C. State University, Raleigh, NC

9:45 **2. FUNCTIONAL GENOMICS APPROACHES TO HARM REDUCTION IN TOBACCO PRODUCTS.** Michael P. TIMKO[1], Paul J. Rushton[2], Marta T. Bokowiec[1] and Hongbo Zhang[1]; [1]Department of Biology, University of Virginia, Charlottesville, VA and [2]Department of Biology and Microbiology, South Dakota State University, Brookings, SD

10:15 **Break**

10:45 **3. ENGINEERING HIGH VALUE OIL PRODUCTION IN TOBACCO.** Joe CHAPPELL, Shuiqin Wu, Satrio Husodo and Robert Williams; Dept. of Plant & Soil Sciences, University of Kentucky, Lexington, KY

11:15 **4. TOXICOGENOMICS AND EMERGING TECHNOLOGIES IN TOXICOLOGICAL RESEARCH.** Wanda R. FIELDS; R. J. Reynolds Tobacco, Co., Research & Development, Winston-Salem, NC

11:45 **Lunch**

1:00 **Posters**

5. EFFECTS OF PAPER PROPERTIES AND TOBACCO BLEND COMPOSITION ON CIGARETTE SELF-EXTINCTION. Alan NORMAN, Charlotte Smith and Curtis Doe; R. J. Reynolds Tobacco Company, Winston-Salem, NC

6. QUANTITATIVE DETERMINATION OF AMADORI COMPOUNDS IN TOBACCO USING ION EXCHANGE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS). Anthony GERARDI, Crystal H. Byrd and Serban C. Moldoveanu; R. J. Reynolds Tobacco Company, Winston-Salem, NC

7. GENE EXPRESSION ANALYSES OF THE LIVER FROM APOE-/- MICE EXPOSED TO MAINSTREAM CIGARETTE SMOKE. Brian K. NORDSKOG, Geoffrey M. Curtin, Joya E. Brown and Betsy R. Bombick; R. J. Reynolds Tobacco Co., Bowman Gray Technical Center, Winston-Salem, NC

8. CLINICAL METHODOLOGY AND RESULTS FOR PHYSIOLOGICAL ASSESSMENTS INCLUDING FLOW-MEDIATED AND EXPIRED CARBON MONOXIDE IN EXCLUSIVE CIGARETTE SMOKERS, EXCLUSIVE MOIST SNUFF CONSUMERS, AND NON-CONSUMERS OF TOBACCO. Buddy G. BROWN[1], Bobbette A. Jones[1], Brian K. Nordskog[1], David L. Heavner[2], Thomas J. Steichen[1] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, [2]Pinnacle, NC

9. AN IMPROVED HPLC METHOD TO DETERMINE PHENOLICS IN CELLULOSE ACETATE FILTERS. Denise FISHER JONES, Steven A. Wilson and Jeremy K. Steach; Eastman Chemical Company, Kingsport, TN

10. STANDARDIZATION OF THE PREPARATION OF SMOKELESS TOBACCO EXTRACTS FOR ASSESSMENT OF BIOLOGICAL EFFECTS. Gaddamanugu L. PRASAD, Kathy W. Fowler, Betsy R. Bombick and Jo Ann Hill; Research & Development, R. J. Reynolds Tobacco, Winston-Salem, NC

11. APPLICATION OF CARBON FIBER MICROELECTRODES FOR MEASUREMENT OF KINETIC CONSTANTS OF NITRIC OXIDE DECAY IN BLOOD. Jay L. ZWEIER, Xiaoping Liu, Gamal A. El-Sherbiny, Eric Collard, Xin Huang, Douglas Follmer, Mohamed A. El-Mahdy and Hassan Talukder; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

12. QUANTITATION OF PESTICIDES IN FLUE-CURED TOBACCO BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY (GCXGC-TOFMS). Joe BINKLEY and Scott Pugh; LECO Corporation Saint Joseph, MI

13. QUALITY DIFFERENCE AND ANALYSIS OF AGING TOBACCO LEAF BY THREE SECTIONS CUTTING. YAN Keliang, Wu Yi, Zeng Xiaoying and Wang Chao; Technology Center of Hongyun Honghe Tobacco (Group) Co. Ltd., Kunming PR China

14. QUANTITATIVE/COMPARATIVE MOUSE LYMPHOMA ASSAY (MLA) FOR THE TESTING OF TOTAL PARTICULATE MATTER (TPM). Mark BALLANTYNE[1], Vicky Stone[1], Ian Crooks[2], Ken Scott[2], Clive Meredith[2], Debbie Dillon[2], Mari Johnson[1] and Jim Saul[1]; [1]Covance Laboratories Ltd, Harrogate, North Yorkshire, UK, [2]British American Tobacco GR&D, Southampton, UK

15. QUANTITATIVE DETERMINATION OF VOLATILE NITROSAMINES (VNA) IN SMOKELESS TOBACCO PRODUCTS. Mary DENNIS, Tianrong Cheng and Darren Steelman; Lancaster Labs, Lancaster, PA

16. QUANTIFICATION OF CITRATE IN CIGARETTE PAPER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. N. PALANI, T.K. Dinesh, S. Suman, P.C.Ajith Kumar and S.V. Dhalewadikar; ITC R&D Centre, Peenya, Bangalore, India

17. COMPARISON OF THE BACTERIAL MUTAGENICITY OF WHOLE-SMOKE, GAS-VAPOR PHASE AND SMOKE CONDENSATES FROM MENTHOLATED AND NON-MENTHOLATED CIGARETTES. Robert LEVERETTE; Lorillard Tobacco Company, A.W. Spears Research Center, Greensboro, NC

18. RESIDUAL ANALYSIS OF CARBONYLS IN TOBACCO AND TOBACCO PRODUCTS BY HPLC. Sharad K MEHTA, S.V. Dhalewadikar and B.J. Rajesh; ITC R&D Center, ITC Ltd Phase 1, Peenya Industrial Area, Bangalore, India

19. OPTIMIZATION OF LEUKOCYTE ISOLATION FOR CLINICAL STUDIES. Subhashini ARIMILLI[1], Brad E. Damratoski[1], Peter Chen[2], Bobbette A. Jones[2], W. Edward Swords[1] and G. L. Prasad[2]; [1]Department of Microbiology & Immunology, Wake Forest University School of Medicine, Winston-Salem, NC, [2]R&D Department, R.J. Reynolds Tobacco Company, Winston-Salem, NC

20. COMPARISON OF REGULAR SMOKERS OF MENTHOL AND NON-MENTHOL CIGARETTES ON SMOKING PROFILE AND EXPOSURE. Valerie TROUDE, Bénédicte Varignon and Xavier Cahours; SEITA Imperial Tobacco Group, Fleury-les-Aubrais, France

21. COMPARISON OF SFA, IC-PAD, AND LC-MS/MS FOR QUANTITATIVE CHARACTERIZATION OF CARBOHYDRATES IN TOBACCO PRODUCTS. John SHIFFLETT and Dawit Z. Bezabeh; Alcohol and Tobacco Tax and Trade Bureau, Scientific Services Division, Beltsville, MD

22. EVALUATION OF CYTOTOXICITY OF DIFFERENT TOBACCO PREPARATIONS. Subhashini ARIMILLI[1], Brad E. Damratoski[1], Betsy Bombick[2], W. Edward Swords[1], Mike Borgerding[2] and G. L. Prasad[2]; [1]Department of Microbiology & Immunology, Wake Forest University School of Medicine, Winston-Salem, NC, [2]R&D Department, R.J. Reynolds Tobacco Company, Winston-Salem, NC

Monday Afternoon, October 4, 2010

Session A
Session Chair: Joseph Wanna

2:20 PM

23. THE EFFECT OF POSITION OF CARBON GRANULES WITHIN A CIGARETTE FILTER ON THE RELEASE OF PARTICLES OR FIBRES FROM THE FILTER. Tony McCORMACK and Mike Taylor; Filtrona Technology Centre, Jarrow, Tyne & Wear, UK

2:40 PM

24. CORRELATION BETWEEN MANUAL AND SEMI AUTOMATIC MEASUREMENTS OF IGNITION PROPENSITY TO ASTME2187-04. Tim MASON and Ian Tindall; Cerulean, Linford Wood East, Milton Keynes, UK

3:00 PM

25. QUANTIFICATION METHOD FOR NNAL, ISO-NNAL AND NNA IN TOBACCO BY LC-MS/MS. Jasper D. H. VAN HEEMST; British American Tobacco, GR&D Centre, Southampton, UK

Session B
Session Chair: Brian Nordskog

2:20 PM

28. CARBON-CENTERED FREE RADICALS AS INTERMEDIATES TO MAINSTREAM SMOKE CARBONYLS. Anthony GERARDI and William Coleman, III; R. J. Reynolds Tobacco Company, Winston-Salem, NC

2:40 PM

29. USE OF TWO GC-MS SCAN TECHNIQUES FOR THE CHARACTERIZATION OF WRAPPERS AND BINDERS TAKEN FROM CIGAR PRODUCTS. John H. LAUTERBACH[1] and Deborah A. Grimm[2]; [1]Lauterbach & Associates, LLC, Macon, GA, [2]Coordinated Instrumentation Facility, Tulane University, New Orleans, LA

3:00 PM

30. ANALYSIS OF SELECTED CARBONYLS IN MAINSTREAM CIGARETTE SMOKE BY UPLC-UV. Sofia A. ESSÉN, Laura Ashmore and Thomas Brice; British American Tobacco, Group R&D, Southampton, UK

3:20 PM BREAK

Monday Afternoon, October 4, 2010

3:50 PM

26. IMPROVEMENT OF ANALYTICAL METHOD FOR QUANTIFICATION OF TSNA IN TOBACCO USING UHPLC-MS/MS. Hiroyuki YOSHIDA, Motomi Yajima and Toshiro Teraoka; Japan Tobacco Inc., Leaf Tobacco Research Center, Oyama, Tochigi, Japan

4:10 PM

27. DETERMINATION OF MALEIC HYDRAZIDE RESIDUE IN TOBACCO AND TOBACCO PRODUCTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. Hongfei ZHANG, Zhaoyang Bian, Zhonghao Li, Fengpeng Zhu, Gangling Tang and Qingyuan Hu; Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China

3:50 PM

31. FACTORS AFFECTING THE DESIGN OF PAPER DIFFUSIVITY MEASUREMENT APPARATUS WITH PARTICULAR REFERENCE TO THE DESIGN OF TRANSFER STANDARDS. James VINCENT and Ian Tindall; Cerulean, Linford Wood East, Milton Keynes, UK

4:10 PM

32. ADSORPTION CHARACTERISTICS OF SILANOL GROUP FOR VOLATILE CONSTITUENTS IN CIGARETTE SMOKE. Noritoshi FUJITA; Japan Tobacco Inc., Yokohama, Kanagawa, Japan

4:30 PM

33. STUDIES ON TRANSITION METALS MODIFIED POROUS MATERIALS FOR REDUCING HYDROCYANIC ACID IN CIGARETTE SMOKE. Le ZHAO[1], Bin Peng[1], Xuehui Sun[1], Jing Zhu[1], Cong Nie[1] and Xuewu Yan[2]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan Province, P. R. China, [2]Nanjing University of Science and Technology, Nanjing, Jiangsu Province, P. R. China

ADJOURN

Tuesday Morning, October 5, 2010

Session A
Session Chair: Michael Dube

8:30 AM

34. A CLINICAL TRIAL OF CARDIOVASCULAR DISEASE BIOMARKERS IN ADULT SMOKERS AND MOIST SNUFF CONSUMERS, PART I: STUDY DESIGN, SUBJECT SELECTION AND COHORT CHARACTERIZATION. Bobbette A. JONES[1], Buddy G. Brown[1], Leanne C. Lee[1], David Heavner[2], Thomas J. Steichen[3] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, NC, [2]Pinnacle, NC, [3]Winston-Salem, NC

8:50 AM

35. CARDIOVASCULAR DISEASE BIOMARKERS STUDY, PART II: TOBACCO-RELATED BIOMARKERS OF EXPOSURE IN EXCLUSIVE CIGARETTE SMOKERS, EXCLUSIVE MOIST SNUFF CONSUMERS, AND NON-CONSUMERS OF TOBACCO. Buddy G. BROWN[1], Leanne C. Lee[1], David Heavner[2], Thomas J. Steichen[3] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, NC, [2]Pinnacle, NC, [3]Winston-Salem, NC

Session B
Session Chair: Lowell Bush

8:30 AM

42. DEVELOPMENT OF TOBACCO LINES WITH ULTRA-LOW LEVELS OF NORNICOTINE. Ralph E. DEWEY[1], Ramsey S. Lewis[1], Steven W. Bowen[1] and Lowell P. Bush[2]; [1]Department of Crop Science, North Carolina State University, Raleigh, NC, [2]University of Kentucky, Lexington, KY

8:50 AM

43. POTENTIAL APPLICATIONS OF 'GM' TECHNOLOGY IN THE PRODUCTION OF TOBACCO FOR ITS TRADITIONAL USES. Orlando CHAMBERS[1], Patrick Thomas[1], H. Maelor Davies[1], Bruce Fortnum[2] and Paul Peterson[2]; [1]Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY, [2]Clemson University, Florence, SC

Tuesday Morning, October 5, 2010

9:10 AM

36. CARDIOVASCULAR DISEASE BIOMARKERS STUDY, PART III: TOBACCO-RELATED BIOMARKERS OF EFFECT IN EXCLUSIVE CIGARETTE SMOKERS, EXCLUSIVE MOIST SNUFF CONSUMERS, AND NON-CONSUMERS OF TOBACCO. Brian K. NORDSKOG[1], Buddy G. Brown[1], Bobbette A. Jones[1], Leanne C. Lee[1], David Heavner[2], Thomas J. Steichen[3] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, NC, [2]Pinnacle, NC, [3]Winston-Salem, NC

9:10 AM

44. THE EFFECT OF POPULATION DENSITY ON TSNA ACCUMULATION IN BURLEY TOBACCO. Anne JACK, Colin R. Fisher, Angela Schoergendorfer, Neil F. Fannin and Lowell P. Bush; University of Kentucky, Plant & Soil Sciences Department, Lexington, KY

9:30 AM

37. A REVIEW OF THE LITERATURE ON THE DEGREE OF COMPENSATORY" SMOKING OF LOW-DELIVERY CIGARETTES. Chris COGGINS[1], Ruth Dempsey[2] and Ewald Roemer[2]; [1]Carson Watts Consulting, King, NC, [2]Philip Morris International Management SA, Neuchâtel, Switzerland

9:30 AM

45. (+)-2'-R-NICOTINE IS ENANTIOSELECTIVELY DEMETHYLATED BY ENZYME CYP82E4 IN *N. TABACUM L.* Bin CAI, F. Fannin, A. Jack and L.P. Bush; Dept of Plant and Soil Sciences, University of Kentucky, Lexington, KY

9:50 AM BREAK

10:20 AM

38. INFLUENCE OF MENTHOL ON THE PERMEABILITY OF ARTIFICIAL CELL MEMBRANES TO ACROLEIN, BENZO(A)PYRENE (BAP), AND 4-(METHYLNITROSAMINO)- 1-(3-PYRIDYL)-1-BUTANONE (NNK). Edward A. ROBINSON and Florian R. Perini; Lorillard Tobacco Company, Greensboro, NC

10:20 AM

46. DESIGN CONSIDERATIONS FOR YIELD IN USE STUDIES. Paul R. NELSON; R.J. Reynolds Tobacco Co, Winston-Salem, NC

Tuesday Morning, October 5, 2010

10:40 AM

39. IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN BLOOD PLASMA OF CONTROL AND CIGARETTE SMOKE-EXPOSED MICE BY 2-D DIFFERENTIAL IN-GEL ELECTROPHORESIS/MASS SPECTROMETRY. Jay L. ZWEIER and Arun K. Tewari; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

10:40 AM

47. MENTHOL SMOKERS DO NOT ACHIEVE HIGHER CIGARETTE YIELDS: RESULTS FROM THREE YIELD IN USE STUDIES. Paul R. NELSON and Peter X. Chen; R.J. Reynolds Tobacco Co, Winston-Salem, NC

11:00 AM

40. A MODEL FOR THE RESPIRATORY RETENTION OF COMPOUNDS FOUND IN CIGARETTE SMOKE. F. Kelley ST. CHARLES; St. Charles Consultancy, Winston-Salem, NC

11:00 AM

48. DETERMINANTS OF SELF-REPORTED ENERGY EFFICIENCY IN WOOD-BASED CURING PRACTICES AMONG GROWERS IN BRAZIL, TANZANIA AND UGANDA. Helmut GEIST[1] and Samuel Mugisha[2]; [1]University of Aberdeen, Department of Geography & Environment, Aberdeen, United Kingdom/Scotland, [2]Makerere University, Department of Zoology, Kampala, Uganda

11:20 AM

41. URINARY TOBACCO SMOKE RELATED METABOLITES IN RELATION TO BRAND YIELD: A 200 SUBJECT CANADIAN STUDY. Mehran SHARIFI, William Rickert, Peter Joza and Wendy Wagstaff; Labstat International ULC,Kitchener, ON Canada

11:20 AM

49. THEORETICAL APPROACH FOR PREDICTING PLASTICIZED FIRMNESS OF CELLULOSE ACETATE FILTER RODS. Kevin NORFLEET; Celanese Acetate LLC, Narrows, VA

LUNCH

Tuesday Afternoon, October 5, 2010

Session A
Session Chair: Edward Robinson

1:30 PM

50. ESTIMATION OF THE RETENTION OF MENTHOL IN THE RESPIRATORY TRACT OF SMOKERS. Melissa HAGAN HUGHES, Kyle Lott and J. Daniel Heck; Lorillard Tobacco Company, A.W. Spears Research Center, Greensboro, NC

1:50 PM

51. CHRONIC CIGARETTE SMOKE EXPOSURE INDUCES VASCULAR DYSFUNCTION THROUGH UPREGULATION OF CYTOGLOBIN IN MOUSE AORTA. Mohamed A. EL-MAHDY, Gamal A. El-Sherbiny, Tamer M. Abdelghany and Jay L. Zweier; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

2:10 PM

52. CHRONIC CIGARETTE SMOKE EXPOSURE IMPAIRS VASCULAR ENDOTHELIAL FUNCTION THROUGH A TETRAHYDROBIOPTERIN-DEPENDENT MECHANISM. Tamer M. ABDELGHANY, Gamal A. El-Sherbiny, Mohamed A. El-Mahdy and Jay L. Zweier; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

Session B
Session Chair: Ray Robertson

1:30 PM

56. TOBACCO COMPOSITION I: A QUANTITATIVE CHEMICAL COMPOSITION OF TOBACCO IN 2008, FROM A NUTRITIONAL PERSPECTIVE. M. F. DUBE, W. M. Coleman, III, D. M. Lawson and W. T. Morgan; R.J. Reynolds Tobacco Company, Winston-Salem, NC

1:50 PM

57. TOBACCO COMPOSITION II: A QUANTITATIVE CHEMICAL COMPOSITION OF 2008 GREEN AND CURED BURLEY AND FLUE CURED TOBACCOS. D. M. LAWSON, M. F. Dube, W. M., Coleman, III and W. T. Morgan; R.J. Reynolds Tobacco Company, Winston-Salem, NC

2:10 PM

58. TOBACCO COMPOSITION III: A QUANTITATIVE CHEMICAL COMPOSITION OF TOBACCO 2008, IMPACT OF STALK POSITION AND GROWING REGIONS. W.M. COLEMAN, III, M. F. Dube, D.M. Lawson and W. T. Morgan; R.J. Reynolds Tobacco Company, Winston-Salem, NC

Tuesday Afternoon, October 5, 2010

2:30 PM

53. MOLECULAR AND CELLULAR RESPONSE OF ORAL CAVITY CELLS TO TOBACCO PREPARATIONS. Wolfgang ZACHARIAS[1], Hong Gao[1], Prasad and Gaddamanugu L[2]; [1]Dept. of Medicine, J.G. Brown Cancer Univ. of Louisville, Louisville KY, [2]RJ Reynolds Tobacco Co., R&D, Winston-Salem, NC

2:30 PM

59. PROFILING OF GLYCERIDES IN TOBACCO SEEDS. Serban MOLDOVEANU and Yiping Chang; R.J. Reynolds Tobacco Co, Winston-Salem, NC

2:50 PM BREAK

3:20 PM

54. BANDS PRINTED ON THE OUTSIDE OF CIGARETTES. Vladimir HAMPL, JR.; Schweitzer-Mauduit, International, Alpharetta, GA

3:40 PM

55. INFLUENCE OF HUMIDITY, NUMBER OF FILTER PAPERS, AND ORIENTATION OF THE FILTER PAPER ON ASTM TEST RESULTS. Joseph WANNA; Schweitzer Mauduit Int., Alpharetta, GA

3:20 PM

60. THE INFLUENCE OF CARBON ON SELECTIVE REMOVAL OF PHENOLIC COMPOUNDS. Jeremy K. STEACH and Denise Fisher-Jones; Eastman Chemical Company, Kingsport, TN

3:40 PM

61. FREE FATTY ACID COMPLEXATION WITH IRON IN CIGARETTE SMOKE. Florian R. PERINI and Edward A. Robinson; Lorillard Tobacco Company Greensboro, NC

4:00 PM

62. INVESTIGATIONS ON FACTORS INFLUENCING THE MOISTURE RETENTION PROPERTIES OF TOBACCO. Shitong ZENG, Jun Hu, Yang Liu, Chuanchuan Gao, Xinliang Bai and Mingyue Zhao; Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan, China

ADJOURN

Wednesday Morning, October 6, 2010
Combined Session

Session Chair: Florian Perini

8:30 AM **63. METABOLOMIC STUDIES WITH URINE OF SMOKERS AND NONSMOKERS.** Gerhard SCHERER, Gerhard Gilch and Wibke Peters; ABF Analytisch-Biologisches Forschungs-labor GmbH, Muenchen, Germany

8:50 AM **64. SENSITIVE METHOD FOR THE DETERMINATION OF ACRYLAMIDE IN TOBACCO FILLER AND ALTERNATIVE TOBACCO PRODUCTS BY UPLC-MS/MS.** Tianrong CHENG[1], Nancy Qian[1], Mary Dennis[1], Serban C. Moldoveanu[2] and Anthony R. Gerardi[2]; [1]Lancaster Laboratories Inc., Lancaster, PA, [2]R. J. Reynolds Tobacco Co., Winston-Salem, NC

9:10 AM **65. LIMITATIONS IN THE CARACTERISATION OF THE CIGARETTE BRANDS USING DIFFERENT MACHINE SMOKING REGIMES.** Valerie TROUDE[1], Stephen W. Purkis[2], Gerald Duputie[1], Christian Teissier[1] and Benedicte Varignon[1]; [1]SEITA Imperial Tobacco Group, Fleury-les-Aubrais France, [2]Imperial Tobacco Limited, Southville, Bristol UK

9:30 AM **66. A STUDY FOR THE IDENTIFICATION OF HYDROGEN PEROXIDE PRECURSORS BY FRACTIONATION OF THE AQUEOUS EXTRACT OF PARTICULATE-PHASE CIGARETTE SMOKE.** Yuichiro TAKANAMI; Japan Tobacco Inc, Yokohama, Kanagawa, Japan

9:50 **Break**

10:20 AM **67. DELIVERIES OF SMOKE CONSTITUENTS FROM CHARCOAL FILTER CIGARETTES WHEN SMOKED WITH VARYING INTENSITIES.** Peter JOZA, William Rickert and Wendy Wagstaff; Labstat International ULC, Kitchener, ON Canada

10:40 AM **68. GLYCOSIDES – FLAVOUR ENHANCERS IN SMOKE.** Namasivayam PALANI, S. Vinutha, T.K. Dinesh, Manoj Kumar Singh, Soumitra Mukherjee and S.V. Dhalewadikar; ITC R&D Centre, Peenya, Bangalore, India

11:00 AM **69. LEVEL IN TOBACCOS OF THREE NITROGENOUS COMPOUNDS (ADENOSINE, DEOXYFRUCTOSAZINE AND GLUCOSAMINE.** S. C. MOLDOVEANU, A. R. Gerardi and C. H. Byrd; R.J. Reynolds Tobacco Co., Winston-Salem, NC

11:20 AM **70. STUDY OF THE DETERMINATION OF LOSS OF TOBACCO FROM THE CIGARETTE ENDS USING VIBRATION.** Feng Qian[1], Wu Xiaosong[2], Liang Wei[1], ZHAO Jijun[1], Zhang Long[2], Li Zhi-Gang[2] and Liu Yong[2]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China and [2]Anhui Institute of Optics and Fine Mechanics of CAS, Hefei, China

Adjourn

64th Tobacco Science Research Conference

MONDAY MORNING, OCTOBER 4, 2010

Symposium

9:00 AM **WELCOME: Linda Crumpler, Cerulean & Amy Walker, Global Laboratory Services, 64th TSRC Chairs**

9:10 AM **SYMPOSIUM: "Tobacco Research in the Era of Biotechnology and Genomics" Chair: Balazs Siminszky**

9:15 AM **MONDAY**

1. GENETICS-BASED MODIFICATION OF TOBACCO CHEMISTRY IN A REGULATORY ENVIRONMENT. Ramsey S. LEWIS and Ralph E. Dewey; Crop Science Department, N.C. State University, Raleigh, NC

Tobacco products contain a number of constituents classified as carcinogens. Concentrations of some of these compounds will soon be regulated by various agencies around the world, including the FDA in the United States. Changes in nicotine levels might also become mandated. Research in the area of tobacco plant genetics will be a component of overall strategies to develop reduced risk products and comply with upcoming regulations. This presentation will focus on history and opportunities for the combined use of traditional plant breeding, genomics, and biotechnology to develop tobacco cultivars carrying traits of relevance to harm reduction. Our own research has focused on the use of genetics-based approaches to reduce levels of nornicotine, the precursor to the tobacco specific nitrosamine, N-nitrosonornicotine (NNN), in cured tobacco leaves. We have used both genetic engineering and mutation breeding to produce tobacco genotypes with dramatically reduced potential for accumulation of this potent carcinogen in cured leaves. In a separate area of research, multiple laboratories have greatly increased knowledge of genes involved in alkaloid biosynthesis within the last fifteen years. This has led to increased opportunities for increasing or decreasing alkaloid levels through conventional or molecular-based approaches. Other historical and current efforts in the area of genetics-based manipulation of harmful tobacco constituents will also be discussed.

9:45 AM **MONDAY**

2. FUNCTIONAL GENOMICS APPROACHES TO HARM REDUCTION IN TOBACCO PRODUCTS. Michael P. TIMKO[1], Paul J. Rushton[2], Marta T. Bokowiec[1] and Hongbo Zhang[1]; [1]Department of Biology, University of Virginia, Charlottesville, VA and [2]Department of Biology and Microbiology, South Dakota State University, Brookings, SD

There is overwhelming evidence that the use of tobacco products plays a major role in the pathogenesis of lung and oral cancers, cardiovascular disease, COPD, and a variety of other human diseases. In the absence of complete elimination of their use, a viable alternative is the development of tobacco products that deliver reduced levels of harmful constituents to the human body. Successful strategies aimed at harm reduction through the manipulation

of tobacco leaf chemistry and composition require a detailed understanding of the cellular processes that control plant development, and the biosynthesis and accumulation of cell components and secondary metabolites that contribute to the formation of known harm components. Our studies aim to define the signaling components and transcription factors (TFs) controlling the formation of nicotine, the most prevalent alkaloid found in cultivated tobacco (*Nicotiana tobaccum L.*), nornicotine, and various minor alkaloids (e.g., anatabine, anabasine, anatalline) that contribute to the production of tobacco specific nitrosamines (TSNAs) during curing and fermentation. Using genomic and transcriptomic sequence data, bioinformatics, and transgenic technologies (ectopic overexpression / RNAi knockdowns) in cultured cells and whole plants we are unraveling the transcriptional circuitry that controls alkaloid formation in response to various developmental, phytohormonal, and environmental cues. We have identified several classes of TFs that directly control expression of genes encoding key enzymes in nicotine synthesis in the response to jasmonates (JAs) and shown that directed manipulation of these TFs and their target genes can substantially alter alkaloid synthesis and accumulation. Using oligonucleotide arrays we have carried out extensive global gene expression analysis to identify novel cellular targets controlling alkaloid biosynthesis and derivation and other characteristics of leaf chemistry and composition important in harm reduction. Our progress towards harm reduced products will be discussed.

10:15 AM ***Break***

10:45 AM **MONDAY**

3. ENGINEERING HIGH VALUE OIL PRODUCTION IN TOBACCO. Joe CHAPPELL, Shuiqin Wu, Satrio Husodo and Robert Williams; Dept. of Plant & Soil Sciences, University of Kentucky, Lexington, KY

Assuming biofuels generated via the fermentation of sugars derived from cellulosic and non-cellulosic constituents of biofuels crops will provide a substantial contribution to our future energy needs, augmenting and amending the productivity of these biofuel crops is now a major research thrust worldwide. One way of enhancing these biofuels crops will be to engineer them for value-added components such as oils that can be used for efficient fuel production and the manufacturing of other high-value products currently derived from petroleum oils. Towards this end, we have developed an engineering strategy for optimized production of long, branched-chain hydrocarbon biosynthesis in plants. Branched chain hydrocarbons, like methylated triterpenes, are readily cracked into paraffins and naphthenes that can either be distilled to combustible fuels (gasoline, JP-8 and diesel), or can be used directly for the synthesis of plastics, nylons, paints and other oil-derived products manufactured by diverse chemical industries. Our working hypothesis has been that success in generating high level production platforms for triterpene oils in plants can be accomplished by targeting this metabolism to the chloroplast compartment of cells, thus eliminating the regulatory mechanisms that normally operate to control this metabolism occurring in the cytoplasm, and providing a means for the direct channeling of photosynthetically fixed CO2 to the biosynthesis of novel, value-added products. Preliminary experiments suggests that engineering this metabolism into chloroplasts of photosynthetically active mesophyll cells can result in plants yielding up to 0.1% of their dry weight as triterpenes. In comparison, plants having this metabolism engineered into

secretory trichomes can yield up to 1% of their dry weight as triterpenes. These results have important ramifications for our understanding of basic metabolism in plants, as well as the development of novel chemical production platforms in plants.

11:15 AM MONDAY

4. TOXICOGENOMICS AND EMERGING TECHNOLOGIES IN TOXICOLOGICAL RESEARCH. Wanda R. FIELDS; R. J. Reynolds Tobacco, Co., Research & Development, Winston-Salem, NC

Research efforts over the last several decades have significantly impacted and accelerated the study of biology. In particular, the early study of macromolecules spawned a revolution in the field of molecular biology. Molecular biology in simple terms is the study of life at the molecular level. Fundamental to molecular biology is the understanding that genetic material is maintained in the form of DNA and can be transcribed to RNA and finally translated into protein, providing a flow of biological information from the genetic code to cellular function. Toxicological testing and biomarker discovery as well as drug development and therapy have been influenced by advances in molecular biological techniques such as "–omics" technologies. These technologies utilize a broad range of molecular tools to support the study of various macromolecules or subcellular components in response to exposures and treatments. The leading -omic disciplines are genomics (DNA), transcriptomics (RNA), proteomics (protein) and metabolomics (metabolites).

Toxicogenomics combines genomics and bioinformatics to characterize and identify mechanisms of toxicity induced by various exposures. Within this scientific discipline, genomics, transcriptomics, proteomics and metabolomics may be applied to assess the toxicological responses of chemicals using *in vitro* and *in vivo* models. Data from such studies can be analyzed by tools that combine biology, computer software and statistics to generate biological information that support efforts in predictive toxicology, mechanistic toxicology, biomarker discovery, risk assessment, identification of mechanisms of disease and drug development.

This presentation will provide a summary of toxicogenomics in toxicology and tobacco research. Standard testing measures, challenges and current advances will be discussed.

11:45 PM ***Lunch***

1:00 PM POSTERS

5. EFFECTS OF PAPER PROPERTIES AND TOBACCO BLEND COMPOSITION ON CIGARETTE SELF-EXTINCTION. Alan NORMAN, Charlotte Smith and Curtis Doe, R. J. Reynolds Tobacco Company, Winston-Salem, NC

A thermal imaging method was used to measure power radiated from the coal during free smolder within, and outside the banded areas of cigarettes made with print-banded papers. Base papers made with a range of CaCO3 (6 gsm to 10 gsm) filler, wood pulp fiber (17.5 gsm to 22.5 gsm), and mixed sodium/potassium citrate (0.65% to 0.95%) contents were printed with sodium alginate bands of two diffusion capacity levels (0.07 cm/s to

0.12 cm/s). Test cigarettes were made with the printed papers using two tobacco blends differing in lamina cut width, and content of expanded and reconstituted tobacco. Radiant coal power outside the banded paper area varied with the blend composition, base paper filler content, and base paper citrate content. Within-band coal power output was lower than that outside the banded area, and it varied with the band diffusion capacity, blend composition, and base paper composition. Cigarette self-extinction rates (SE) measured with the ASTM method were strongly and inversely correlated with the within-band coal power regardless of the blend or paper composition. These observations suggest that SE is governed by the within-band power output. Because the within-band power output is influenced by the band diffusion capacity, blend and base paper composition, all of these features must be considered when developing specifications for fire standards compliant product designs.

6. QUANTITATIVE DETERMINATION OF AMADORI COMPOUNDS IN TOBACCO USING ION EXCHANGE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS). Anthony GERARDI, Crystal H. Byrd and Serban C. Moldoveanu; R. J. Reynolds Tobacco Company, Winston-Salem, NC

Amadori compounds are formed during non-enzymatic browning of reducing sugars and amino acids and are important in the aroma of agricultural and food products. These compounds form naturally in tobacco and are of importance to the flavor profile of smoke. The analysis developed herein demonstrates the quantitation of 13 different Amadori compounds, including those derived from glucose and rhamnose. An LC-MS/MS procedure allowed for simple determination of the Amadori compounds using SRM transitions corresponding to the parent mass of the Amadori compounds transitioning to the largest single fragment, usually a loss of water or $[M+H]^+$ to $[M-H2O+H]^+$. The separation of compounds was performed on a IonPac CS-17 cation exchange column (250 x 2 mm, Dionex) and an IonPac CG-17 guard column (50 x 2 mm, Dionex) using a gradient of 0.1% aqueous formic acid and 0.1% formic acid in methanol/water (80:20). The analytical separation was applied to a series of standards at known concentration (ranges of ~100 ng/mL-2000ng/mL) and to various tobacco samples. Two flue-cured tobacco samples, one with added standard and one without, were studied to determine matrix effects. The method proved suitable for the determination of Amadori compounds in tobacco.

7. GENE EXPRESSION ANALYSES OF THE LIVER FROM APOE-/-MICE EXPOSED TO MAINSTREAM CIGARETTE SMOKE. Brian K. NORDSKOG, Geoffrey M. Curtin, Joya E. Brown and Betsy R. Bombick; R. J. Reynolds Tobacco Co., Bowman Gray Technical Center, Winston-Salem, NC

The goal of this study was to identify key molecular alterations induced by mainstream smoke exposure and diet in a genetically susceptible mouse model of atherosclerosis. ApoE-deficient female mice were exposed nose-only to mainstream cigarette smoke for 18 weeks (3h/day, 5day/week) at concentrations of 0, 0.16, 0.32 and 0.48 mg WTPM/L +/- high-fat diet. Total RNA from the liver was isolated and prepared for hybridization onto Affymetrix 430 mouse 2.0 arrays. Using a Benjamini and Hochberg False Discovery Rate for multiple testing correction ($p<0.05$) and a 2-fold threshold, ANOVA analysis identified 1233 differentially expressed genes compared to control samples. Genes having the greatest change from basal expression included: SQLE, GPNMB, IDI1, CYP51, LEPR, SC4MOL,

SULT1E1, FAM19A2, CYP2C55, and FDPS. The top gene ontologies included lipid and immune/inflammatory processes. Gene expression in the liver from mice fed a high-fat diet was significantly modulated compared to chow-fed controls. Livers from mice exposed to cigarette smoke and consuming the high-fat diet had the highest number of differentially expressed genes compared to controls, whereas the chow-fed mice exposed to smoke had the fewest. In summary, the combination of diet and mainstream cigarette smoke exposure had the biggest effect on molecular alterations in the livers of ApoE-/- mice.

8. CLINICAL METHODOLOGY AND RESULTS FOR PHYSIOLOGICAL ASSESSMENTS INCLUDING FLOW-MEDIATED AND EXPIRED CARBON MONOXIDE IN EXCLUSIVE CIGARETTE SMOKERS, EXCLUSIVE MOIST SNUFF CONSUMERS, AND NON-CONSUMERS OF TOBACCO. Buddy G. BROWN[1], Bobbette A. Jones[1], Brian K. Nordskog[1], David L. Heavner[2], Thomas J. Steichen[1] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, [2]Pinnacle, NC

Several physiological assessments have been reported in the literature as measures for detecting, predicting and monitoring CVD. These non-invasive techniques were investigated in three exclusive all male cohorts (cigarette smokers, moist snuff consumers, and non-consumers of tobacco) to evaluate potential differences in CVD status. Flow-mediated dilation (FMD), carotid intima-media thickness (CIMT), and ankle brachial index (ASI) were selected to assess CVD endpoints. Secondarily, spirometry and expired carbon monoxide (ECO) were measured to assess lung function. The three cohorts were age-stratified into four groups: 26-31; 32-37; 38-43; and 44-49. FMD and ASI were measured on Days 1 and 2; the change between days was calculated. CIMT was measured on Day 2 only. For CIMT, a significant age group main effect was observed, demonstrating a tendency toward higher CIMT with age. For FMD, no significant age or cohort main effects were observed at any time-point. For ASI, the only significant difference was observed on Day 1 between smokers and non-consumers of tobacco, with smokers having the lower ASI mean value. Day 2 spirometry measures (% predicted FVC and % predicted FEV1) were significantly lower in smokers compared to moist snuff consumers and non-consumers. ECO and derived COHb were significantly higher in smokers compared to snuff consumers and non-consumers. No differences were observed between snuff consumers and non-consumers for either spirometry or ECO. Although some cohort or age differences were observed in CVD endpoints, the results are consistent with "within normal ranges" for each physiological assessment. ECO measures replicate data reported in other tobacco studies. Spirometry measures support lung function changes expected in smokers compared to smokeless tobacco consumers or non-consumers of tobacco.

9. AN IMPROVED HPLC METHOD TO DETERMINE PHENOLICS IN CELLULOSE ACETATE FILTERS. Denise FISHER JONES, Steven A. Wilson and Jeremy K. Steach; Eastman Chemical Company, Kingsport, TN

Analysis of phenolic compounds has been a standard test for assessing selective removal by cellulose acetate filters. The phenolic compounds of phenol, o-cresol, m,p-cresol, and catechol have been typically analyzed to understand selective removal in filters. These compounds are determined by high pressure liquid chromatography (HPLC) after extraction of Cambridge pads or cellulose acetate filters in an alcohol solution. Direct analysis of extracts enables the phenolic compounds to be determined from the same smoking session

as used to determine NFDPM, nicotine, and CO. In this work, improvements to the method have been developed for the injection program, fluorescence detector, eluent gradient, and LC column. These improvements allow the simultaneous analysis of previously studied phenolics as well as some additional components of interest including 2-methoxyphenol, dimethylphenols, resorcinol, and hydroquinone. Both precision and resolution have been improved over the previous method. Results with 2R4F Kentucky reference cigarettes agree well with published literature values. With improved precision, resolution, and the detection of additional components, this HPLC method will prove to be a more valuable tool for investigating selective removal.

10. STANDARDIZATION OF THE PREPARATION OF SMOKELESS TOBACCO EXTRACTS FOR ASSESSMENT OF BIOLOGICAL EFFECTS. Gaddamanugu L. PRASAD, Kathy W. Fowler, Betsy R. Bombick and Jo Ann Hill; Research & Development, R. J. Reynolds Tobacco, Winston-Salem, NC

Exposure to smokeless tobacco (ST) has been reported to elicit diverse biological effects at the cellular level. Currently, there is no consensus regarding parameters that will impact cellular response. Various conditions have been described for 1) preparation methods of ST extracts 2) dosage in cell culture or 3) detailed analyses of the ST preparation that is used for the treatment of cells. Hence, we have evaluated methods for preparation and analysis of ST extracts using 2S3 reference smokeless tobacco. Ten percent (w/v) preparations of ST were extracted in DMSO (ST/DMSO) or complete artificial saliva with enzymes (ST/CAS) for up to 24h, and analyzed for the presence of nicotine, 21 polycyclic aromatic hydrocarbons (PAHs) and four tobacco specific nitrosamines (TSNAs). Nicotine extraction efficiency was ~90% under all conditions. While PAHs were extracted equally in DMSO at 2 vs. 24 hours, their extraction was found to be generally higher in DMSO compared to ST/CAS. TSNA extraction efficiency was equivalent for both 2 and 24 h ST/DMSO. However, TSNAs were significantly elevated in the 24h ST/CAS-extracted sample, compared to the 2h ST/CAS-extracted sample. We hypothesize that the increase in TSNA levels in the 24h ST/CAS extractions is artifactual due to microbial action occurring during sample preparation. A time course of ST/CAS extracts prepared with and without antibiotics was performed. Nicotine and PAH levels were unaltered by the presence of antibiotics. Significantly, antibiotics prevented increases over time in the levels of microbes, TSNAs and nitrite/nitrate ratio. Thus, for extraction times greater than 2 h in physiologically relevant aqueous buffers such as ST/CAS, consideration must be given to potential microbial-driven artifact formation (e.g., TSNAs) which may skew biological assay results. When conducting extractions longer than 2 h, the addition of an antibiotic, or other appropriate mitigant is recommended, as necessary, to minimize artifact formation.

11. APPLICATION OF CARBON FIBER MICROELECTRODES FOR MEASUREMENT OF KINETIC CONSTANTS OF NITRIC OXIDE DECAY IN BLOOD. Jay L. ZWEIER, Xiaoping Liu, Gamal A. El-Sherbiny, Eric Collard, Xin Huang; Douglas Follmer, Mohamed A. El-Mahdy and Hassan Talukder; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

Endogenous nitric oxide (NO) is a potent vasodilator that regulates vascular tone. There is evidence that cigarette smoking increases superoxide production in blood vessels by rapidly inactivating NO. This impaired NO bioavailability is a critical cause of endothelial

dysfunction and a major risk factor for vascular disease, such as hypertension and atherosclerosis. Quantitative measurement of the NO decay rate in the blood of cigarette smoke treated animals is important for understanding the effect of cigarette smoking on NO decay kinetics in the vasculature. Carbon fiber microelectrodes (CFM) have been used for measurements of NO concentration. However, the time course of recorded current changes (*I-t* curves) by a CFM is different from the actual time course of NO concentration changes (*c-t* curves) due to CFM's response time (several seconds). This complicates the determination of rate constants for NO decay from the *I-t* curves. To find a simple method for analyzing experimental data, we present a mathematical model to describe the relationship between the recorded currents at the CFM and the NO concentrations in the solution. Using computer simulations based on the mathematical model, an approximation method was developed for determining the rate constants of NO decay from *I-t* curves, and the measurement accuracy was determined. This method was tested in several simple reaction systems with known rate constants, and applied to measure the rate constants of NO decay in blood samples of cigarette smoke exposed and control unexposed mice. These measurements demonstrate that smoking exposure increases the rate of NO decay in blood due to leukocyte activation with superoxide generation.

12. QUANTITATION OF PESTICIDES IN FLUE-CURED TOBACCO BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY (GCXGC-TOFMS). Joe BINKLEY and Scott Pugh; LECO Corporation, Saint Joseph, MI

Flue-curing is a tobacco curing process used to produce cigarette tobacco. The process typically takes approximately a week and generates a tobacco that is high in sugar with a medium to high nicotine content. Tobacco is known to contain thousands of analytes making it an extremely difficult matrix in which to identify pesticide residues. Current methodologies incorporate the use of complex, time consuming sample cleanup techniques to eliminate much of the matrix interference prior to GC-MS analysis.

This poster will show how the increased peak capacity of comprehensive two-dimensional gas chromatography provides the ability to effectively separate pesticides from the complex tobacco sample matrix while minimizing the need for extensive sample cleanup methods. In addition, the cryo-focussing effects of thermal modulation increases detectability allowing lower levels of these pesticide residues to be determined. The use of a Time of Flight Mass Spectrometer provides the ability to acquire full mass range spectra without sacrificing sensitivity. This is beneficial for detecting not only target pesticide analytes, but also new and emerging contaminants.

Quantitative GCxGC-TOFMS data from two flue-cured tobacco samples will be discussed. Matrix matched GCxGC-TOFMS calibration curves were generated for this work and will also be shown in this poster.

13. QUALITY DIFFERENCE AND ANALYSIS OF AGING TOBACCO LEAF BY THREE SECTIONS CUTTING. YAN Keliang, Wu Yi, Zeng Xiaoying and Wang Chao; Technology Center of Hongyun Honghe Tobacco (Group) Co. Ltd., Kunming PR China

Quality difference of aging tobacco leaf by three sections cutting was investigated in this study. Electron microscopy analysis indicated that the difference of tobacco leaf surface structure was significant and there was tighter structure on surface of tobacco leaf tip. Surface groups difference was detected by fourier transform infrared spectrometry and content of carboxyl group and nitrogen group was difference. For the purpose of enhancing spectra resolution, the difference the second-derivative spectra was selected for evaluating the difference of tobacco leaf and obvious difference was observed in region of 700~1200cm^{-1}, which suggested that varieties of sugar content among the three samples. Furthermore, the analysis of chemical component, pigment and aroma constituents showed that the sugar content of tobacco leaf center was highest, while corresponding total nitrogen and chlorophylls was lowest. However, the content of aroma constituents of tobacco leaf base was highest. In addition, the correlation of sensory evaluation with chemical component, pigment and aroma constituents was established and correlation exists among them. The final results showed that the quality of leaf center was best, next are leaf tip and the last is leaf base.

14. QUANTITATIVE/COMPARATIVE MOUSE LYMPHOMA ASSAY (MLA) FOR THE TESTING OF TOTAL PARTICULATE MATTER (TPM). Mark BALLANTYNE[1], Vicky Stone[1], Ian Crooks[2], Ken Scott[2], Clive Meredith[2], Debbie Dillon[2], Mari Johnson[1] and Jim Saul[1]; [1]Covance Laboratories Ltd, Harrogate, North Yorkshire, UK, [2]British American Tobacco GR&D, Southampton, UK

The objective of this study was to statistically measure the resolving power of the MLA for tobacco smoke TPMs. TPMs were produced under ISO smoking conditions from 3R4F and M4A cigarettes. 3R4F is a blended product containing 22% Burley tobacco. M4A contains only flue-cured tobacco. Four experiments were performed, to statistically estimate and then confirm the resolving power of the assay. In all experiments appropriate toxicity was considered achieved and increases in mutant frequency (MF) that exceeded the Global Evaluation Factor (GEF, 126 mutants/106 viable cells) were observed. Statistical analysis of linearity proved that an appropriate range of concentrations was analysed. 3R4F TPM was tested in Experiment 1. The number of replicates required to detect a 30% difference in response at the 5% (two-sided) level of significance with 80% power was determined to be six. Six replicates were used in Experiments 2-4. Experiment 2 compared two dilutions of 3R4F TPM, to simulate a 30% difference in mutagenicity. Experiments 3 and 4 compared 3R4F and M4A TPMs. The data generated in these experiments are considered sufficient evidence that a 30% difference in mutagenic response can reliably be detected using 6 replicates at each concentration and a 30 40% difference can be detected using 4 replicates. The data from this experimentation demonstrates and characterises the suitability of the MLA for quantitative comparison of mutagenic strengths of cigarette tobacco smoke TPMs when using the methodology employed in this study.

15. QUANTITATIVE DETERMINATION OF VOLATILE NITROSAMINES (VNA) IN SMOKELESS TOBACCO PRODUCTS. Mary DENNIS, Tianrong Cheng and Darren Steelman; Lancaster Labs, Lancaster, PA

Volatile nitrosamines have been classified by the International Agency for Research on Cancer as probable or possible carcinogenic compounds to humans. (1) Exposure to volatile nitrosamines can come from the environment, drinking water, diet and tobacco products.

Due to potential for health concerns it is necessary to develop a method that is selective and sensitive enough to determine ppb levels in tobacco products.

This method was developed to quantitatively determine the concentration of N-Nitrosodimethylamnie, N-Nitrosoethylmethylamine, N-Nitrosodiethylamine, N-Nitrosomorpholine, Nitrosopyrrolidine, and N-Nitrosopiperdine, in smokeless tobacco.

Tobacco samples are extracted with aqueous 0.01N potassium hydroxide using Chem Elut cartridges. The VNA's are separated on a DB-1701 30 meter by 0.25 mm column. Positive chemical ionization is used to obtain the molecular ion of each VNA. Identification and quantitation is performed on a TSQ Quantum GC triple quadrupole mass spectrometer from ThermoFisher. Quantitation was performed by multiple reaction monitoring (MRM) of the precursor ion to a product ion specific for each compound. Deuterium analogs of the VNA's are used as internal standards.

VNA's in smokeless tobacco products were not detected at levels below 0.5 ng/g (dry tobacco weight).

1) IARC Some N-nitroso compounds. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 17 (1978)

16. QUANTIFICATION OF CITRATE IN CIGARETTE PAPER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. N. PALANI, T.K. Dinesh, S. Suman, P.C. Ajith Kumar and S.V. Dhalewadikar; ITC R&D Centre, Peenya, Bangalore, India

Alkali metal salts of Organic acids can be applied as a burn modifier to the paper. Levels of these additives are typically between 0.5% and 3 % of the paper weight. These additives will also play a role in taste and flavour of smoke. The alkali metal salts provide a whiter ash and increase static burn rates. Hence it is necessary to quantify the citrate salts to monitor the quality of the Cigarette paper.

There are several methods for quantification of citrate in Cigarette paper reported in the literature viz titration method and enzymatic method. All the above methods involve lengthier and laborious procedures. We have developed a simple method using HPLC which does not involve any sample preparation and it is very simple. The Cigarette paper was extracted with 0.1% orthophosphoric acid, filtering the solution and injecting in HPLC. Citrate was converted into citric acid and separated using 5µ C18 column using 0.1% orthophosphoric acid as mobile phase and quantified at 210 nm. The recovery of citric acid from cigarette paper was greater than 95% and there is no matrix effect found in the above method. The results from the above method were also compared with the literature method and the results are in close agreement.

17. COMPARISON OF THE BACTERIAL MUTAGENICITY OF WHOLE-SMOKE, GAS-VAPOR PHASE AND SMOKE CONDENSATES FROM MENTHOLATED AND NON-MENTHOLATED CIGARETTES. Robert LEVERETTE; Lorillard Tobacco Company, A.W. Spears Research Center, Greensboro, NC

Menthol is widely used in the pharmaceutical, cosmetic, food and tobacco industries and is generally regarded as safe (GRAS) for these applications. Menthol itself is non-mutagenic in the Ames Assay. This study was conducted to compare the mutagenicity of wet total particulate matter (WTPM) and gas-vapor phase (GVP) as well as whole-smoke from a non-mentholated and mentholated cigarette to determine if the presence of menthol has an effect on this biological endpoint. The cigarettes used were comparable in construction, composition, and WTPM deliveries. The mentholated cigarette contained menthol at a normal user level. Cigarettes were smoked with a 35mL puff volume, 2 second puff duration and a 1 minute puff interval on a VITROCELL® VC10 smoking robot. WTPM from each cigarette type was pad collected and extracted in dimethylsulfoxide. *Salmonella* strains TA98 and TA100 were exposed to WTPM with metabolic activation (S9+). Whole-smoke (TA98 and TA100, S9+) and GVP (TA100, S9-) exposures utilized the VITROCELL® exposure modules and smoke dilution system. No differences were detected in the specific activities (revertants / μg) of the WTPM (TA98, $p = 0.8335$; TA100, $p = 0.7889$) or the GVP ($p = 0.4595$). Differences were observed in the whole-smoke activities, with the mentholated sample having significantly lower specific activity, less mutagenic, than the non-mentholated cigarette (TA98, $p = 0.0027$; TA100, $p = 0.0297$), only from cigarettes used from a freshly opened pack. No differences in whole-smoke cytotoxicity were observed. No differences in whole-smoke activities occurred when cigarettes were opened and conditioned (23°C, 60% RH) for approximately 18 hours prior to smoking. Analytical data suggests these results may be partially explained by the loss of menthol upon cigarette conditioning.

18. RESIDUAL ANALYSIS OF CARBONYLS IN TOBACCO AND TOBACCO PRODUCTS BY HPLC. Sharad K MEHTA, S.V. Dhalewadikar and B.J. Rajesh; ITC R&D Center, ITC Ltd Phase 1, Peenya Industrial Area, Bangalore, India

Carbonyl compounds have been drawing more and more attention because some carbonyls have been proven to be carcinogenic or risk for human health. Tobacco leaves are important source of carbonyls. It is hypothesized that these carbonyls are formed from lipid and wax constituents in tobacco leaves. Although methods are available for the determination of carbonyls in cigarette smoke, however methods are not available for tobacco leaves.
A simple method is developed for the quantitative determination of Carbonyls residues in tobacco and tobacco products.

The method uses HPLC with Photo-diode Array (PDA) detector involves derivitization and ultra sonication for the rapid and complete extraction of carbonyl compounds from tobacco leaves. Tobacco leaves were minced and ultra sonicated in acidic 2,4-dinitrophenylhydrazine (DNPH acidified with phosphoric acid) in acetonitrile for 2hrs and then holding for 30 min to allow for the complete reaction of aldehydes and ketones with DNPH and subsequent chromatographic separation on Lichrospher 100 RP- 18e(250mm x 4mm x 5 micron). A 20ul sample was injected into the HPLC system and analyzed by a ternary gradient mobile phase with a flow rate of 1.5 ml per minute and monitored at 365 nm.

Mobile phase A –(30% Acetonitrile, 10 % THF and 1% IPA) in water, mobile phase B-(65% Acetonitrile,1% THF,1% IPA) in water, mobile phase C - acetonitrile. Various parameters like concentration and derivitization of carbonyl with DNPH, sample extraction technique and extraction time were optimized. Quantification is based on external standard technique.

The method has been validated by standard validation protocols i.e. limit of detection, limit of quantification, recovery, repeatability and reproducibility. Recoveries of 73% – 103.4 % were obtained with a linear regression coefficient of 0.9998 for the range of 0.022- 4.52 mg/Kg of selected 8 carbonyls. Tobacco samples of various grades were analyzed.

19. OPTIMIZATION OF LEUKOCYTE ISOLATION FOR CLINICAL STUDIES. Subhashini ARIMILLI[1], Brad E. Damratoski[1], Peter Chen[2], Bobbette A. Jones[2], W. Edward Swords[1] and G. L. Prasad[2]; [1]Department of Microbiology & Immunology, Wake Forest University School of Medicine, Winston-Salem, NC, [2]R&D Department, R.J. Reynolds Tobacco Company, Winston-Salem, NC

Isolation and processing blood into its constituent cell types is an important process in basic and clinical research. Although a number of reagents are available and methods have been developed to fractionate blood, there is a need to optimize methods for clinical study samples involving larger volumes of blood. Therefore, we tested different methods and reagents. Here, we describe a simple and reproducible method for processing larger volumes of blood rapidly and consistently. We found that the following cost-effective methodology yielded reproducible leukocyte populations with better yields and purity. Briefly, freshly collected blood (350-400 ml) from healthy donors (under IRB approval) was subjected to gradient centrifugation to isolate peripheral blood mononuclear cells (PBMCs). By employing a semi-automatic method of fractionation we isolated 97% pure T cell and monocytes and 85% pure B cells and NK cells from the freshly prepared PBMCs. We also found that RNA isolated from these cells was intact and is suitable for downstream applications. Storing blood for 24h prior to fractionation resulted in significantly lower yields of PBMCs, T cells, B cells and monocytes, whereas the purity of the fractionated cells was comparable to that obtained from fresh blood. Further, we found that isolating leukocyte subtypes from frozen PBMCs resulted in overall improved yields and purity compared to isolations from 24h stored blood. Therefore, isolating PBMCs from fresh blood and freezing them for fractionation at a later date appears to be a better approach when processing multiple samples of larger volumes of blood. In summary, we have described simple and reproducible methods to isolate different leukocyte populations with a high degree of homogeneity which are suitable for a variety of assays in clinical studies.

20. COMPARISON OF REGULAR SMOKERS OF MENTHOL AND NON-MENTHOL CIGARETTES ON SMOKING PROFILE AND EXPOSURE. Valerie TROUDE, Bénédicte Varignon and Xavier Cahours; SEITA Imperial Tobacco Group, Fleury-les-Aubrais, France

As menthol's cooling effect might affect puffing and smoke inhalation, possible adverse effects of cigarette mentholation have been suggested. Since only few publications in this topic include smoking topography results, we performed a cross-sectional study in regular Caucasian smokers of American blended mentholated and non-mentholated cigarettes (n=64). As it is not possible to find two brands with exactly the same specification (tobacco blend and design) with and without menthol, we selected two brands having similar tar (12 mg ISO) and nicotine (0.7 mg ISO) levels. The purpose was to determine whether these two groups exhibit differences in smoking profile and biomarkers of exposure.

The Smoking Topography measurements, measured by Puff Analyser (D-80 Sodim), provided higher values ($p<0.05$) of average puff volume, average flow rate, total smoking

duration and total volume of smoke for smokers of non-menthol cigarettes. No substantive differences in puff number, puff interval and puff duration were found.

Smokers of menthol and non-menthol cigarettes exhibit identical levels of biomarkers of exposure (Carboxyhemoglobin and nicotine metabolites measurements). Mouth levels of exposure (calculated using filter tips from natural smoking conditions) were higher for the non-mentholated cigarette group: 56% and 47% for tar and nicotine respectively.

We worked out the inhalation index (as a proxy in the estimate of the volume of the smoke in the lung). We confirmed no differences in daily consumption parameters between the two groups excepted in the daily inhaled CO (18.0±1.2 and 14.3±0.9 cig/day for mentholated and non-mentholated cigarettes, respectively).

21. COMPARISON OF SFA, IC-PAD, AND LC-MS/MS FOR QUANTITATIVE CHARACTERIZATION OF CARBOHYDRATES IN TOBACCO PRODUCTS. John SHIFFLETT and Dawit Z. Bezabeh; Alcohol and Tobacco Tax and Trade Bureau, Scientific Services Division, Beltsville, MD

The U.S. Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for determining proper tax classification of tobacco products. Tobacco products in the U.S. may fall into several taxable categories including cigars, cigarettes, snuff, chewing tobacco, pipe tobacco and roll-your-own. As major components of tobacco, carbohydrates are valuable for product characterization and differentiation.

The p-hydroxybenzoic acid hydrazide (PAHBAH) method is widely used for the determination of carbohydrates, specifically reducing sugars, in tobacco. It was adapted to Segmented Flow Analysis (SFA) as a high throughput application and is available commercially though several manufacturers as an analytical cartridge. Ion chromatography with pulsed amperometric detection (IC-PAD) is also widely utilized for the determination of carbohydrates. IC separations of glucose, fructose, and sucrose are achieved using a Dionex CarboPac PA1 analytical column. It is more selective than the SFA method but does not share its high throughput capabilities. LC-MS/MS is also used as a technique for quantification of carbohydrates in tobacco extracts. LC-MS/MS separations of glucose, fructose, and sucrose are achieved using a LC-NH2 column. Negative ion electrospray with multiple reactions monitoring (MRM) mode is utilized for detection. Of the three methods presented here, LC-MS/MS appears to be the most selective. Results obtained from the analysis of tobacco and tobacco products using SFA, IC-PAD, and LC-MS/MS will be presented for comparison and differences in the selectivity of these methods will be discussed.

22. EVALUATION OF CYTOTOXICITY OF DIFFERENT TOBACCO PREPARATIONS. Subhashini ARIMILLI[1], Brad E.Damratoski[1], Betsy Bombick[2], W. Edward Swords[1], Mike Borgerding[2] and G. L. Prasad[2]; [1]Department of Microbiology & Immunology, Wake Forest University School of Medicine, Winston-Salem, NC, [2]R&D Department, R.J. Reynolds Tobacco Company, Winston-Salem, NC

Current debate regarding the potential for significant reductions in human health risk associated with smokeless tobacco (ST) use compared to cigarette smoking suggests further

examination of the relative cellular/molecular effects of non-combustible and combustible tobacco products is warranted. Available *in vitro* data regarding the potential toxicological effects of ST lacks consensus, which prompted an investigation into the relative cytotoxic potential of ST and whether ST elicits differential cellular/molecular responses compared to combustible tobacco preparations. Cigarette smoke condensate (CSC) and whole smoke conditioned medium (WS-CM) were prepared from 3R4F cigarettes, while 10% ST extract in artificial saliva with enzymes (ST/CAS) was prepared from 2S3 moist tobacco. Two different human cell lines, HL60 and THP1, and freshly isolated human peripheral blood mononuclear cells (PBMCs) were used to examine the relative cytotoxic effects of the tobacco preparations, as well as nicotine in short-term cell culture assays. Corresponding EC_{50} values, normalized for nicotine content, suggest that combustible tobacco preparations induced markedly higher cytotoxicity, as follows: WS-CM ≥ CSC> ST> nicotine. WS-CM and CSC similarly induced time-dependent cytotoxicity in PBMCs. While all three tobacco preparations induced some level of DNA damage, IL-8 secretion and oxidative stress, the combustible tobacco preparations were significantly more potent than ST/CAS. Moreover, tobacco preparations appeared to elicit differential responses in dendritic cell maturation assays. These findings suggest that the relative cytotoxic and other cell biological effects of tobacco preparations are dose-dependent, but that ST-induced cytotoxic effects, inflammatory responses, oxidative stress and DNA damage responses are evident only at substantially higher doses in this study.

MONDAY AFTERNOON, OCTOBER 4, 2010

SESSION A ***Session Chair: Joseph Wanna***

2:20 PM MONDAY

23. THE EFFECT OF POSITION OF CARBON GRANULES WITHIN A CIGARETTE FILTER ON THE RELEASE OF PARTICLES OR FIBRES FROM THE FILTER. Tony McCORMACK and Mike Taylor; Filtrona Technology Centre, Jarrow, Tyne & Wear, UK

In recent years, there has been debate regarding the release of particles from filter cigarettes during smoking and numerous studies have been carried out to quantify the number and type of particles or fibres released from cigarettes or filters. We have now developed a routine method for particle counting based on a modified laser particle counter and switching system. This method allows particles eluted from cigarettes or filters in the size range 0.5 to 50 microns over a range of different flow rates to be rapidly counted.

Cigarette filters containing activated carbon have the potential to give greater levels of particle release than filters where no granular additive is present. Recent innovations in filter technology enable the carbon to be located in particular regions of the filter, either evenly spread across the body of the filter as in traditional triple granular or active acetate ("dalmation") type filters or localized in particular cross-sectional regions of the filter. Notable examples of this latter category are "Active Patch" filters in which the carbon granules are located around the periphery or "Smooth Core" filters that feature a centrally located pocket of granules.

The developed laser particle counting technique has been used to measure the release of particles from four different types of carbon filters - 'Active Acetate', 'Triple Granular', 'Active Patch' and 'Smooth Core'. The results from these experiments are presented together with our findings on the effect of different carbon cigarette filter design variables on particle or fibre release.

2:40 PM MONDAY

24. CORRELATION BETWEEN MANUAL AND SEMI AUTOMATIC MEASUREMENTS OF IGNITION PROPENSITY TO ASTME2187-04. Tim MASON and Ian Tindall; Cerulean, Linford Wood East, Milton Keynes, UK

The current ASTME2187-04 method for ignition propensity is both time consuming and subject to variability due to the human element of the test. In order to reduce this inherent variability a semi automatic method was developed that relies upon a camera with pattern recognition technology.

The use of such camera technology highlights the difficulty of deciding the length of a burn and several examples are presented where the burn length could be in dispute. Consequently a "rules based" decision making process was adopted to determine a burn as being full length, terminated or questionable. The apparatus was tested by comparing the full length burn (FLB) of commercially available cigarettes tested under semi automatic conditions

with the same brands tested using a manual tool. Good agreement was achieved for the brands tested. Estimation of the repeatability of the apparatus was also made using FLB and residual length (RL) statistics on commercial cigarettes. The repeatability was found to be brand dependant. The significance of residual burn length and ASTME2187-04 pass /fail criteria are noted and discussed.

3:00 PM MONDAY

25. QUANTIFICATION METHOD FOR NNAL, ISO-NNAL AND NNA IN TOBACCO BY LC-MS/MS. Jasper D. H. VAN HEEMST; British American Tobacco, GR&D Centre, Southampton, UK

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-4-(3-pyridyl)-butanal (NNA) are both possible nitrozation products of nicotine. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) are the reduced forms of NNK and NNA respectively. Although NNAL, iso-NNAL and NNA are potentially carcinogenic, only limited information is available about their relative amounts in tobacco. Furthermore, they have mainly been determined using historic and non-specific techniques such as GC-TEA, combined with extensive sample clean-up and concentration procedures that may impact on the accuracy of data.

A simple, selective and sensitive HPLC-MS/MS method was developed for the quantification of NNAL, iso-NNAL and (separately) NNA. The methods use a common sample extraction procedure and different LC/MS conditions. No further sample clean-up or concentration is required prior to analysis. The selectivity of both methods was achieved by using specific MRM transitions in conjunction with optimized chromatographic separation. Deuterated analogs of NNAL and iso-NNAL were used as internal standards.

The method has been validated for the determination of the target compounds in a range of tobacco products.

3:20 PM ***Break***

3:50 PM MONDAY

26. IMPROVEMENT OF ANALYTICAL METHOD FOR QUANTIFICATION OF TSNA IN TOBACCO USING UHPLC-MS/MS. Hiroyuki YOSHIDA, Motomi Yajima and Toshir Teraoka; Japan Tobacco Inc., Leaf Tobacco Research Center, Oyama, Tochigi, Japan

Method improvement was performed for quantification of tobacco specific nitrosoamins (TSNAs) in tobacco. The objective was to establish a new analytical method being able to shorten analysis time with better or equal level of accuracy and reproducibility compared to the previous method and without unduly influence of matrix effect. Validation was done for the evaluation of method performance, and the results of the validation indicated that the matrix effect, the reproducibility and the recovery of the new method were more improved than those of the previous method.

In the previous method, quantification was performed with a LC-MS/MS (LC: Agilent 1100, MS/MS: Applied Biosystems API 4000). In the new method, while quantification was done by using a UHPLC-MS/MS (LC: ACQUITY UPLC, MS/MS: Quattro Premier XE, Waters) with electrospray ionization in the new method. The UHPLC (UPLC) method was optimized for the separation of four common TSNAs by gradient elution profiling and a reversed-phase column. In both methods, samples were extracted with 0.1M ammonium acetate buffer.

The effect of matrix was depressed by appropriately setting the column equilibrium time. The runtime of the new method was approximately 65% shorter than that of the previous method. Overall the reproducibility of new method was improved in validation. The recovery rates of NNN, NAT, NAB and NNK in Laboratory Fortified Blanks (LFB) were 103.2%, 101.7%, 101.6% and 104.7% respectively, those in Laboratory Fortified Matrix (LFM) of burley were 105.2%, 104.3%, 113.2% and 103.7% respectively, and those in LFM of flue-cured were 102.0%, 95.6%, 102.1% and 98.7% respectively.

4:10 PM MONDAY

27. DETERMINATION OF MALEIC HYDRAZIDE RESIDUE IN TOBACCO AND TOBACCO PRODUCTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.
Hongfei ZHANG, Zhaoyang Bian, Zhonghao Li, Fengpeng Zhu, Gangling Tang and Qingyuan Hu; Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China

Maleic hydrazide has been used as a growth regulator for grass and tobacco and as a sprout suppressant for onions and potatoes. Its popularity for growth regulator of tobacco has increased over the last few years. Maleic hydrazide has a systemic mode of action, translocating to the tubers by way of the vascular system, after being applied to the plant's leaves. According to the CORESTA, the maximum residue level (MRL) of maleic hydrazide permitted in tobacco and tobacco products is 80mg/kg. The literature contains several methods for the extraction of maleic hydrazide from a range of commodities, including garlic, onions and potatoes. However, few of them are fully appropriate for the extraction of maleic hydrazide from tobacco and tobacco products.

So a method for the determination of maleic hydrazide in tobacco and tobacco products was established by high performance liquid chromatography. Tobacco samples were separated with Varian Microsorb-MV 100-5 C18 column(250*4.6mm) using 0.1M acetic acid as mobile phase, after extracted by 4mol/L hydrochloric acid (in which a mixture of free and bound maleic hydrazide is extracted), and cleaned up by C18 solid-phase cartridge. It was quantified by external standards method and determined at UV 313 nm. Results showed that the linear range for the maleic hydrazide was 0.1μg /ml – 100μg /ml and the limit of quantify was 1μg /g. A blank tobacco sample was spiked at 8, 80, 160μg/g for maleic hydrazide, and the average recoveries ranged from 94.18% to 96.32%, with the relative standard deviations(RSD) below 13.60%(n=6). This method could be used for determine the maleic hydrazide in tobacco and tobacco products.

4:30 PM ADJOURN

MONDAY AFTERNOON, OCTOBER 4, 2010

SESSION B ***Session Chair: Brian Nordskog***

2:20 PM MONDAY

28. CARBON-CENTERED FREE RADICALS AS INTERMEDIATES TO MAINSTREAM SMOKE CARBONYLS. Anthony GERARDI and William Coleman, III; R. J. Reynolds Tobacco Company, Winston-Salem, NC

Free radicals in cigarette smoke may contribute to harm associated with cigarette smoking, more specifically, contribute to oxidative stress and subsequent biological activity. A recent high throughput procedure for relative determination of 14 different carbon-centered free radicals (CCFR), both acyl and alkylaminocarbonyl type, was used in conjunction with traditional carbonyl determination to demonstrate the intermediary role of CCFR in mainstream smoke carbonyl generation. This procedure used the radical scavenger 3-cyanoproxyl radical (3-CNP), which was diluted in acetonitrile and spiked onto a 44 mm fiberglass Cambridge filter pad, having the acetonitrile subsequently evaporated. Directly after the 3-CNP coated pad was an impinger containing acidified 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile: water (80:20). Fresh whole smoke from various cigarettes was passed through 3-CNP coated Cambridge filter pads and impingers. Cigarettes evaluated afforded a representative range of standardized 'tar' yields (by the Cambridge filter method), straight grade (Burley, Flue-Cured, and Oriental) cigarettes and included Kentucky reference cigarettes 3R4F and 1R5F. Cigarette smoke was exposed to coated filter pads and impingers using the 35 cc puff/ 60 sec interval/ 2 sec puff duration / 0% vent block (35/60/2/0) smoking regime. Liquid chromatography tandem mass spectrometry (LC-MS/MS) with precursor ion monitoring was used for detecting the large array of radicals, while HPLC with UV detection was used for the determination of carbonyls trapped by DNPH. Two controls, a blank pad and no pad placed in-line between the cigarette and impinger, were used for comparison. The range of CCFR concentration was related to 'tar' delivery and in selected instances, carbonyl delivery was found to be influenced by the presence of the free radical trap by as much as 74%.

2:40 PM MONDAY

29. USE OF TWO GC-MS SCAN TECHNIQUES FOR THE CHARACTERIZATION OF WRAPPERS AND BINDERS TAKEN FROM CIGAR PRODUCTS. John H. LAUTERBACH[1] and Deborah A. Grimm[2]; [1]Lauterbach & Associates, LLC, Macon, GA, [2]Coordinated Instrumentation Facility, Tulane University, New Orleans, LA

The proper classification of tobacco products is very important for regulatory and taxation purposes. Cigars products present a special problem because of the wide variation in sizes, blends, and additives used in the tobacco filler, and the nature of the wrapper and binder (if used). Proper classification of little cigars (also known as small cigars or cigarillos) can present problems because of their similarity to some cigarette products, and the inability of routine and some nonroutine assays to discriminate among products. Consequently, we characterized the wrappers and binders (if used) from several varieties of little cigars with two GC-MS scan techniques: 1) the Direct Silylation Scan (in situ silylation of

tobacco before analysis), which provides identifications and semi-quantitative data, on acids, humectants, sugars, and certain other compounds (Moldoveanu et al., 46th TCRC, Paper #28); and 2) the HFP Scan (in situ extraction of tobacco with hexafluoroisopropanol or methanol before analysis), which allows the analysis of the semivolatile compounds ranging, from low molecular-weight ketones to neophytadiene and some sterols (Dong et al., 47th TCRC, Paper #16). Both GC-MS techniques were performed on an Agilent 6890 GC coupled with an Agilent 5972 MS. A DB-5MS capillary GC column (25 m X 0.25 μm film thickness and 0.25 mm ID) was used. The data from our analyses allowed us to distinguish between natural and reconstituted materials and apparent transfer of tobacco constituents to wrappers when no binders are used.

3:00 PM MONDAY

30. ANALYSIS OF SELECTED CARBONYLS IN MAINSTREAM CIGARETTE SMOKE BY UPLC-UV. Sofia A. ESSÉN, Laura Ashmore and Thomas Brice; British American Tobacco, Group R&D, Southampton, UK

Carbonyl compounds, including acetaldehyde, acrolein and crotonaldehyde, are toxicants of potential concern that are formed during the combustion of tobacco. Derivatisation using 2,4-dinitrophenylhydrazine (DNPH) and high performance liquid chromatography are common techniques used in their determination in cigarette smoke. Since the introduction of UPLC (ultra performance liquid chromatography), many HPLC methods have been transferred to UPLC in order to reduce the analysis time and/or to improve separation efficiency. In the present study, a new UPLC method was developed to determine eight selected carbonyl compounds, *i.e.* formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, methyl ethyl ketone and butyraldehyde. The separation of the selected carbonyls from potentially interfering artefacts was improved and the analysis time was decreased from 35 to 14 minutes. The method utilizes a polar, reversed-phase column, a water/acetonitrile mobile phase and detection of the DNPH-derivatives at 380 nm. The method has been demonstrated to be applicable to cigarette smoke samples produced using ISO and Health Canada Intense smoking regimes. Method performance has been compared to the currently used HPLC-UV method and to data produced by other laboratories.

3:20 PM ***Break***

3:50 PM MONDAY

31. FACTORS AFFECTING THE DESIGN OF PAPER DIFFUSIVITY MEASUREMENT APPARATUS WITH PARTICULAR REFERENCE TO THE DESIGN OF TRANSFER STANDARDS. James VINCENT and Ian Tindall; Cerulean, Linford Wood East, Milton Keynes, UK

The measurement of CO_2 diffusion through paper substrates has been demonstrated to correlate well with the ability of a cigarette to pass the ASTME2187-04 test for ignition propensity. The design of the apparatus making such measurements must be such that a high degree of reproducibility and linearity is achieved. Comparisons have been hampered in this by a lack of a suitable transfer standard. To overcome this deficiency a laser drilled

strip was developed to repeatedly mimic papers of differing diffusivity. The significant design parameters of the strip are presented.

A number of factors affect performance, a key factor being the design of the gassing head. This paper describes two alternative gassing head designs and compares their performance. Using finite element analysis of flow and practical measurements the ability of the two heads to provide linear, repeatable results on paper and standards are explored.
Recommendations are made as to the preferred geometry of the test head and further development and use of transfer standards.

4:10 PM MONDAY

32. ADSORPTION CHARACTERISTICS OF SILANOL GROUP FOR VOLATILE CONSTITUENTS IN CIGARETTE SMOKE. Noritoshi FUJITA; Japan Tobacco Inc., Yokohama, Kanagawa, Japan

We report the influence of the silanol group (Si-OH) of silica gel and mesoporous silica on the adsorption characteristics for various constituents in cigarette smoke. Comparing activated carbon, siliceous porous materials that have silanol groups selectively adsorbed the hydrophilic volatile constituents with saturated vapor pressure from 10^3 to 10^5 Pa at 298 K. Preparing the modified silica gel, the trimethylsilyl group (hydrophobic) replaced the silanol group (hydrophilic) to decrease the amount of hydrophobic groups. Adsorption efficiencies of hydrophilic volatile constituents on the modified silica gel were clearly lower than on unmodified silica gel. This result suggests the silanol groups play an important role in adsorption of the constituents, i.e. the groups adsorb the constituents by chemical interaction such as hydrogen bonding, electric dipole-dipole interaction. The adsorption performances of various silica gels and mesoporous silicas with the different number of silanol groups per gram were examined for volatile constituents. As a result, the larger the number of silanol groups, n, goes, the smaller the penetration efficiency, *1-E*, in logarithmically, *Ln(1-E)=-K n*. The *K* is the adsorption factor and shows a different value for each hydrophilic constituent. The correlation between *K* and the octanol–water partition coefficient, log P_{ow}, is expressed by $K = -1.4 \times 10^{-22}\ Log\ P_{ow} + 3.5 \times 10^{-22}$ ($r = -0.85$). Consequently, it is found that (i) silanol groups selectively adsorb the hydrophilic volatile constituents by chemical interaction, and (ii) adsorption efficiency of each volatile constituent can be predicted by two parameters of the number of silanol groups of siliceous porous materials and log P_{ow} of each constituent.

4:30 PM MONDAY

33. STUDIES ON TRANSITION METALS MODIFIED POROUS MATERIALS FOR REDUCING HYDROCYANIC ACID IN CIGARETTE SMOKE. Le ZHAO[1], Bin Peng[1], Xuehui Sun[1], Jing Zhu[1], Cong Nie[1] and Xuewu Yan[2]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan Province, P. R. China, [2]Nanjing University of Science and Technology, Nanjing, Jiangsu Province, P. R. China

In this study, different transition metals such as Zn, Co, Fe, Cu modified HZSM-5 and SBA-15 materials have been prepared by impregnation method for the reducing hydrocyanic acid in cigarette smoke. The porous materials were characterized by powder X-ray diffraction

(XRD) and N_2 sorption isotherms. The characterization results showed that most transition metal oxides were well dispersed on HZSM-5 or SBA-15. The different supports, transition metal oxides and the amount of those were investigated using the model reactor in detail. SBA-15 loading 5%mol CuO was found to be most effective for reducing hydrocyanic acid in cigarette smoke. The effects for decreasing hydrocyanic acid in cigarette smoke were investigated using a dual-filter cigarette. Compared to the control cigarette, the released amount of hydrocyanic acid for experimental sample reduced by about 23.0 . While the nicotine and tar deliveries were almost the same, indicating that this material had good selective filtration behavior for decreasing hydrocyanic acid in cigarette smoke. Moreover, the taste of the experimental sample was similar to the control cigarette.

4:50 PM ADJOURN

TUESDAY MORNING, OCTOBER 5, 2010

SESSION A ***Session Chair: Michael Dube***

8:30 AM TUESDAY

34. A CLINICAL TRIAL OF CARDIOVASCULAR DISEASE BIOMARKERS IN ADULT SMOKERS AND MOIST SNUFF CONSUMERS, PART I: STUDY DESIGN, SUBJECT SELECTION AND COHORT CHARACTERIZATION. Bobbette A. JONES[1], Buddy G. Brown[1], Leanne C. Lee[1], David Heavner[2], Thomas J. Steichen[3] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, NC, [2]Pinnacle, NC, [3]Winston-Salem, NC

A single site, three cohort, age-stratified, cross-sectional study was conducted in the U.S. in subjects who were exclusive cigarette smokers (n=60), exclusive moist snuff consumers (n=48), and non-consumers of tobacco (n=60) to identify potential CVD-related endpoints that differed among the three cohorts. Enrolled subjects were generally healthy, adult males (ages 26-49) who were free of clinically significant health problems, measured "' 70% of predicted for FEV, (spirometry) and were willing to undergo all study procedures. Tobacco-use cohorts provided their usual brand (US cigarettes or moist snuff) for use during an overnight clinical confinement. On Day 1, a 45-minute product abstention period was followed by a US tobacco product "challenge" appropriate to the cohort. A 10-12 hour overnight "fast" from food/drink and all tobacco products preceded the start of Day 2 procedures. Clinical endpoints, measured 15-minutes Postchallenge- Day 1 and/or Fasting-Day 2, included tobacco- (and potentially CVD-) related biomarker evaluations of spot-urine and blood sample; physiological assessments (FMD, ASI, CIMT, spirometry and expired CO); and self-reported product use, nicotine dependence and diet/health status measures. Cohort-specific inclusion/exclusion criteria were well-defined to create exclusive use groups with the expectation that the biomarkers of exposure and effect could differentiate the three cohorts. In this study, comparison of the three cohorts revealed that, within each tobacco-use cohort, comparable product usage among the different age groups was reported. No significant differences in the Fagerstrom nicotine dependence scores were observed within or between the tobacco-use cohorts. Overall, the subjects rated themselves as healthier than the population norm on the health questionnaire (SF-36v2™), with no significant age effects observed and only one significant difference between the tobacco cohorts and non-consumers.

8:50 AM TUESDAY

35. CARDIOVASCULAR DISEASE BIOMARKERS STUDY, PART II: TOBACCO-RELATED BIOMARKERS OF EXPOSURE IN EXCLUSIVE CIGARETTE SMOKERS, EXCLUSIVE MOIST SNUFF CONSUMERS, AND NON-CONSUMERS OF TOBACCO. Buddy G. BROWN[1], Bobbette A. Jones[1], Leanne C. Lee[1], David Heavner[2], Thomas J. Steichen[3] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, NC, [2]Pinnacle, NC, [3]Winston-Salem, NC

A CVD biomarkers study conducted in the US measured putative tobacco-related biomarkers in three exclusive cohorts: cigarette smokers, moist snuff consumers and nonconsumers of tobacco. Subjects were generally healthy males between the ages of 26-49.

Samples for spoturine and blood (Day 1-Challenge and Day 2-Fasting) were collected and analyzed. Urinary biomarkers of exposure for the following tobacco-related components were determined: nicotine + nine metabolites (NicEQT), NNK, benzene, acrolein, PAHs, 1,3-butadiene, acrylamide, crotonaldehyde, o-toluidine, 2aminonapthalene, 4-ABP and 3-ABP. Urinary biomarkers of effect (isoprostanes iPF2a-1I1 and iPF2a-VI) were measured to determine oxidative stress. All urinary biomarkers were normalized to urinary creatinine. Blood exposure biomarkers included COHb (carboxyhemoglobin), nicotine and cotinine. COHb was significantly higher in smokers compared to both moist snuff consumers and non-consumers on Days 1 and 2. Serum nicotine, measured on Day 1, showed smokers>moist snuff consumers>non-consumers; and on Day 2, moist snuff consumers>smokers>non-consumers. Serum cotinine differed significantly among all cohorts with moist snuff consumers>smokers>non-consumers on both days. All urinary biomarkers principally derived from tobacco combustion by-products were significantly higher in smokers compared to both moist snuff consumers and non-consumers. NicEQ-T differed significantly among all cohorts (moist snuff>smokers>non-consumers) on both days. NNAL (an NNK biomarker) was significantly higher in moist snuff consumers compared to cigarette smokers and non-consumers on Days 1 and 2. The urinary isoprostanes were significantly higher in smokers compared to moist snuff consumers and non-consumers. In this study, these data indicate: 1) urinary biomarkers of tobacco combustion by-products are significantly reduced in smokeless tobacco consumers over smokers, 2) urinary NNAL results for smokeless consumers are consistent with values in the literature, and 3 blood exposure biomarkers adequately characterize the three cohorts studied.

9:10 AM TUESDAY

36. CARDIOVASCULAR DISEASE BIOMARKERS STUDY, PART III: TOBACCO-RELATED BIOMARKERS OF EFFECT IN EXCLUSIVE CIGARETTE SMOKERS, EXCLUSIVE MOIST SNUFF CONSUMERS, AND NON-CONSUMERS OF TOBACCO.

Brian K. NORDSKOG[1], Buddy G. Brown[1], Bobbette A. Jones[1], Leanne C. Lee[1], David Heavner[2], Thomas J. Steichen[3] and Michael F. Borgerding[1]; R J Reynolds Tobacco Company, [1]Winston-Salem, NC, [2]Pinnacle, NC, [3]Winston-Salem, NC

A three cohort, age-stratified cross-sectional study was conducted in the U.S. in cigarette smokers (n=60), moist snuff consumers (n=48) and non-consumers of tobacco (n=60) to evaluate several known cardiovascular biomarkers of effect. Subject enrollment was restricted to generally healthy males (26-49 years) who were free of clinically significant health problems, not taking medication for chronic medical disorders, willing to undergo all study procedures including one overnight confinement, tested negative for drugs and alcohol, and measured ~70% of predicted for FEV1 on spirometry. Physiological assessments included measures of flow mediated dilation (FMD), anklebrachial index (ABI) and expired CO (ECO) on Days 1 and 2; carotid intimal media thickness (CIMT) and spirometry on Day 2. Forty-five blood biomarkers of tobacco exposure/effect and fourteen clinical hematology indices were also evaluated. For the cardiovascular-related physiological assessments, no significant differences were found between cohorts for FMD or CIMT although a significant age group main effect was observed for CIMT. Smokers had a lower mean ABI value following normal smoking behavior, but this difference was lost after a 10-12 hour overnight tobacco abstinence period. ECO was significantly different between the

cohort that smoked and the two that did not. Approximately half (n=22) of the measured blood biomarkers of effect showed differences in cohort comparisons. IL-12(p70), ICAM1, IL8 and MCP1 were the biomarkers that best differentiated the three cohorts. Age effects were also seen. In conclusion, significant cohort and age effect differences were identified. Concentrations of measured biomarkers were consistent with normal clinical ranges; however, differences between cohorts (within normal ranges) were observed.

9:30 AM TUESDAY

37. A REVIEW OF THE LITERATURE ON THE DEGREE OF COMPENSATORY" SMOKING OF LOW-DELIVERY CIGARETTES. Chris COGGINS[1], Ruth Dempsey[2] and Ewald Roemer[2]; [1]Carson Watts Consulting, King, NC, [2]Philip Morris International Management SA, Neuchâtel, Switzerland

To review trends in measures of compensatory smoking behavior over time and by method of assessment.

Introduction. An analysis of the papers published on "compensatory" smoking, observed when smokers switch to lower-yielding products from higher-yielding products (yields determined on smoking machines), indicates that compensation clearly does occur. However, differences in the degree of compensation reported, according to the approach taken for the measurement of compensation, have not previously been reviewed.

Results. Almost all studies using the "voluntary switching" concept (smokers selecting long-term their own brands, with no experimental switching) show tobacco-specific biomarker (e.g. serum or urine cotinine) responses that are intermediate between those that would be expected for complete compensation (compensation index, CI, =1), and for absorption of the ISO yields of nicotine (CI=0). The "voluntary switching" data show that daily exposure in smokers of lower-yielding cigarettes may be somewhat higher than would be expected, based on nominal yields, but that the exposures are much lower than would be expected, if compensation were complete. These conclusions are further compared to a number of studies that have looked at different aspects of compensation such as puffing behavior, vent blocking and numbers of cigarettes smoked per day.

Implications. An overall judgment is for a CI of ~0.6, considerably less than complete compensation. Smokers of lower-yielding cigarettes do indeed have lower daily exposures of nicotine.

9:50 AM ***Break***

10:20 AM TUESDAY

38. INFLUENCE OF MENTHOL ON THE PERMEABILITY OF ARTIFICIAL CELL MEMBRANES TO ACROLEIN, BENZO(A)PYRENE (BAP), AND 4-(METHYLNITROSAMINO)- 1-(3-PYRIDYL)-1-BUTANONE (NNK). Edward A. ROBINSON and Florian R. Perini; Lorillard Tobacco Company, Greensboro, NC

Azzi *et al.* (*Carcinogenesis* 2006) explored the impact of menthol on membrane permeability in a porcine esophagus model. The authors found that menthol did not affect the permeation of test compounds, but did increase the membrane reservoir formation for NNK. We examined the influence of menthol on the permeation of acrolein, BaP, and NNK using a PAMPA (parallel artificial membrane permeability assay) system (pION, Inc.). The PAMPA system allows determination of the permeability of an artificial membrane of dioleoylphosphatidyl-choline to test compounds based on the diffusion of those compounds from a donor compartment containing the compound to an acceptor compartment with no test compound. A single concentration of each test compound was assessed both without added menthol and with three concentrations of menthol. All experiments were conducted using a solvent system consisting of pION Prisma™ buffer and dimethylsulfoxide. Concentrations were chosen to reflect their relative concentrations in smoke. Diffusion of the analytes through the artificial membrane was followed by fluorescence (BaP) and ultraviolet-visible spectroscopy (acrolein and NNK) at the test compounds' respective maxima. Some statistically significant differences were noted between samples, but, in contrast to previously reported results, these were not consistent with an effect of menthol on permeability or membrane reservoir formation.

10:40 AM TUESDAY

39. IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN BLOOD PLASMA OF CONTROL AND CIGARETTE SMOKE-EXPOSED MICE BY 2-D DIFFERENTIAL IN-GEL ELECTROPHORESIS/MASS SPECTROMETRY. Jay L. ZWEIER and Arun K. Tewari; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

Cigarette smoke exposure is known to induce chronic obstructive pulmonary disease, atherosclerosis, and several types of cancer in humans and also in animal models. Smoking leads to oxidative stress and inflammation that are important in triggering pulmonary and cardiovascular disease. The objective of the current study was to quantify differences in expression levels of plasma proteins of cigarette smoke-exposed and control mice, and identify these proteins for use as potential biomarkers of smoking-induced disease. We utilized two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry (MS) technology to characterize these proteomic changes. 2D-DIGE of plasma samples identified 11 differentially expressed proteins. Out of these 11 proteins, 9 of them were down-regulated and two were up-regulated. The proteins identified are involved in vascular function, coagulation, metabolism, and immune function. Among these, the alterations in fibrinogen (2.2 fold decrease), alpha-1-antitrypsin (1.8 fold increase) and arginase (4.5 fold decrease) are of particular interest since these have been directly linked to cardiovascular and lung pathology. Thus, we observe that chronic cigarette smoke exposure in mice leads to prominent changes in the protein expression profile of blood plasma and these

changes in turn can potentially serve as markers predictive of the onset and progression of cardiovascular and pulmonary disease.

11:00 AM TUESDAY

40. A MODEL FOR THE RESPIRATORY RETENTION OF COMPOUNDS FOUND IN CIGARETTE SMOKE. F. Kelley ST. CHARLES; St. Charles Consultancy, Winston-Salem, NC

For the risk assessment of cigarette smoke, an estimate of the mass of chemical compounds retained in the body is needed. Filter studies provide estimates of mouth exposure to compounds in cigarette smoke, but do not account for mouth spill and respiratory retention. Urinary biomarkers provide the relative uptake of certain compounds when comparing products, but generally do not provide absolute uptake values. Nicotine is the exception since the multiple urinary biomarkers currently measured can account for over 90% of the retained nicotine. In addition, deposition information may be needed for compounds for which no biomarker has been developed. Knowledge of the respiratory retention of smoke compounds allows mouth exposure to be converted to a more realistic estimate of dose although mouth spill is still neglected. Data from multiple studies have been combined and respiratory retention has been determined as a function of vapor pressure. Averaging data from multiple studies reduces both subject and method variability. A plot of the average respiratory retention versus the log (vapor pressure at 20-25° C) gives a sigmoid shape with three distinct regions. Compounds with vapor pressure greater than 10^{-4} pascal (Pa) generally have respiratory retentions of 90% or greater. Compounds with vapor pressure less than 10 Pa, generally have respiratory retentions of 60% or less. A transition region lies between these ranges. Solanesol, with a vapor pressure of about 10^{-20} Pa, is generally assumed to represent smoke particulate deposition. Solanesol retention increases as both inhalation volume and lung exposure time increase. A model for solanesol retention versus these variables is also presented as well as a technique to account for mouth spill.

11:20 AM TUESDAY

41. URINARY TOBACCO SMOKE RELATED METABOLITES IN RELATION TO BRAND YIELD: A 200 SUBJECT CANADIAN STUDY. Mehran SHARIFI, William Rickert, Peter Joza and Wendy Wagstaff; Labstat International ULC, Kitchener, ON Canada

The purpose of this study was to investigate exposure to tobacco smoke related constituents based on the concentration of metabolites in 'spot' urine samples. A total of 200 healthy subjects were recruited over a 9 month period by a CRO from a Canadian phase 1 clinical trial. Subjects were selected based on smoking status, gender and ISO tar yield resulting in 20 male and 20 female non smokers and 40 males and 40 females in each of the tar categories of ≤ 9 mg and > 9 mg. In addition to tar yield, questionnaire data included age, cigarette brand, number of cigarettes smoked, and years of smoking. Urine samples were collected and shipped frozen to the laboratory where, nicotine equivalents, levels of creatinine, TSNA's, PAH's, and arylamines were determined. In comparison with non smokers, smokers had increased (median) urinary concentrations of o-toluidine (196 vs. 178 ng/mg creatinine), 1-aminonaphthalene (62 vs. 29 ng/mg creatinine), 2-aminonaphthalene (20 vs. 14 ng/mg creatinine), and 4-aminobiphenyl (17 vs. 9.9 ng/mg creatinine). With respect to PAHs,

non smokers in comparison with smokers had lower median urinary concentrations of hydroxynaphthalenes (6.3 vs. 17 ng/mg creatinine), hydroxyphenanthrenes (0.26 vs. 0.61 ng/mg creatinine), 2-hydroxyfluorene (1.2 vs. 2.1 ng/mg creatinine), and 1-hydroxypyrene (0.08 vs. 0.25 ng/mg creatinine). Considering TSNA's, the results for smokers were consistent with those reported by others. For each of the biomarkers in this study, notable differences between "≤ 9 mg" and "> 9 mg" tar categories for both male and female subjects were not apparent.

11:40 AM LUNCH

TUESDAY MORNING, OCTOBER 5, 2010

SESSION B ***Session Chair: Lowell Bush***

8:30 AM TUESDAY

42. DEVELOPMENT OF TOBACCO LINES WITH ULTRA-LOW LEVELS OF NORNICOTINE. Ralph E. DEWEY[1], Ramsey S. Lewis[1], Steven W. Bowen[1] and Lowell P. Bush[2]; [1]Department of Crop Science, North Carolina State University, Raleigh, NC, [2]University of Kentucky, Lexington, KY

Our goal is to develop tobacco lines that display a greatly reduced propensity for producing tobacco-specific nitrosamines (TSNAs) during the curing, storage and processing of the leaf. Specifically, we are targeting the genes responsible for the demethylation of nicotine as a strategy for minimizing the synthesis and accumulation of nornicotine, the alkaloid precursor of N-nitrosonornicotine (NNN), a TSNA classified as a Group I carcinogen by the International Agency for Research on Cancer.

Several years ago we identified *CYP82E4* as the genetic locus responsible for the exceptionally high levels of nicotine demethylation observed in tobacco plants possessing an activated converter locus (termed "converters"). We subsequently discovered two "minor" nicotine demethylase genes, *CYP82E5v2* and *CYP82E10*, the combined activity of which is responsible for the majority of the low level of nornicotine observed in a typical nonconverter tobacco plant (2 – 4% of total alkaloid content). By combining debilitating EMS-induced mutations for all three nicotine demethylase loci into single genotypes, we have been able to develop lines that accumulate exceptionally low levels of nornicotine. These materials promise to provide a facile and effective means of producing tobacco products with greatly reduced NNN content. In this presentation we will provide an update of our latest research results.

8:50 AM TUESDAY

43. POTENTIAL APPLICATIONS OF 'GM' TECHNOLOGY IN THE PRODUCTION OF TOBACCO FOR ITS TRADITIONAL USES. Orlando CHAMBERS[1], Patrick Thomas[1], H. Maelor Davies[1], Bruce Fortnum[2] and Paul Peterson[2]; [1]Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY, [2]Clemson University, Florence, SC

The flue-cured and burley tobacco industries have thus far relied solely on the intrinsic gene-pool of the tobacco plant itself for traits that enhance yield, disease resistance, leaf quality etc. Meanwhile, over the last 25 years the tobacco plant has been widely used as a convenient research species for developing transgenic (genetically modified; GM) strategies that employ genes from other sources for crop improvement, resulting in the demonstration of many new performance and input traits of potential use for tobacco production. We are conducting a comprehensive survey of this work, and also considering how and whether GM tobacco could be safely and securely produced in the open-field environment without compromising production of the conventional, non-GM crop. We anticipate that this study will be valuable to growers and their organizations as a base of knowledge concerning those GM technologies that might be employed in traditional burley and flue-cured varieties in the

future. Our progress to-date has comprised extensive literature searches in several scientific/technical databases, followed by sorting and filtering of the many thousands of publications to remove replicated material. Patents, government-regulated field trials, and interviews with industry and growers are also contributing useful information. A classification format has been designed that will enable the final search results to be interpreted easily in terms of what traits have been demonstrated in tobacco plants through GM technology, how effective they are, whether they have been expressed in commercial tobacco varieties and field-tested, etc. Our findings will be summarized in a report which will be openly available on-line.

9:10 AM TUESDAY

44. THE EFFECT OF POPULATION DENSITY ON TSNA ACCUMULATION IN BURLEY TOBACCO. Anne JACK, Colin R. Fisher, Angela Schoergendorfer, Neil F. Fannin and Lowell P. Bush; University of Kentucky, Plant & Soil Sciences Department, Lexington, KY

Lower alkaloids generally result in lower TSNAs, and high population tobacco generally has lower alkaloids than standard population tobacco. The objective of this study was to quantify the effect of population density on TSNAs, other nitrogenous components, physical characteristics, yield and quality. Because the effects of population density and available nitrogen are inextricably confounded, several nitrogen treatments were included. Experimental design was a split-split plot, with three nitrogen treatments as main plots, two population densities as subplots and two varieties as sub-subplots. Nitrogen treatments were high nitrogen per hectare (336 kg N /ha), standard nitrogen per hectare (252 kg N/ha) and the standard nitrogen level applied on a per plant basis. Populations were standard (18,040 plants/ha) and high (27,060 plants/ha). Varieties were a high converter selection of TN 90 (TN 90H) and commercial low converter TN 90 (TN 90LC). NNN, NAT and total TSNAs were lower in the high population. Total TSNAs were reduced from 3.3 ppm to 2.3 ppm in TN 90LC, and from 11.0 ppm to 7.9 ppm in TN 90H. TSNAs were reduced in the high population regardless of nitrogen rates. Leaves were smaller and thinner in the high population, which also had a higher yield (3527 kg/ha vs. 3147 kg/ha). There were few differences in quality variables, but the curing conditions were unfavorable, and it is likely that under more favorable curing conditions, the differences between populations would be greater. Although the high population reduced TSNAs by 30% and increased yield by 12%, stripping and handling costs would be 50% more with high density tobacco.

9:30 AM TUESDAY

45. (+)-2'-R-NICOTINE IS ENANTIOSELECTIVELY DEMETHYLATED BY ENZYME CYP82E4 IN N. TABACUM L. Bin CAI, F. Fannin, A. Jack and L.P. Bush; Dept of Plant and Soil Sciences, University of Kentucky, Lexington, KY

In tobacco (*N. tabacum L.*), a family of cytochrome P450 (CYP) proteins (nicotine demethylase enzymes) N-demethylate nicotine to nornicotine. CYP82E4 (E4) and CYP82E5 (E5) are two nicotine demethylases which have been reported to be active in tobacco. Naturally occurring (+)-2'-R-nicotine accounts for less than 1% of total nicotine, while after demethylation 4 to 75% of nornicotine is (+)-2'-R-nornicotine and the percentage varies among lines and tissues. The reason for this high and variable percentage of

R-nornicotine resulting from low and constant R-nicotine substrate is unknown. In a yeast system, we have shown R-nicotine was demethylated faster by E4 than (-)-2'-S-nicotine, which can partially explain the high percentage of R-nornicotine. The objective of study is to confirm this preference for R-nicotine in tobacco plants. Leaf disks and root segments from converter parent, E4 and E5 mutant and RNAi tobacco plants were used in this study. 2'-^{14}C- R, -S or racemic nicotine were fed to tissues to measure the enzyme enantioselectivity. In converter parent plants, both leaves and roots produced 10-fold more R-nornicotine than (-)-2'-S-nornicotine. From mutant plants, roots also produced more R-nornicotine, but the amount of R-nornicotine produced was lower compared with converter plants. No detectable radiolabeled nornicotine was recovered from leaf disks of mutant plants. No detectable radiolabeled nornicotine was recovered from either root or leaf tissues of RNAi plants. These results confirm our previous yeast work which showed E4 preferentially demethylated 2'-R-nicotine. Our results also suggest that E4 is responsible for most, if not all, nicotine demethylation in leaf tissue, and in roots there are other nicotine demethylase activities besides E4 and E5, which also has preference for 2'-R-nicotine compared with 2'-S-nicotine.

9:50 AM ***Break***

10:20 AM **TUESDAY**

46. DESIGN CONSIDERATIONS FOR YIELD IN USE STUDIES. Paul R. NELSON; R.J. Reynolds Tobacco Co, Winston-Salem, NC

R.J. Reynolds has incorporated yield in use (YIU) measurement in three large-scale studies (>800 subjects/study). A number of challenges have been identified that may be of interest to those contemplating commissioning of YIU and other consumer-based studies. The most significant problems are in the areas of subject recruitment (incorrect brand style, test product source) and sampling (uncollected filters, unusable filters).

Actual vs. self-reported consumption was compared between smokers who provided their own cigarettes and those who received *gratis* cigarettes. Provision of *gratis* cigarettes appears to have led to increased consumption, by as much as 36%, on the collection day. When collection kits allowed for collection of large numbers of used cigarettes many smokers have asked if they should fill the collector. To ensure collection of typical filters, it was important to emphasize to the participants that they should smoke normally.

Failure of smokers to collect all cigarette butts on the collection day (6-8% of subjects) and their collection of unusable butts (3-30+% of subjects) are other issues that arose during the course of studies. Failure to collect butts was addressed by providing subjects with a mechanism to self-report uncollected cigarettes. Final estimates of YIU per day have been adjusted by correction for uncollected and unusable cigarette filters.

Another problem encountered was the collection of an incorrect brand style. Unfamiliarity of study staff, many of whom are non-smokers, with subtle differences among cigarette brand-styles was the most likely cause of this problem. This issue may worsen with the removal of descriptors from product labels to comply with FDA regulations. Providing a pictorial guide to site staff for use during enrollment and identification of brand-style by

persons familiar with cigarette packaging prior to analysis are two solutions that have been identified to minimize the impact of brand-style misidentification.

10:40 AM TUESDAY

47. MENTHOL SMOKERS DO NOT ACHIEVE HIGHER CIGARETTE YIELDS: RESULTS FROM THREE YIELD IN USE STUDIES. Paul R. NELSON and Peter X. Chen; R.J. Reynolds Tobacco Co, Winston-Salem, NC

In recent years, R.J. Reynolds has conducted three national yield in use (YIU) studies involving a total of 2962 smokers of 64 different FSC and non-FSC brand styles. The data from those studies has been examined to determine whether menthol appears to influence yields of "tar" and nicotine achieved by smokers in their normal smoking environment.

Results from individual studies have been examined using a combination of regression analysis, one-way ANOVA and two-way ANOVA depending upon the data structure. In general, regression analysis has shown no relationship between menthol inclusion and YIU "tar" or nicotine. Two-way ANOVA, examining "tar" category and menthol as main factors, showed statistically significantly lower "tar" and nicotine from menthol cigarettes on a per-cigarette and per-day basis from one study. For the same study, one-way ANOVA within each "tar" band also showed that menthol was associated with statistically significantly lower "tar" and nicotine on a per-cigarette and per-day basis for lights cigarettes. No statistically significant differences were observed between full-flavor menthol and non-menthol cigarettes.

Two of the studies shared similar designs, and the results were pooled to increase statistical power. Once again, regression analysis showed no influence of menthol on YIU "tar" or nicotine. Two-way ANOVA ("tar" category and menthol as main factors) showed that menthol was statistically significantly associated with decreases in all YIU measurements. One-way ANOVA within "tar" category showed statistically significantly lower "tar" per-cigarette from menthol cigarettes for the full flavor and lights categories and in nicotine per-cigarette from the full flavor category.

Overall, there was no evidence that inclusion of menthol in a cigarette leads to greater "tar" or nicotine yields (per-cigarette or per-day), and some evidence that menthol smokers may generate less "tar" and nicotine from their cigarettes than non-menthol smokers.

11:00 AM TUESDAY

48. DETERMINANTS OF SELF-REPORTED ENERGY EFFICIENCY IN WOOD-BASED CURING PRACTICES AMONG GROWERS IN BRAZIL, TANZANIA AND UGANDA. Helmut GEIST[1] and Samuel Mugisha[2]; [1]University of Aberdeen, Department of Geography & Environment, Aberdeen, United Kingdom/Scotland, [2]Makerere University, Department of Zoology, Kampala, Uganda

Relating to a decrease in the environmental footprint of leaf production to meet anticipated requirements of Article 18 of the Framework Convention on Tobacco Control (FCTC), companies with agricultural supply chains are targeted by their investors for management

of risks related to biodiversity and ecosystem services. In the search for a standardised, internationally comparative system of evaluation and monitoring, self-reported energy efficiency in wood-based flue-curing systems turns out to be cost-effective and reliable if compared to other approaches such as direct or laboratory measurements. Insights are presented from surveys carried out in the 2007-2009 period in major operational areas where wood-based flue-curing dominates the cropping pattern (southern Brazil, northwest Uganda, central Tanzania). A common data protocol was applied with a limited set of basic questions (source of wood, type of trees, amount of wood, quantification of consumption per unit weight of cured tobacco). The Specific Fuelwood Consumption (SFC), i.e., kg of wood/1 kg of tobacco), was found to range from as low as 3 (Brazil) to as high as 13 (Tanzania) and 17 (Uganda). The range is related to differential determinants such as farmer's experience, curing technology, source availability, self-owned/shared barn, and indigenous/exotic tree species used. Farmers' self-reported data will be discussed in relation to other study results and databases such as those of the Natural Value Initiative (NVI), The Economics of Ecosystems and Biodiversity (TEEB), Forest Footprint Disclosure Project (FFDP), and the Cooperation Centre's for Scientific Research Relative to Tobacco (CORESTA) Task Force on curing technology.

11:20 AM TUESDAY

49. THEORETICAL APPROACH FOR PREDICTING PLASTICIZED FIRMNESS OF CELLULOSE ACETATE FILTER RODS. Kevin NORFLEET; Celanese Acetate LLC, Narrows, VA

The firmness of a cellulose acetate filter rod depends on a variety of factors that make predicting firmness challenging. Obvious parameters such as time and plasticizer application play a significant role but variables such as circumference, denier per filament, total denier and weight also impact filter firmness. Adding to the challenge, firmness exhibits highly non-linear behaviors that make predictions using standard least-squares regressions difficult and often highly limited in effective range. Celanese has developed an improved model for predicting the effect of plasticizer and time on dry filter rod firmness across the range of commercial filters, from super-slim to standard sizes. Experimental data and previous studies have shown that firmness increases in a logarithmic fashion with time after plasticizer is applied. Other work has shown that varying plasticizer results in a quadratic relationship between firmness and plasticizer level. This study combines the separate equations using experimental work and mathematical derivations to develop a highly accurate equation form for predicting firmness as a function of time and plasticizer level.

11:40 AM ***Lunch***

TUESDAY AFTERNOON, OCTOBER 5, 2010

SESSION A ***Session Chair: Edward Robinson***

1:30 PM TUESDAY

50. ESTIMATION OF THE RETENTION OF MENTHOL IN THE RESPIRATORY TRACT OF SMOKERS. Melissa HAGAN HUGHES, Kyle Lott and J. Daniel Heck; Lorillard Tobacco Company, A.W. Spears Research Center, Greensboro, NC

Estimating the respiratory tract retention of cigarette smoke-delivered menthol would be informative in the development of calculations that provide a realistic perspective on the effect that menthol may have in smokers. A small sample of 15 smokers participated in a study designed to determine the efficiency with which menthol is retained in the lung. The mean age of the smokers was 34.4 years and the test cigarette delivered 13.3 mg tar. Carbon monoxide levels were measured before the smoke session to confirm their smoking status (Mean ± SD: 18.1 ppm ± 1.9 ppm). Menthol, nicotine, and solanesol were collected and analyzed from each participant. For menthol, an average of 0.250 mg/cig was retained (94%); for nicotine an average of 0.491 mg/cig was retained (96%) and for solanesol an average of 0.180 mg/cig (59%) was retained. The values for the retention of nicotine and solanesol by smokers in this study were in agreement with the few reported results in the literature. Since this is one of the first studies to examine menthol lung retention in human smokers, the present study extends the knowledge on lung retention of smoke constituents.

1:50 PM TUESDAY

51. CHRONIC CIGARETTE SMOKE EXPOSURE INDUCES VASCULAR DYSFUNCTION THROUGH UPREGULATION OF CYTOGLOBIN IN MOUSE AORTA. Mohamed A. EL-MAHDY, Gamal A. El-Sherbiny, Tamer M. Abdelghany and Jay L. Zweier; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

Cigarette smoking is a risk factor for cardiovascular disease. The relationship between smoking and cardiovascular disease results from multiple mechanisms that interact to contribute to several pathological conditions, including vascular dysfunction. In chronic smokers, reduced endothelial-derived nitric oxide (NO) bioactivity and impairment of endothelium-dependent vasodilation have been shown to be associated with cardiovascular disease risk factors. Here, we tested the hypothesis that cytoglobin, a newly discovered globin that degrades NO, plays a role in the regulation of NO bioactivity in aortas of cigarette smoke-exposed mice. Male C57BL/6 mice were exposed for a period of 48 weeks to the whole body mainstream and the side stream cigarette smoke generated from 3R4F reference research cigarettes using the TE-10 cigarette smoking machine (Teague Enterprises, California). Chronic exposure of C57BL/6 mice to cigarette smoke resulted in elevation of the blood pressure in a time dependent manner. Such an effect was accompanied with impairment of acetylcholine-induced vascular response. Electrochemical measurement of NO revealed that the decay of NO was faster in smoke-exposed mice compared to non-exposed controls. Interestingly, immunohistochemical analysis of cytoglobin in mouse aortas showed that cytoglobin expression was higher in the smoke-exposed mice compared to the controls.

Moreover, these data were supported by immunoblotting analysis of cytoglobin in aortic tissue homogenate. In conclusion, the data suggest that chronic cigarette smoke exposure induces vascular dysfunction and faster rate of NO decay, in part, through the upregulation of cytoglobin. Our study provides an important insight toward understanding how smoking contributes to the genesis of cardiovascular disease.

2:10 PM TUESDAY

52. CHRONIC CIGARETTE SMOKE EXPOSURE IMPAIRS VASCULAR ENDOTHELIAL FUNCTION THROUGH A TETRAHYDROBIOPTERIN-DEPENDENT MECHANISM. Tamer M. ABDELGHANY, Gamal A. El-Sherbiny, Mohamed A. El-Mahdy and Jay L. Zweier; Center for Environmental and Smoking Induced Diseases, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH

Cigarette smoke exposure (CSE) is one of the major risk factors of cardiovascular disease. Although tobacco smoking is associated with the onset of atherosclerosis, coronary disease, and ischemic heart disease, the precise mechanism by which these deleterious effects occur is not fully elucidated. In the current study, we provide evidence that CSE greatly impairs vascular endothelial function and induces cardiovascular changes in C57BL/6 male mice. Animals were exposed for a period of up to 48 weeks to the whole body mainstream and the side stream cigarette smoke generated from 3R4F reference research cigarettes using the TE-10 cigarette smoking machine (Teague Enterprises, California). HPLC analysis revealed a significant decrease in tetrahydrobiopterin (BH4) level following CSE, along with a decrease in the expression levels of endothelial nitric oxide synthase (eNOS) and its Ser^{1177} phosphorylated (activated) form in the aorta of smoke-exposed mice. Consistently, immunohistochemical analysis showed a clear decrease in eNOS expression in the endothelium of aorta from smoke-exposed mice compared to controls. Smoke-exposed mice also showed overexpression of NADPH oxidase, giving rise to reactive oxygen species (ROS) production and inducing BH4 depletion. CSE impaired acetylcholine-induced vascular relaxation and induced persistent hypertension and cardiac hypertrophy in smoke-exposed mice. Our data indicate that CSE decreases BH4 bioavailability, resulting in eNOS uncoupling, which triggers ROS generation and decreased NO production leading to vascular endothelial dysfunction, hypertension and cardiac hypertrophy. Overall, our study provides important insights toward understanding how smoking contributes to the genesis of cardiovascular disease.

2:30 PM TUESDAY

53. MOLECULAR AND CELLULAR RESPONSE OF ORAL CAVITY CELLS TO TOBACCO PREPARATIONS. Wolfgang ZACHARIAS[1], Hong Gao[1], Prasad and Gaddamanugu L[2]; [1]Dept. of Medicine, J.G. Brown Cancer Univ. of Louisville, Louisville KY, [2]RJ Reynolds Tobacco Co., R&D, Winston-Salem, NC

To examine the effects of standardized (reference) tobacco preparations on human oral cavity cells, two oral squamous cell carcinoma cell lines (101A and 101B) and normal gingival epithelial cells (HGEC) were treated with standardized cigarette smoke condensate (CSC), smokeless tobacco extracted with complete artificial saliva (ST/CAS), or whole-smoke conditioned media (WS-CM; 20%, 2 cigarettes/10 ml media). EC-50 values, as

determined using sulforhodamine B assays, varied among the cell types and agents. When normalized to nicotine content, cytotoxicity for WS-CM and CSC appears to be higher compared to that observed with ST/CAS.

For mechanistic analysis, activation of pro-apoptotic caspase-3, caspase-8 and caspase-9 were examined in all cell types at their respective EC-50 doses for the three agents. CSC significantly activated caspase-3 in all three cell types, whereas caspase-8 and caspase-9 activities did not significantly increase. Neither ST/CAS nor 20% WS-CM stimulated activation of caspase-3, caspase-8, or caspase-9. Data will also be presented on the effects of CSC, ST/CAS, and WS-CM on motility and invasiveness of exposed oral cavity cells using Matrigel invasion chambers, and on activation of endogenous proteases (lysosomal cathepsins and metalloproteases MMP-2 and MMP-9). High-density microarray-based expression profiling was used to identify any gene expression patterns that may be altered in either tumor cells (101A, 101B) or non-malignant control cells (HGEC) after treatment with these tobacco preparations.

These studies characterize any differential molecular and cellular responses of normal and malignant oral cells after exposure to CSC, ST/CAS, or WS-CM, and will assist in identifying novel biomarkers for tobacco smoke exposure in the oral cavity.

2:50 PM ***Break***

3:20 PM **TUESDAY**

54. BANDS PRINTED ON THE OUTSIDE OF CIGARETTES. Vladimir HAMPL, JR.; Schweitzer-Mauduit, International, Alpharetta, GA

Fire safety compliant cigarettes which utilize printing technology for cigarette papers typically have the polymeric solutions applied in bands to the wire side of cigarette paper, which is facing the tobacco. The main reason is to keep the bands less visible to the consumer. However, there are some advantages (as well as disadvantages) to having the printed bands on the outside of the cigarette.

During the manufacture of cigarette papers, water is removed from the bottom side, called wire side. This one-sided water removal of water creates an uneven distribution of filler particles, fibers and air spaces through the thickness of the cigarette paper. The opposite side, called top or felt side, has a greater fraction of filler particles, fiber fines and small pores. In a burning cigarette the ash curls toward the wire side and therefore the wire side is the preferred side to have facing the tobacco column and hold-in the coal. When bands are on the outside of the cigarette, they are slightly farther away from the coal than when they are on the inside; and are also somewhat insulated by the cigarette paper. Hence, the bands are exposed to less heat from the burning coal and their efficacy is improved, which results in higher pass rate in the ASTM test.

This presentation contrasts some of the differences in both band properties and cigarette performance relative to alginate bands being on the wire side vs. top side; including ASTM performance, carbon monoxide deliveries, porosity and diffusivity in the band region and uniformity of bands. Same base papers were printed under the same process conditions on

the wire and also top side. The band properties were evaluated. Cigarettes made with these papers were tested in the ASTM test and for deliveries. In addition to higher pass rate in the ASTM test, other benefits observed with alginate bands on the top side of cigarette paper were higher band porosity, less variable bands, and slightly lower CO deliveries.

3:40 PM TUESDAY

55. INFLUENCE OF HUMIDITY, NUMBER OF FILTER PAPERS, AND ORIENTATION OF THE FILTER PAPER ON ASTM TEST RESULTS. Joseph WANNA; Schweitzer Mauduit Int., Alpharetta, GA

The ASTM test method E-2187 specifies conditioning cigarettes and filter paper at 55 ± 5% RH and a temperature of 23 ± 3ºC for at least 24 hours prior to testing. The recent version of the ASTM test method (E2187-09) also specifies the orientation of the filter paper that the cigarette is placed on, the rough side. All LIP standards established in many countries use the ASTM test method and specify no more than 25% full length burns on 10 layers of Whatman #2 filter paper. Very little if any data is available on the influence of relative humidity on the test results and recent modification specifies the orientation of the paper filter. This paper will provide an update on the changes to the ASTM test method, explore the influence of changes in relative humidity in increments of 5% on 10 and 3 layers of filter paper, and test the impact of filter paper surface orientation. Two cigarette brands were identified to perform this study. According to the ASTM test method one of the brands gave 20% and the second 15% full length burns on 10 layers of filter paper. Physical properties of another filter paper will be compared to those of Whatman filter paper #2 and with ASTM results.

4:00 PM ADJOURN

TUESDAY AFTERNOON, OCTOBER 5 2010

SESSION B ***Session Chair: Ray Robertson***

1:30 PM TUESDAY

56. TOBACCO COMPOSITION I: A QUANTITATIVE CHEMICAL COMPOSITION OF TOBACCO IN 2008, FROM A NUTRITIONAL PERSPECTIVE. M. F. DUBE, W. M. Coleman, III, D. M. Lawson and W. T. Morgan; R.J. Reynolds Tobacco Company, Winston-Salem, NC

A definitive quantitative breakdown of the components of tobacco is provided. Specific quantitative analyses were completed on a range of tobaccos, including, for example, flue-cured, burley and oriental tobaccos. The data provides a comprehensive chemical breakdown of the chemical constituents comprising a range of tobaccos in 2008. Standard nutritional analyses were also completed. Chemical differences, on a molecular level, between tobacco types are clearly evident and discussed. Most importantly, mass balances based on these quantitative assessments were consistently greater than 90%. When possible, direct analyte comparisons were made between historical data and data produced within this study. In a number of cases indications as to significant changes in average values were noted, for example, changes in average nicotine, sugars and acids.

1:50 PM TUESDAY

57. TOBACCO COMPOSITION II: A QUANTITATIVE CHEMICAL COMPOSITION OF 2008 GREEN AND CURED BURLEY AND FLUE CURED TOBACCOS. D. M. LAWSON, M. F. Dube, W. M., Coleman, III and W. T. Morgan; R.J. Reynolds Tobacco Company, Winston-Salem, NC

This second tobacco composition study provides a definitive quantitative breakdown of the components of tobacco both green and cured. Specific analytical analyses were completed on a range of green and cured burley and flue-cured tobaccos by internal and external laboratories. The data provides a comprehensive chemical breakdown of the chemical constituents comprising a range of tobaccos for crop year 2008. Chemical differences between tobacco types and as a result of curing were clearly evident. Most importantly, mass balances consistently greater than 90% are possible. In addition, notable differences in composition were noted between the tobaccos reported herein and the historical values describing the tobacco composition.

2:10 PM TUESDAY

58. TOBACCO COMPOSITION III: A QUANTITATIVE CHEMICAL COMPOSITION OF TOBACCO 2008, IMPACT OF STALK POSITION AND GROWING REGIONS. W.M. COLEMAN, III, M. F. Dube, D.M. Lawson and W. T. Morgan; R.J. Reynolds Tobacco Company, Winston-Salem, NC

This third tobacco composition study provides a definitive quantitative breakdown of the components of tobacco both green and cured with special emphasis on the impact of

stalk position and growing regions. Specific analytical analyses were completed on a range of green and cured burley and flue-cured tobaccos by internal and external laboratories. The data provides a comprehensive chemical breakdown of the chemical constituents comprising a range of tobaccos for crop year 2008. Chemical differences between tobacco types, and stalk position were clearly evident. Most importantly, mass balances consistently greater than 90% are possible.

2:30 PM TUESDAY

59. PROFILING OF GLYCERIDES IN TOBACCO SEEDS. Serban MOLDOVEANU and Yiping Chang; R.J. Reynolds Tobacco Co, Winston-Salem, NC

Tobacco seeds from several tobacco species (*N. tabacum, N. rustica, N. langsdorfii, N. suaveolens, N. sylvestris, N. glutinosa,* and *N. alata*), and from three types of *N. tabacum* (flue-cured, burley, and oriental) were extracted with hexane using an accelerated solvent extraction system (ASE). The instrument was an ASE 350 (Dionex Corporation, Sunnyvale, CA 94085) and the extraction was performed at 105° C in three cycles of 10 min each. The oils from the extracts were analyzed by two techniques, one to generate a chromatographic profile of the glycerides, and another to profile the fatty acids from these triglycerides. The glyceride profile was obtained using a GC/MS instrument (Agilent 7890A/5975 system, Wilmington, DE 19808) equipped with a cold on column injection port, a Varian CP-TAB column (Varian, Walnut Creek, CA 94598), and using H2 as a carrier gas. The fatty acid profile was obtained as trimethylsilyl derivatives of each acid. For this purpose, 0.1 to 0.3 mg oil was hydrolyzed with KOH in ethanol. The resulting mixture was neutralized, extracted with CH_2Cl_2, derivatized to trimethylsilyl esters, and analyzed by GC/MS with the GC equipped with a DB-5 MS column. The quantitations were performed based on peak areas and using calibration curves. The oil yields from flue-cured tobacco seeds were around 38% which is close to the expected level of oil in tobacco seeds. The oil consisted mainly of trilinolein (about 68%), palmito-di-linolein (about 16%), triolein (about 14%), di-palmito-linolein (about 1%), tripalmitin (less than 1%), and a small proportion of tristearin. The oils from the other tobacco types or species had basically similar qualitative composition, but the proportion of different constituents showed some variations. The results provide additional information on tobacco seed oils, enhancing the previous knowledge published regarding this subject.

2:50 PM ***Break***

3:20 PM TUESDAY

60. THE INFLUENCE OF CARBON ON SELECTIVE REMOVAL OF PHENOLIC COMPOUNDS. Jeremy K. STEACH and Denise Fisher-Jones; Eastman Chemical Company, Kingsport, TN

Selective filtration is a unique property of cellulose acetate filters. The selective removal of phenolic compounds (phenol, catechol, and cresols) has been detailed for mono acetate filters. Also, the selective removal was determined to be enhanced with the addition of plasticizer to the filters. Although selective removal has been extensively evaluated for

mono-acetate filters, the addition of filter additives and their effect on selective removal have not been studied extensively.

For this experiment, carbon was selected for the study of the effect of an additive on selective removal, specifically of phenolic compounds. Three different types of filters were used for this experiment, which were dalmation (carbon on cellulose acetate), cavity, and mono-acetate filters. The delivery and selective removal of the phenolic compounds were monitored over time to determine aging effects for each filter type. It was determined that the use of carbon as an additive in filters does not impact the selective removal of phenolic compounds from the mainstream cigarette smoke. It does impact the enhancement that triacetin has on selective removal and is dependent upon the type of filter type. Dalmation and cavity filters displayed different removal efficiencies for phenolic compounds.

3:40 PM TUESDAY

61. FREE FATTY ACID COMPLEXATION WITH IRON IN CIGARETTE SMOKE. Florian R. PERINI and Edward A. Robinson; Lorillard Tobacco Company Greensboro, NC

It has been previously reported (Qian *et al. Am J Pathol.* 1991) that both organic extracts of cigarette smoke and free fatty acids (FFA) can transfer ferrous iron into isolated red cell membranes and human red blood cells. Iron alone was nontoxic and palmitic acid alone was minimally cytotoxic to human fibroblasts. The two species together result in 100% cell death (Gao *et al. Free Radical Biology and Medicine* 2006). In this study, the behavior of FFA (palmitic, stearic, myristic, oleic and linoleic) was explored with iron to determine whether species were formed from the reaction between FFA and Fe that could lead to intracellular iron translocation and oxidative injury. Complex formation was tested by cyclic voltammetry (CV), a novel method as applied to smoke, by thin layer chromatography (TLC) and ultraviolet/visible (UV/vis) spectroscopy. Evidence for complex formation was provided by the appearance of stable, distinctive CV peaks for each complex, which were not found in the individual FFA or the Fe salt. Furthermore, TLC revealed a new spot detected by iodine vapor for each complex, and UV/vis showed the appearance of a new absorbance peak associated with complex formation. Whole mainstream smoke also was spiked with FFAs and/or Fe salts to establish whether the putative Fe-FFA complexes were formed in smoke. When CV was applied to smoke samples, comparable peaks, which were enhanced by the Fe spike, appeared in the voltammogram for the smoke alone. These results are consistent with the formation of Fe-FFA complexes, which may play a role in the biological damage produced by cigarette smoke.

4:00 PM TUESDAY

62. INVESTIGATIONS ON FACTORS INFLUENCING THE MOISTURE RETENTION PROPERTIES OF TOBACCO. Shitong ZENG, Jun Hu, Yang Liu, Chuanchuan Gao, Xinliang Bai and Mingyue Zhao; Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan, China

To investigate the factors influencing the moisture retention property of tobacco, we tested the moisture retention property of different tobacco types following epicuticular wax removal and CO_2 super-critical extraction. Chemical components of tobacco, epicuticular

wax and CO_2 super-critical extracts were analyzed by Continuous Flow Analysis and GC/MS. The microstructure of tobacco leaves were observed by TEM. The influence of sucrose, fructose and glucose on moisture retention property of tobacco was investigated by adding them to cut tobacco. The results indicated that the moisture retention property varied by tobacco types due to their chemical and structure differences. Compared to flue-cued and oriental tobacco, burley tobacco had a better moisture retention property despite of lower sugar content, maybe because of its unique microstructure. The degradation of moisture retention property of tobacco following scrubbing the leaves with methylene chloride confirmed that the epicuticular wax of tobacco played a crucial role in moisture retention. After CO_2 super-critical extraction, the moisture retention property of tobacco in low humidity condition was enhanced, despite the decrease in epicuticular wax, demonstrating that a change in structure was the determinant of the variation in the moisture retention property. Water soluble sugars had a positive effect on the moisture retention property under low humidity conditions, but they exerted a negative effect under high humidity conditions.

4:20 PM ADJOURN

WEDNESDAY MORNING, OCTOBER 6, 2010

COMBINED SESSION ***Session Chair: Florian Perini***

8:30 AM WEDNESDAY

63. METABOLOMIC STUDIES WITH URINE OF SMOKERS AND NONSMOKERS.
Gerhard SCHERER, Gerhard Gilch and Wibke Peters; ABF Analytisch-Biologisches Forschungs-labor GmbH, Muenchen, Germany

The urinary metabolome represents the entity of all 'small' compounds (molecular weight < 1000 Da) in this biological matrix. The metabolites can be of both endogenous and exogenous origin. Differences in the metabolome between well defined groups of individuals allow the identification of biomarkers of exposure and effect, responsible for these differences. We started our investigations with a manageable part of the urinary metabolome, namely the volatile compounds. As an analytical platform, we used headspace-solid-phase-microextraction (HS-SPME)-GC-MS. For further substance identification purposes, GC combined with time of flight (TOF) MS was also applied. Forty (40) urine samples (derived from 20 smokers, 20 nonsmokers) were analyzed by this method under acidic and basic conditions. Peak finding and alignment procedures yielded in total about 1000 signals (mass fragments), characterized by m/z-values, retention times and intensities. Statistical methods such as t-test and partial least square discriminant analysis (PLS-DA) of the data set revealed significant differences between the groups of smokers and nonsmokers, which were basically caused by 100 – 150 signals. About 40 compounds, mostly smoke components and flavoring agents, meaningful for the observed differences, were identified by comparisons to reference mass spectra libraries. Presently we extend our studies to non-volatile urinary metabolites by applying GC-MS to derivatized urine extracts. Taken together, the results of our feasibility study demonstrate that metabolomic investigations are potentially powerful tools for identifying smoking- and tobacco use-related biomarkers of exposure and effect. Further applications of this methodology for tobacco product evaluation purposes will be also briefly discussed.

8:50 AM WEDNESDAY

64. SENSITIVE METHOD FOR THE DETERMINATION OF ACRYLAMIDE IN TOBACCO FILLER AND ALTERNATIVE TOBACCO PRODUCTS BY UPLC-MS/MS.
Tianrong CHENG[1], Nancy Qian[1], Mary Dennis[1], Serban C. Moldoveanu[2] and Anthony R. Gerardi[2]; [1]Lancaster Laboratories Inc., Lancaster, PA, [2]R. J. Reynolds Tobacco Co., Winston-Salem, NC

A sensitive method for the analysis of acrylamide in tobacco cut filler and various alternative tobacco products was developed, optimized and fully validated. The method LOD was 1ng/mL (S/N = 3.7) and LOQ was 2.5 ng/mL (S/N=10.5). The method had a linear range with R^2 of 0.9990 from 1 ng/mL to 500 ng/mL. The method was developed using an Acquity UPLC system coupled with an API 5000 MS/MS, monitoring the transition m/z 72 to 55 for acrylamide and m/z 75 to 58 for $^{13}C_3$-acrylamide in electrospray ionization mode. $^{13}C_3$-Acrylamide was used as an internal standard. The column used for the separation was an Acquity RP18 BEH Shield 1.7um, 2.1mm x 100mm and the separation was obtained using

isocratic conditions water/MeOH 98/2 (v/v). The sample preparation involved the removal of interference from valine that is present in tobacco as well as reported in food. Valine has a very similar chromatographic behavior to acrylamide, and its removal enhanced baseline separation and improved sensitivity. Careful selection of the solvent injected with the sample is critical for obtaining a good peak shape in this method. This method enabled the accurate measurement of acrylamide in tobacco cut filler and alternative tobacco products at levels as low as 25ng/g of material. The method was applied to 3R4F cut filler, tobacco blends and alternative tobacco products.

9:10 AM WEDNESDAY

65. LIMITATIONS IN THE CARACTERISATION OF THE CIGARETTE BRANDS USING DIFFERENT MACHINE SMOKING REGIMES. Valerie TROUDE[1], Stephen W. Purkis[2], Gerald Duputie[1], Christian Teissier[1] and Benedicte Varignon[1]; [1]SEITA Imperial Tobacco Group, Fleury-les-Aubrais France, [2]Imperial Tobacco Limited, Southville, Bristol UK

Public health representatives have proposed that the intense regime mandated for testing in Canada with 100% vent blocking, should be used for product characterisation. However the conditions generated in the cigarette during such intense machine smoking do not fit well with most human smoking as shown from this study on ventilated (50%) "3 mg" and unventilated "12 mg" ISO tar yielding cigarettes.

Cigarettes were smoked by four machine smoking regimes (ISO, Massachusetts, ISO Working Group 9 Option B and Canadian) and by machine-duplicating human smoking (Sodim DFC D87) from data on 30 smokers of each cigarette type. The puffing conditions of the "average smoker" under laboratory conditions were selected using statistical tools (kernel density computing) and shown to be equivalent to the 90th percentile when the studied smokers smoked under natural conditions (Yield-in-Use protocol by the analysis of spent filters from human-smoked cigarettes).
In this study we show that machine smoking particularly the Canadian regime with the 100% vent blocking does not well reflect how smokers modify their behaviour, on a per puff basis, reducing their smoking intensity in response to increases in draw resistance, smoke concentrations and smoke temperatures.

In fact the Canadian regime gives neither smoke temperature nor pressure drop values which were readily achieved by most human smokers.

9:30 AM WEDNESDAY

66. A STUDY FOR THE IDENTIFICATION OF HYDROGEN PEROXIDE PRECURSORS BY FRACTIONATION OF THE AQUEOUS EXTRACT OF PARTICULATE-PHASE CIGARETTE SMOKE. Yuichiro TAKANAMI; Japan Tobacco Inc, Yokohama, Kanagawa, Japan

It is well known that an aqueous extract of cigarette smoke generates reactive oxygen species. The method of analyzing hydrogen peroxide in smoke extract has been reported at TSRC2008, and a mechanism for the generation of those species has been proposed at

TSRC2009. These studies suggested that the hydrogen peroxide accumulated in the extract is an important precursor for the generation of hydroxyl radicals. To elucidate the origin of hydrogen peroxide in cigarette smoke, the particulate phase of the smoke was extracted and the extract was fractionated with reverse-phase HPLC under acidic conditions. After the pH of the fractions was adjusted to basic values, hydrogen peroxide was detected not only in the fractions containing hydroquinone and catechol but also in fractions containing other components. These components were eluted using longer retention times than those applied for hydroquinone and catechol. The concentration of phenolic compounds in the fractions was evaluated by a colorimetric test. The absorbance at 750nm in the test and the yield of hydrogen peroxide showed a positive correlation. These results support the idea that phenolic components are possible precursors of hydrogen peroxide; precursors other than hydroquinone and catechol would be more hydrophobic than the two components.

9:50 AM ***Break***

10:20 AM **WEDNESDAY**

67. DELIVERIES OF SMOKE CONSTITUENTS FROM CHARCOAL FILTER CIGARETTES WHEN SMOKED WITH VARYING INTENSITIES. Peter JOZA, William Rickert and Wendy Wagstaff; Labstat International ULC, Kitchener, ON Canada

The addition of charcoal to cigarette filters has been reported to reduce concentrations of many volatile compounds found in cigarette smoke. The purpose of this investigation was to evaluate the effectiveness of charcoal addition on the yields of Hoffman analytes when cigarettes are smoked under multiple regimes. Seven Canadian products with charcoal filters (CFC), along with 3 acetate filter cigarettes (CAC), were evaluated under 6 smoking conditions; puff volume (25, 35, 45, 55mL), puff frequency (30, 50, 60 seconds), vent blocking (0, 100%). Charcoal in the filters varied from 0.05 to 0.09mg/mm^3. Tip ventilation, pressure drop and paper porosity were also measured in order to identify additional factors important in filter effectiveness.

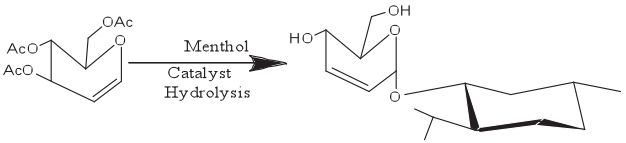
A comparison of slopes from the smoke constituent yield plotted against the total volume of smoke was used to assess effectiveness. As expected, deliveries of Hoffmann analytes increased as the total smoke volume increased for both CFC and CAC. For most of the volatiles examined, increased charcoal filter length and increased tip ventilation correlated with a lower concentration (yield/litre) and were found to be significant factors in filter effectiveness. In a comparison of CFC's versus CAC's, it was found that yields of most compounds by a popular CAC were either similar or slightly increased compared with the highest yielding CFC and largely independent of smoking condition. When the same CAC was compared with the lowest yielding CFC, the yield ratio for a number of compounds, pyridine and styrene in particular, was not independent of smoking regime. Under the 'lightest' smoking condition, the CAC delivered 10 times more pyridine than the comparator CFC decreasing to a factor of 2 under intensive smoking (no vent blocking).

10:40 AM WEDNESDAY

68. GLYCOSIDES – FLAVOUR ENHANCERS IN SMOKE. Namasivayam PALANI, S. Vinutha, T.K. Dinesh, Manoj Kumar Singh, Soumitra Mukherjee and S.V. Dhalewadikar; ITC R&D Centre, Peenya, Bangalore, India

Glycosides in food materials have been extensively studied in recent years, and interest has been focused on their role as flavor precursors. Glycosidic components of tobacco leaves could also contribute to generating aroma and taste in the curing and aging process of tobacco. Sugars when combined with alcohols, phenols, polyphenols and sterols to form glycosides which are non volatile flavor precursors present in tobacco. These on pyrolysis release aglycones (flavor compounds) which enhances the smoke flavor.

Menthol, because of its inherent mint flavour and refreshing feeling, has been used as additives to medicines, tobaccos, foodstuffs etc. The volatility limited its wider application in the high temperature processing and it is overcome by converting menthol to its glycoside which is non volatile and stable. Menthyl glycoside was synthesized using Menthol and readily available triacetyl glucal.



The above menthyl glycoside was added into a cigarette and cooling effect due to menthol release was felt while smoking. We also synthesized other glycosides using viz Phenylethylalcohol, Citronellol and benzyl alcohol. These glycosides were added in the cigarette and they enhanced the smoke flavour. All these molecules are well characterized ^{1}HNMR and ^{13}C NMR. These glycosides gave a positive smoke flavor in the smoke.

11:00 AM WEDNESDAY

69. LEVEL IN TOBACCOS OF THREE NITROGENOUS COMPOUNDS (ADENOSINE, DEOXYFRUCTOSAZINE AND GLUCOSAMINE. S. C. MOLDOVEANU, A. R. Gerardi and C. H. Byrd; R.J. Reynolds Tobacco Co., Winston-Salem, NC

The knowledge on the level of nitrogenous compounds in tobacco is important since they may be precursors for undesirable toxicants in cigarette smoke. Besides alkaloids that are rather stable to heating, tobacco contains proteins, amino acids, and also other nitrogenous compounds such as deoxyfructosazines, glucosamine, adenosine, Amadori type compounds, Maillard browning polymers, etc. While alkaloids, proteins and amino acids are commonly analyzed in tobacco, the measurement of the level of the other nitrogenous compounds have received less attention. A LC/MS/MS procedure allowing a simple analysis of adenosine, deoxyfructosazine and glucosamine has been developed and applied to a variety of blended tobaccos from cigarettes, to single grade cured tobaccos, as well as to green (dry) tobacco. The separation of the compounds was performed on a Luna 3µ HILIC column (Phenomenex) in isocratic mode (with 78% CH_3CN, 22% water to which

was added 0.1% HCOOH and 0.154 g/L NH_4OOCCH_3). The detection was done using ES ionization and MRM mode for glucosamine (transition m/z 180 to 162) and for adenosine (transition m/z 268 to 136), while for deoxyfructosazines (2,5 and 2,6) the detection was done for ion m/z 305 without generating daughter ions. The glucosamine level was found to be as high as 1500 mg/g tobacco, and that of 2,5 and of 2,6-doxyfructosazines as high as 500 mg/g tobacco. Adenosine level in tobaccos was found to be around 25 mg/g.

11:20 AM WEDNESDAY

70. STUDY OF THE DETERMINATION OF LOSS OF TOBACCO FROM THE CIGARETTE ENDS USING VIBRATION. Feng Qian[1], Wu Xiaosong[2], Liang Wei[1], ZHAO Jijun[1], Zhang Long[2], Li Zhi-Gang[2] and Liu Yong[2]; [1]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China and [2]Anhui Institute of Optics and Fine Mechanics of CAS, Hefei, China

The loss of tobacco from the cigarette ends is an important quality index characterising the stability of the cigarette end. The two methods described in ISO3550 reflect the loss of tobacco from the cigarette ends in the different processes, But they can not fully reflect the current actual situation during the cigarette manufacturing, packaging processes, the distribution network, in the smoker's pocket and the quality of cigarette. Through the observation and analysis of the cigarette's state of motion in each section, this paper presents a new method of determination the loss of tobacco from the cigarette ends based on the vibration that can simulate the cigarette's motion state in the manufacturing, packaging, transportation and consumption process, reflect synthetically the situation of the loss of tobacco from the cigarette ends. This paper describes the schematic diagram and the technical parameters design of the device of determination the loss of tobacco from the ends using a vibro-bench, it was verified the effectiveness of the method of vibration by the test of blending design, the distribution process and the collaborative test.

11:40 AM ADJOURN

Author Index

Author Index
(Continued)

Author Index
(Continued)

ACKNOWLEDGEMENTS:

The Local Arrangements Committee of the 64th TSRC expresses its appreciation to our sponsors for providing generous support to the conference.
The participating organizations are:

Welcome Reception
Cerulean
Global Laboratory Services

Diamond Sponsors
Altria
Japan Tobacco
R.J. Reynolds Tobacco

Platinum Sponsors
Arista Laboratories
Covance
Hertz & Selck
Labstat International
Lorillard Tobacco
Tobacco Technology

Gold Sponsors
American Snuff
Celanese Acetate
delfortgroup
Eastman Chemical
Imperial Tobacco Canada
Mane
Mother Murphy's Laboratories
RBH
Santa Fe Natural Tobacco

Additional Financial Support
Astra Tobacco
Schweitzer-Mauduit International

64th TOBACCO SCIENCE RESEARCH CONFERENCE
OCTOBER 3-6, 2010
Hilton Head Island, South Carolina USA

Chairpersons
Linda Crumpler – *Cerulean*
Amy Walker – *Global Laboratory Services*

Editorial Committee
Balazs Siminszky – *Philip Morris International*
Ray Robertson – *Celanese Acetate*
Ed Robinson – *Lorillard Tobacco Company*

Policy Committee
Rob Stevens – *Lorillard Tobacco Company (64th Chair)*
John Bunch – *American Snuff Company (65th Chair)*
Rodney Schluter – *R.J. Reynolds Tobacco Company (66th Chair)*
Sean Kelly – *Schweitzer-Mauduit International (67th Chair)*
J. Michael Moore – *University of Georgia (68th Chair)*

Host Committee

Cerulean	*Global Laboratory Services*
Linda Crumpler	David Gillespie
Steve Frankham	Amy Walker

Conference Management
Global Connections

Golf Tournament
Buddy Brown
Mike Ogden
R.J. Reynolds Tobacco Company

65th TOBACCO SCIENCE RESEARCH CONFERENCE
SEPTEMBER 18-21, 2011
HYATT REGENCY HOTEL
LEXINGTON, KENTUCKY USA

Host:
University of Kentucky

www. tsrcinfo.com