PROGRAM BOOKLET AND ABSTRACTS

Volume 62

62nd Tobacco Science Research Conference

September 21-24, 2008
Nashville, Tennessee USA

Host:
U.S. Smokeless Tobacco Manufacturing Company

METROPOLITAN GOVERNMENT OF NASHVILLE AND DAVIDSON COUNTY

KARL F. DEAN
MAYOR

OFFICE OF THE MAYOR
METROPOLITAN COURTHOUSE
NASHVILLE, TENNESSEE 37201
PHONE: (615) 862-6000
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September 21, 2008

Dear Participants:

On behalf of Nashville, I want to welcome the participants of the 62nd Tobacco Science Research Conference to our great city. We are honored to host such an important gathering.

While you are here, I encourage you to take advantage of our city's diverse offerings. I am extremely proud of our brand as Music City, but you will quickly find we have more to offer than music venues. Downtown is home to an expanse of cultural activities and entertainment options from the historic to the state of the art.

It is my sincere wish that your visit with us is unique and that you enjoy the hospitality of our city. Thank you for coming to Nashville and being a part of this special conference. We hope to see you back many times in the future.

Sincerely,

Karl F. Dean
Mayor

GENERAL PROGRAM

Sunday, September 21, 2008

2:00 pm – 6:00 pm	Registration	Ballroom Level Desk
2:00 pm – 6:00 pm	Speaker Ready Room	Belmont
6:30 pm – 9:30 pm	Welcome Reception	Country Music Hall of Fame®
	Buses depart beginning 6:00 from main entrance of the hotel	
	Hosted by - U.S. Smokeless Tobacco Manufacturing Company	

Monday, September 22, 2008

7:30 am – 8:45 am	Session Chairs Breakfast	Oaklands
7:30 am – 8:45 am	TAG ISO Breakfast	Evergreen
7:30 am – 5:00 pm	Registration	Ballroom Level Desk
7:30 am – 5:00 pm	Speaker Ready Room	Belmont
8:30 am – 9:00 am	Morning Coffee	Ballroom Lobby
9:00 am – 11:45 am	Symposium	Hermitage Ballroom
	"Smokeless Tobacco – What's New in an Old Product?"	
	Chairman, Paul Nelson, R.J Reynolds Tobacco Company	
10:15 am – 10:45 am	Coffee Break	Ballroom Lobby
11:45 am – 1:00 pm	Lunch	McGavock's Ballroom
1:00 pm – 2:00 pm	Poster Session	Tulip Grove F
2:00 pm – 5:10 pm	Session A	Hermitage Ballroom
	Session B	Hermitage Ballroom
3:20pm – 3:50 pm	Coffee Break	Ballroom Lobby
5:15 pm – 7:00 pm	TITL Technical Advisory Board Meeting	Evergreen
5:15 pm – 7:00 pm	TSRC Analytical Methods Meeting	Two Rivers

Tuesday, September 23, 2008

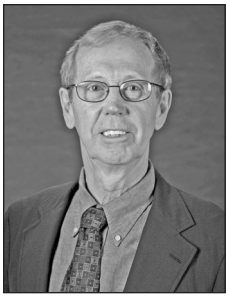
7:30 am – 8:45 am	Policy Committee Breakfast	Oaklands
7:30 am – 5:00 pm	Registration	Ballroom Level Desk
7:30 am – 5:00 pm	Speaker Ready Room	Belmont
8:30 am – 9:00 am	Morning Coffee	Ballroom Lobby
9:00 am – 12:10 pm	Session A	Hermitage Ballroom
	Session B	Hermitage Ballroom
10:20 am – 10:50 am	Coffee Break	Ballroom Lobby
11:50 am – 1:40 pm	Lunch	McGavock's Ballroom
12:10 pm – 1:30 pm	Tobacco Science Council Meeting	Oaklands
1:40 pm – 4:50 pm	Session A	Hermitage Ballroom
	Session B	Hermitage Ballroom
3:00 pm – 3:30 pm	Coffee Break	Ballroom Lobby
5:00 pm – 5:45 pm	TSRC Business Meeting	McGavock's B
6:30 pm – 7:15 pm	Social Hour	Ballroom Lobby
7:30 pm – 9:30 pm	Award Banquet	Plantation Ballroom

Wednesday, September 24, 2008

8:30 am – 9:00 am	Morning Coffee	Ballroom Lobby
8:00 am – 11:50 am	Speaker Ready Room	Belmont
9:00 am – 11:50 am	Combined Session	Hermitage Ballroom
10:00 am – 10:30 am	Coffee Break	Ballroom Lobby
11:50 am	Adjourn	

LIFETIME ACHIEVEMENT AWARD

Dr. Lowell P. Bush



Lowell Bush grew up on a farm in southwestern Minnesota near Russell where his mother and brothers are still actively involved in farming. He received a B.A. degree in biology from Macalester College and the M.S. and Ph.D. degrees in plant physiology from Iowa State University. In 1966, after a post-doctoral position in plant pathology at the University of Minnesota, he joined the Department of Plant and Soil Sciences (Agronomy Department) at the University of Kentucky where he conducted research on physiology of tobacco. Those efforts quickly focused on tobacco alkaloids and subsequently included alkaloids of tall fescue. Lowell has had sabbaticals to strengthen his alkaloid expertise with Dr. J.A.D. Jeffreys in the Pure and Applied Chemistry Department of Strathclyde University in Glasgow, Scotland and with Dr. Manuel do valle Ribeiro with Teagasc in Ireland.

Lowell not only conducted research on tobacco alkaloids but also taught plant biology and crop production courses at both undergraduate and graduate levels. Most recently he taught a required freshmen level course on "Issues in Agriculture Development". He has advised several undergraduate students, graduate students, and post-doctoral fellows as well as hosted many visiting scientists, including some on sabbatical leave. Among those studying in his laboratory have been seven CORESTA Study Grant Awardees who conducted research on tobacco alkaloids and TSNAs.

His early tobacco alkaloid research involved identification of the alkaloids in tobacco seeds, their metabolism and accumulation during germination and seedling establishment. This led to studies on enzymes involved in alkaloid biosynthesis and enzymes limiting alkaloid accumulation. Most recent results of these investigations have been the characterization of nicotine demethylase and the development of the LC (low converter) protocol. TSNA research determined a positive correlation between soil nitrogen fertility and accumulation of secondary amine alkaloids with TSNA accumulation. Source of nitrite formation for TSNA synthesis during curing was found to be of microfloral origin.

Lowell received the Award for Distinguished Achievement in Tobacco Science from TCRC in 1982. In 1987 he was appointed the Philip Morris Professor of plant biology at the University of Kentucky and has served on the Advisory Board of Beiträge Tabakforschung since 1998. In 2000 he was the recipient of the CORESTA Prize. Lowell was selected as a Fulbright Research Scholar to Ireland as well as Fellow of the American Society of Agronomy and Crop Science of America. He has served terms on the Policy and Program Committee's of TSRC, co-chaired three TSRC meetings and authored four TSRC Symposium presentations. Significant mentors have been T.C. Tso, James Chaplin, Inger Wahlberg and A.J. Hiatt.

Joan and Lowell have resided in Lexington for 42 years and have three children and five grandchildren.

62nd TOBACCO SCIENCE RESEARCH CONFERENCE

Monday Morning, September 22, 2008
Combined Session

9:00 **WELCOME:** Cliff Bennett, 62nd TSRC Chair

9:10 **SYMPOSIUM: "Smokeless Tobacco – What's New in an Old Product?"**
Chair: Paul Nelson

9:15 **1. SMOKELESS TOBACCO IN THE WESTERN WORLD: A JOURNEY FROM THE PAST TO THE PRESENT.** James A. STRICKLAND and Barbara B. Doonan, U.S. Smokeless Tobacco Company, Nashville, TN 37203

9:45 **2. TOBACCO HARM REDUCTION: AN ALTERNATIVE APPROACH TO SMOKING CONTROL.** Brad RODU, University of Louisville, Louisville, KY

10:15 **Break**

10:45 **3. DIFFERENTIATING THE HEALTH RISKS OF CATEGORIES OF TOBACCO PRODUCTS.** Michael C. FALK, Amy M. Brownawell, Fabiana F. de Moura, Robin S. Feldman, Heather E. Gorby, and Kara D. Lewis, Life Sciences Research Office (LSRO), 9650 Rockville Pike, Bethesda, MD

11:15 **4. ANALYTICAL CHALLENGES IN SMOKELESS TOBACCO.** James E. FRANKE and Cliff B. Bennett, U.S. Smokeless Tobacco Manufacturing Company, Nashville, TN 37203 USA

11:45 **Lunch**

1:00 **Posters**

5. A HIGHLY SENSITIVE AND SPECIFIC ANALYTICAL PROCEDURE FOR THE DETERMINATION OF A TOBACCO SPECIFIC NITROSAMINE, 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANOL IN HUMAN URINE USING MOLECULAR IMPRINTED POLYMER EXTRACTION, HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRIC DETECTION. Brian BAILEY, Covance Laboratories Ltd, Harrogate, North Yorkshire, HG3 1PY, UK

6. ANALYSIS OF FREE AMINO ACIDS IN CIGARETTE TOBACCO BY CAPILLARY ELECTROPHORESIS COUPLING WITH NOVEL MULTI-PHOTON EXCITED FLUORESCENCE DETECTION. Sheng CHEN, Dan Li, Wei Zhu, Long Huang, Tonglin Zhao, Xin Liu, Technology Center of Wuhan Tobacco Company, Shisheng Road, Hanyang District, Wuhan City, China, 430051

7. A RAPID METHOD FOR THE DETERMINATION OF MALEIC HYDRAZIDE IN TOBACCO. B.J. Rajesh, S.V. Dhalwadekar and S.K. MEHTA, ITC R&D Centre, Bangalore, India

8. FLAVOR PROFILING OF TOBACCO BY HEADSPACE TECHNIQUE USING GC-MS. S.K. MEHTA, Nalini Dilip Sangli, Raghu H.S., S.V. Dhalewadikar, ITC R&D Centre, Bangalore, India

9. A RAPID METHOD FOR QUANTIFICATION OF DICAMBA, 2,4-D AND 2,4,5-T IN TOBACCO BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. H.S. Raghu, A. Devaraj, T.K. Dinesh., N. PALANI and S.V.Dhalewadikar. Organic Chemistry Lab, ITC R&D Centre, Peenya, Bangalore-560058, India

10. SINGLE PHOTON IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY: QUANTIFICATION OF SELECTED HOFFMANN COMPOUNDS USING THE BORGWALDT LM2X-PHOTON-TOFMS. Ralf ZIMMERMANN, Helmholtz Zentrum Muenchen, Neuherberg, Germany, Stefan Mitschke, University of Augsburg, Germany, Nils Rose, Borgwaldt KC, Hamburg, Germany

11. A NEW METHOD FOR MEASURING THE APPARENT DENSITY OF SHRED AND VOID FRACTION IN A TOBACCO COLUMN. In-Hyeog OH, Han-Joo Chung, Byong-Kwon Jeh, Do-Young Ra, Dae-Keun Kwak, Byeoung-Ku Kim, Si-Hyung Jo and Moon-Soo Rhee, KT&G Central Research Institute, 302 Shinseong-Dong, Yuseong-Gu, Daejeon, 305-805 South Korea

12. DESIGN OF TOBACCO FLAVOR QUALITY CONTROLLING SOFTWARE. Xiong Guoxi, WANG Na, Si Hui, ZhuWei, Xiong Bin, LI Dan Technology Center of China Tobacco Hubei Industrial Corporation; No. 22, Road Shisheng, District Hanyang, Wuhan City, P.R. China, 430051

13. AN IMPROVED UV METHOD TO DETERMINE FILTRATION EFFICIENCY OF CELLULOSE ACETATE FILTERS. V. S. WILLIAMS, A.S. Watts and L. W. Renfro, Eastman Chemical Company, Kingsport, TN 37662

14. COMPARISON OF LOOSE SNUS AND POUCHED SNUS CONSUMPTION BEHAVIOUR IN SWEDEN. Helena DIGARD, Delcio Sandi and Rudi Hartmann, Group R&D, British American Tobacco, Southampton, SO15 8TL, UK

15. PROPOSAL FOR A STANDARDIZED METHOD FOR WATERPIPE SMOKING. Juergen HAHN, Chemisches und Veterinäruntersuchungsamt Sigmaringen, Sigmaringen Germany, Nils Rose, Borgwaldt KC GmbH, Hamburg, Germany

16. CHARACTERIZATION OF THE MAINSTREAM AND THE SIDESTREAM CIGARETTE SMOKE SIMULTANEOUSLY GENERATED USING AUTOMATED CIGARETTE SMOKE EXPOSURE SYSTEM. Vladimir MIKHEEV, Alec Hitchman, Ronald Rhoads, Tessa Oxford, K. Monica Lee, Bruce Westerberg, Battelle Toxicology Northwest, Richland, WA 99354 USA

17. TRAP MARKER STUDY OF THE CHARACTERISTIC AROMA OF ORIENTAL TOBACCO-LIKE IN N.TABACUM L. REN Min, Wang Rixin, Jia Xinghua, Fen Quanfu, Wang Shaomei, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao

17a. FACTORS AFFECTING THE EFFECTIVENESS OF SMOKE TRAPPING BY MEANS OF AN ELECTROSTATIC TRAP. Tim MASON and Ian Tindall, Cerulean, Milton Keynes, MK14 6LY United Kingdom

17b. INFLUENCE OF CURING PRACTICES ON TSNA PRODUCTION IN DARK FIRE-CURED TOBACCO. Andy BAILEY, University of Kentucky, Princeton, KY 42445, Bill Pitt and Barry Sims, University of Tennessee, Springfield, TN

Monday Afternoon, September 22, 2008

Session A
Session Chair: Balazs Siminszky

2:00 PM

18. BREEDING FOR REDUCED TSNA IN BURLEY TOBACCO. R.D. MILLER, University of Kentucky and University of Tennessee, Lexington, KY 40546-0312

2:20 PM

19. TOWARD DEVELOPMENT OF LOW NORNICOTINE DARK TOBACCO LINES THROUGH MUTAGENESIS. Yanxin SHEN, Dongmei Xu, David Norman, and Mark Nielsen, USSTC, Winchester, KY 40391

2:40 PM

20. TOBACCO NICOTINE DEMETHYLASE GENE DYSFUNCTION IS AN EFFECTIVE AND PRACTICAL MEANS OF REDUCING NORNICOTINE LEVELS IN TOBACCO. Dongmei XU, Yanxin Shen, David Norman, Marcos Lusso, Greg Davis, Frank Hart, Mingwu Cui and Mark Nielsen, USSTC, Winchester, KY 40391

Session B
Session Chair: Randy Hudson

2:00 PM

26. EVALUATION OF HPLC MOBILE PHASES AND EXTRACTING SOLUTIONS FOR THE DETERMINATION OF GLYCYRRHIZIC ACID IN LICORICE AND TOBACCO. Charles H. RISNER, R. J. Reynolds Tobacco Company, Winston-Salem, NC

2:20 PM

27. DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE DETERMINATION AND QUANTITATION OF HETEROCYCLIC AROMATIC AMINES (HAAs) IN MAINSTREAM SMOKE USING A SIMPLE EXTRACTION AND SAMPLE PREPARATION. Anthony GERARDI, RJ Reynolds Tobacco Company, Winston-Salem, NC

2:40 PM

28. DEVELOPMENT OF A METHOD FOR QUANTITATIVE ANALYSIS OF HYDROGEN PEROXIDE GENERATED FROM AQUEOUS EXTRACT OF CIGARETTE SMOKE. Yuichiro TAKANAMI, Takako Moriyama and Yasutaka Kosaka, Japan Tobacco Inc. 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan

Monday Afternoon, September 22, 2008

3:00 PM

21. THE VIRULENCE, BIOVARS AND PATHOTYPES OF RALSTONIA SOLANACEARUM IN TOBACCO PLANT IN YUNNAN PROVINCE, CHINA. Wang Min[1], LIU Yong[1], Li Meiyuan[1], Ji Guanhai[2], Li Yongping[1], [1]Yunnan Institute of Tobacco Science, China Tobacco Breeding Research Southern Center, Yuxi 653100, China; [2]College of Plant Protection, Yunnan Agriculture University, Kunming 650202, China

3:00 PM

29. PRODUCTIVITY IMPROVEMENTS THROUGH CHROMATOGRAPHY AND AUTOMATION FOR GC/TEA TSNA ANALYSIS OF TOBACCO LEAF. W. Eric HARRIS, Stephen Gibson, Jamie N. Finch, Frank Hart, Daniel Heltsley, Jennifer Johnson, Cecil Ray, and Thomas Thorburn, U. S. Smokeless Tobacco Manufacturing Company, Nashville, TN 37203 USA

3:20 PM BREAK

3:50 PM

30. REDUCING TSNAs IN AIR-CURED TOBACCO – BY WHAT MEASURE? Anne JACK, Neil Fannin, Xiaolong Li and Lowell Bush, University of Kentucky, Lexington, KY 40546 USA

3:50 PM

30. Moved to Session A.

31. SEPARATION AND DETERMINATION OF P-CRESOL AND M-CRESOL IN MAINSTREAM CIGARETTE SMOKE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC USING β-CYCLODEXTRIN AS MOBILE PHASE ADDITIVE. Qian-Rong PENG[1], Jie Zhang[2], Min Yang[2], Jian-Ling Xie[2], Zhong-Xiang Liu[1], Yuan-Qing Cai, [1]Technology Center of China Tobacco Guizhou Industry Company, Guiyang 550003, China; [2]Chemical Engineering Department, Guizhou University, Guiyang 550003, China

4:10 PM

22. EFFECTS OF CONTINUOUS MONO-CROPPING OF FLUE-CURED TOBACCO ON NITROGEN RELATIONS IN THE SOIL AND PLANT. PAN Wenjie, Tang Yuanju, Wang Maoshen, Cheng Yi, Xue Xiaoping, Guizhou Tobacco Research Institute, Guiyang, China

4:10 PM

32. DETERMINATION OF ALKALOID IN TOBACCO BY UPLC. LU Sheming, Ni Caoming, Yang Liu, Li Zhongchang, Wang Di, Miao Mingming, R&D Center of Hong Ta Tobacco Group Co., Ltd., Yuxi 653100, China

Monday Afternoon, September 22, 2008

4:30 PM

23. TRANSCRIPT REGULATION RELATED TO POTASSIUM UPTAKE GENES IN TOBACCO ROOTS. Zhaokui GUO, Xiuqing Wan, Peiqiang Yan, Heilongjiang Tobacco Institute, China

4:50 PM

24. CLONING AND SEQUENCING OF HELPER COMPONENT PROTEINASE GENE OF A NEW ISOLATE OF POTATO VIRUS Y. WANG Yanying[1], Huang Yingchun[2], Wang Fenglong[1], Chen Wansheng[1], Shen Lili[1], Gong Daping[1], [1]Tobacco Research Institute, Chinese Academy of Agricultural Science, Qingdao 2600101, China; [2]College of Biochemistry Engineering, Beijing United University, Beijing 1000023, China

5:10 PM

25. PHYLOGENETIC ANALYSIS OF MICROORGANISMS IN FLUE-CURED TOBACCO AND ITS RELATIONSHIP WITH BIO-ENZYMES. DUAN Yanqing[1], Wu Yi[1],Yang Jinkui[2], Li Qinghua[1], Zhang Kejin[2], [1]Hongyun Tobacco(Group) Co., Ltd.; [2]Laboratory for Conservation and Utilization of Bio-resources, Yunnan University

4:30 PM

33. TRANSFER OF SOME FATTY ACID FLAVORS IN CIGARETTE. CAI Junlan, Zhang Xiaobing, Zhao Xiaodong, Xie Jianping, and Liu Kejian, Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China

ADJOURN

Tuesday Morning, September 23, 2008

Session A
Session Chair: James Strickland

9:00 AM

34. APPLICATION OF THE DIRECT SILYLATION GC-MS SCAN TECHNIQUE TO REFERENCE SMOKELESS TOBACCO PRODUCTS. John H. LAUTERBACH, Lauterbach & Associates, LLC, Macon, GA, USA and Deborah A. Grimm, Tulane University Coordinated Instrumentation Facility, New Orleans, LA

9:20 AM

35. DETERMINATION OF ACRYLAMIDE IN TOBACCO SMOKE AND SMOKELESS TOBACCO PRODUCTS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY. Jingcun WU, Peter Joza, Bill Rickert, Labstat International ULC, Kitchener ON Canada

9:40 AM

36. MARKET SURVEY OF CHEMICAL CHARACTERISTICS OF SMOKELESS TOBACCO PRODUCTS SOLD IN CANADA. Bill Rickert, Peter Joza, Wendy WAGSTAFF, Labstat International ULC., Kitchener ON Canada

Session B
Session Chair: Victor Little

9:00 AM

42. SIMULTANEOUS DETERMINATION OF 1,3-BUTADIENE, ETHYLENE OXIDE, VINYL CHLORIDE , PROPYLENE OXIDE, ACRYLONITRILE, BENZENE AND ISOPRENE IN MAINSTREAM VAPOR PHASE CIGARETTE SMOKE. Ji-Zhou DONG, R.J. Reynolds Tobacco, Winston-Salem, NC 27105 USA

9:20 AM

43. SELECTIVE DETECTION AND CLASSIFICATION OF COMPOUNDS IN TOBACCO SMOKE BY GCXGC-TOFMS. Donald C. HILTON, LECO Corporation, Fort Myers, FL and Jean-Marie D. Dimandja, Spelman College, Atlanta, GA

9:40 AM

44. DEVELOPMENT AND VALIDATION OF A METHOD FOR THE DETERMINATION OF 17 POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN MAINSTREAM TOBACCO SMOKE. J. Evan TARRANT, Ken Mills, Cynthia Williard, Lorillard Tobacco Company, Greensboro, NC 27420

Tuesday Morning, September 23, 2008

10:00 AM

37. QUANTIFICATION OF DMNA IN SNUS BY LC-MS/MS. Jasper VAN HEEMST, British American Tobacco, Group R&D, Southampton, SO15 8TL, UK

10:00 AM

45. THE STUDY ON THE GENERATION OF 17 POLYCYCLIC AROMATIC HYDROCARBONS DURING PUFFING. Shinya YOSHIDA and Yoji Uwano, Japan Tobacco Inc., Yokohama, Kanagawa, Japan

10:20 AM BREAK

10:50 AM

38. IN VITRO MICRONUCLEUS ASSAY FOR CIGARETTE SMOKE USING A WHOLE SMOKE EXPOSURE SYSTEM. Kosuke OKUWA, Yasuo Fukano and Tomoki Nishino, Japan Tobacco Inc. Tobacco Science Research Center, Yokohama, Kanagawa 227-8512, Japan

10:50 AM

46. FACTORS AFFECTING REPEATABILITY AND YIELD WHEN SMOKING BIDI CIGARETTES. Ian TINDALL and Tim Mason, Cerulean, Milton Keynes, MK14 6LY United Kingdom

47. Moved to poster session #17a.

11:10 AM

39. CONTRIBUTION OF FIVE NITROGEN-CONTAINING COMPOUNDS TO TPM MUTAGENIC POTENCIES IN SALMONELLA TYPHIMURIUM TA98. Yasunari OTSU, Toshiro Fukushima and Hideki Takahashi, Japan Tobacco Inc. Tobacco Science Research Center, Yokohama, Kanagawa 227-8512, Japan

11:10 AM

48. IS THERE PENTOBARBITAL IN TOBACCO? Brian E. Rood, Department of Chemistry, Mercer University, Macon, GA, and John H. LAUTERBACH, Lauterbach & Associates, LLC, Macon, GA USA

Tuesday Morning, September 23, 2008

11:30 AM

40. CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF BASE FRACTIONS DERIVED FROM CIGARETTE SMOKE CONDENSATE (CSC). Lijia YANG, Larry T. Taylor, Virginia Tech, Blacksburg, VA 24061-0212 and Michael F. Borgerding, William M. Coleman III, Betsy R. Bombick, Jeremy B. Mabe, Kathy P. Putnam, R.J. Reynolds Tobacco Company, Inc., Winston Salem, NC 27102-1487

11:50 AM

41. EFFECTS OF PARTICLE SIZE DISTRIBUTION ON CIGARETTE PHYSICAL QUALITY. SHEN Xiaofeng[1], Du Jinsong[1], LuoDengshan[1], Li Yuefeng[2], Li Huajie[2], [1]Zhengzhou Tobacco Research Institute of CNTC; [2]Fujian Branch of China Tobacco Industry Co.

11:30 AM

49. STUDY ON THE ANALYSIS OF TOBACCO COMPONENTS BY USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY/TIME-OF-FLIGHT MASS SPECTROMETER. ZHANG Jianxun, Zhengzhou Tobacco Research Institute, No.2,Fengyang Street, Zhengzhou Hi-Tech Industrial Development Zone, 450001, P.R. China

LUNCH

Tuesday Afternoon, September 23, 2008

Session A
Session Chair: John Lauterbach

1:40 PM

50. CURRENT AND HISTORICAL TRENDS IN YIELDS OF FREE-BASE NICOTINE BY CANADIAN CIGARETTES IN RELATION TO SMOKE PH AND CIGARETTE DESIGN PROPERTIES. Bill Rickert, Peter Joza, Mingliang BAO, Labstat International ULC., Kitchener ON Canada, N2C 1L3

2:00 PM

51. DETERMINATION OF SOLANESOL IN EXHALED SMOKE FROM THREE CIGARETTES WITH DIFFERENT HUMECTANT LEVELS. Serban MOLDOVEANU and William Coleman III, R.J. Reynolds Tobacco Co., Winston-Salem, NC 27105

2:20 PM

52. RESULTS FROM A NATIONAL SURVEY OF YIELD IN USE. Paul R. NELSON and Thomas J. Steichen, R.J. Reynolds, Winston-Salem, NC 27102-1487

Session B
Session Chair: Joe Wanna

1:40 PM

58. SYSTEMATIC STUDY: SHOULD DIFFUSIVITY BE CONSIDERED AS A MAIN PARAMETER OF CIGARETTE PAPER? Dietmar VOLGGER, Dieter Möhring, Roland Zitturi, and Irene Rohregger, Papierfabrik Wattens GmbH & Co KG, A-6112-Wattens

2:00 PM

59. DETERMINATION OF ACETATE TOW CAPABILITY RESPONSES ACROSS STANDARD, SLIMS AND SUPER-SLIMS FILTER ROD CIRCUMFERENCES. Francisco RUVALCABA, Celanese Acetate LLC, Narrows VA 24124

2:20 PM

60. AT LINE MEASUREMENT OF TRIACETIN PLASTICIZER CONTENT IN MONOACETATE FILTERS USING THE MICROWAVE METHOD. James VINCENT and Ian Tindall, Cerulean, Milton Keynes, MK14 6LY, United Kingdom

Tuesday Afternoon, September 23, 2008

2:40 PM

53. SIMULTANEOUS DETERMINATION OF NICOTINE AND UV ABSORBANCE OF TIP AND PAD EXTRACTS USING A CIRCULAR DICHROISM / UV SPECTROMETER. P. CLAYTON, British American Tobacco, Group R&D Centre, Southampton, SO15 8TL UK, A.F. Drake and R.J. Fielding, Applied Photophysics Limited, Leatherhead, KT22 7PB UK

2:40 PM

61. THE INFLUENCE OF WATER ON THE SELECTIVE FILTRATION OF PHENOL IN THE UPSTREAM VERSUS DOWNSTREAM FILTER SEGMENTS. A. S. WATTS and S. A. Wilson, Eastman Chemical Company, Kingsport, TN 37662

3:00 PM BREAK

3:30 PM

54. QUANTIFICATION OF METABOLITES OF ARYLAMINES, ACROLEIN and CROTONALDEHYDE IN URINE SAMPLES. Mehran SHARIFI, Peter Joza, Bill Rickert, Labstat International ULC, Kitchener Ontario, Canada, N2C 1L3

3:30 PM

62. THE PERFORMANCE OF CARBON FILTERS AT DIFFERENT SMOKING REGIMES. Tony McCORMACK and Mike Taylor, Filtrona Technology Centre, Jarrow, Tyne & Wear NE32 3UP, UK

3:50 PM

55. OPTIMAL QUANTITATIVE ANALYSIS FOR URINARY BIOMARKERS OF EXPOSURE OF POLYCYCLIC AROMATIC HYDROCARBONS THAT VARY IN RING NUMBER: 1-HYDROXY PYRENE, 3-HYDROXYPHENANTHRENE, AND 2-HYDROXY BENZ[c] PHENANTHRENE. Larry T. TAYLOR, Mehdi Ashraf-Khorassani, Virginia Tech, Blacksburg, VA 24061-0212 and Michael F. Borgerding, William M. Coleman III, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

3:50 PM

63. CONSTRUCTION OF A MODEL FOR BENZENE ADSORPTION FOCUSED ON THE DISTRIBUTION OF CHARCOALS IN CIGARETTE FILTERS. Akihiko SUZUKI, Takashi Hasegawa and Yoichiro Yamashita, Japan Tobacco Inc., Yokohama, Kanagawa, Japan

Tuesday Afternoon, September 23, 2008

4:10 PM

56. THE USE OF MAGNITUDE ESTIMATION TO ASSESS THE ODOUR AND IRRITATION OF SIDESTREAM SMOKE - (PART 1) WITH THE FABRIC METHOD. Virginie M.E. COTTE, Vanessa Lovell, David A. Saich, Teresa May and Julie Cote and Paul D. Case, Group R & D, Millbrook, Southampton SO15 8TL, UK

4:30 PM

57. THE USE OF MAGNITUDE ESTIMATION TO ASSESS THE ODOUR AND IRRITATION OF SIDESTREAM SMOKE - (PART 2) WITH THE CUBICLE METHOD. Virginie M.E. COTTE, Vanessa Lovell, David A. Saich, Teresa May and Julie Cote and Paul D. Case, Group R & D, Southampton SO15 8TL, UK

ADJOURN

Wednesday Morning, September 24, 2008
Combined Session

Session Chair: Vlad Hampl

9:00 AM **64. NITROGEN COMPOUNDS ON MAINSTREAM SMOKE AND TOBACCO PRECURSORS.** Bernard BREGEON, Micheline Coupé, Valérie Troude, Nabil Bouzaidi-Tiali, Sophie Gadois-Pommereul, Altadis Research Center, Fleury les Aubrais 45404 - France

9:20 AM **65. A STUDY OF THE REACTION BETWEEN QUINONE AND 2R4F CIGARETTE SMOKE CONDENSATE.** W. M. COLEMAN, III, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

9:40 AM **66. A DIELS-ALDER REACTION AMONG CIGARETTE MAINSTREAM SMOKE COMPONENTS.** W. M. COLEMAN, III, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

10:00 Break

10:30 AM **67. ANALYSIS OF ACROLEIN AND ACETONE GENERATED BY BLENDED C13-LABELED GLYCEROL IN A BURNING CIGARETTE VIA HPLC-MS.** Larry T. TAYLOR, Shiu-Hang Yip, Mehdi Ashraf-Khorassani, Jianxin Yu,Virginia Tech, Blacksburg, VA 24061-0212 and M. F. Borgerding, W. M. Coleman III, J. A. Bodnar, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

10:50 AM **68. INVESTIGATION ON THE NON-ISOTHERMAL BEHAVIOR OF TOBACCO STEM RELATED WITH THE CHEMICAL COMPOSITION.** Yong Joo SUNG, Young-Lim Han, Yong-Ok Kim, Chung Ryul Kim and Moon-Soo Rhee, KT&G Central Research Institute, 302 Shinseong-Dong, Yuseong-Gu, Daejeon, 305-805, Korea

11:10 AM **69. NOVEL SYNTHESIS OF POLYOL ESTERS OF C2-C6 ACIDS AND THEIR FLAVORING IN TOBACCO.** Shitong ZENG, Peng Li, Jun Hu, Zhengzhou Tobacco Research Institute of CNTC

11:30 AM **70. DETERMINATION OF VOLATILE ORGANIC ACIDS IN FLUE-CURED TOBACCO BY DERIVATIZATION HEADSPACE LIQUID-PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY/MASS SPECTROMETRY.** SUN Shihao, Xie Jian-ping, Zong Yongli, Xie Fuwei, Zhengzhou Tobacco Research Institute, China National Tobacco Corporation, 450001, China

11:50 AM Adjourn

62nd Tobacco Science Research Conference

MONDAY MORNING, SEPTEMBER 22, 2008

Symposium

9:00 AM **WELCOME: Cliff Bennett, 62nd TSRC Chair**

9:10 AM **SYMPOSIUM: "Smokeless Tobacco – What's New in an Old Product?"**
Chair: Paul Nelson

9:15 AM **MONDAY**

1. SMOKELESS TOBACCO IN THE WESTERN WORLD: A JOURNEY FROM THE PAST TO THE PRESENT. James A. STRICKLAND and Barbara B. Doonan, U.S. Smokeless Tobacco Company, Nashville, TN 37203

The smokeless tobacco category has experienced significant growth in the United States in recent years. Although first thought to have been discovered by Ramon Paine, a Franciscan monk who accompanied Christopher Columbus on his second trip to North America, tobacco had been used by Native Americans in a smokeless form for many centuries before his arrival. From its introduction in Europe in the early 1500s, the use of snuff migrated across the European continent as well as its former colonies as a form of tobacco enjoyed by both the aristocracy and the common man. Today smokeless tobacco is enjoyed by millions around the world, although with many different names and formulations. Smokeless tobacco in the western world is predominately made from dark air cured and dark fired cured tobacco and is commonly found in two formats: moist snuff, called "snus" in Sweden, and dry snuff. Moist snuff products include fine cut, long cut and pouch versions. In the United States the moist snuff category has experienced a compounded annual growth rate of about 5 to 7% in recent years. Additionally, a number of mergers and acquisitions over the years have broadened this flourishing category with various companies introducing new smokeless tobacco products. As with other consumer goods categories, application of science and technology continues to be instrumental to the development and characterization of new smokeless tobacco products.

9:45 AM **MONDAY**

2. TOBACCO HARM REDUCTION: AN ALTERNATIVE APPROACH TO SMOKING CONTROL. Brad RODU, University of Louisville, Louisville, KY

After one of the most intense public health campaigns in history, now over 40 years old, about 45 million Americans continue to smoke. Each year 400,000 smokers die from smoking-related diseases because they are unable – or at least unwilling – to achieve cessation through complete nicotine and tobacco abstinence. This presentation reviews the substantial scientific evidence for tobacco harm reduction, which involves the replacement of cigarettes with alternative sources of nicotine, including modern smokeless tobacco (ST) products. It summarizes the epidemiologic evidence that ST use confers only 0.1% to 10% of the risks of smoking with respect to all smoking-related diseases, including oral and other

cancers and cardiovascular diseases. The presentation also describes evidence that ST has served as an effective substitute for cigarettes among Swedish men, who consequently have among the lowest smoking-related mortality rates in the developed world. The presentation concludes by discussing the growing interest in tobacco harm reduction among tobacco research and policy experts from around the world.

10:15 AM ***Break***

10:45 AM MONDAY

3. DIFFERENTIATING THE HEALTH RISKS OF CATEGORIES OF TOBACCO PRODUCTS. Michael C. FALK, Amy M. Brownawell, Fabiana F. de Moura, Robin S. Feldman, Heather E. Gorby, and Kara D. Lewis, Life Sciences Research Office (LSRO), 9650 Rockville Pike, Bethesda, MD

There has been considerable debate in the scientific and public health community about the impact of tobacco harm reduction on the health burden of cigarette smoking and, in particular, the proposal to replace cigarettes with a potentially less harmful tobacco product for smokers who cannot or will not stop using tobacco. One facet of this debate has been the disagreement about how the risk of categories of tobacco products can be measured. The Life Science Research Office (LSRO) will present the results of its independent review to determine whether the scientific evidence is sufficient to distinguish between (1) the health risks of cigarette smoking and the use of one type of potential reduced risk tobacco product (PRRTP), smokeless tobacco (ST), and (2) the health risks of different categories of ST products. LSRO used the comparative risk assessment framework described in the LSRO report *Scientific Methods for Evaluating Potential Reduced-Risk Tobacco Products* which considers data about product characteristics, cigarette smoke and ST extract composition, preclinical studies (*in vitro* and animal studies), clinical studies of biomarkers of exposure and effect, health outcomes (epidemiology), and behavioral studies to draw conclusions about individual and population risk. LSRO focused on the potential for ST to decrease the risks of lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease, which account for the vast majority of deaths from cigarette smoking, but also compared the risks of oral cancer and other diseases. An Expert Panel, comprising scientists and physicians with relevant expertise provided scientific oversight and guidance for the Differentiating Tobacco Risks (DTR) project. The report will be published in fall, 2008.

11:15 AM MONDAY

4. ANALYTICAL CHALLENGES IN SMOKELESS TOBACCO. James E. FRANKE and Cliff B. Bennett, U. S. Smokeless Tobacco Manufacturing Company, Nashville, TN 37203 USA

Smokeless tobacco products fall into distinct categories and are produced in a variety of forms utilizing several tobacco leaf types. There is an absence of standardized sampling and measurement protocols for smokeless tobacco analytes both in the U. S. and internationally. The absence of such analytical standards presents numerous challenges, including the comparison of constituent levels of different smokeless tobacco products. The absence of such standards also presents challenges in connection with potential regulatory issues. Our

purpose here is to identify and discuss the existing analytical protocols in the scientific literature with respect to a variety of smokeless tobacco product analytes. In addition, this paper will discuss the next steps toward the goal of establishing a science-based set of standards for use with smokeless tobacco products. Specifically, we conclude that there is a need for the smokeless tobacco analytical community to establish priorities for development of standard methods for specific analytes, to initiate appropriate collaborative studies for these prioritized methods, and to submit the resultant analytical methods to international standards-producing bodies for approval.

11:45 PM LUNCH

1:00 PM POSTERS

5. A HIGHLY SENSITIVE AND SPECIFIC ANALYTICAL PROCEDURE FOR THE DETERMINATION OF A TOBACCO SPECIFIC NITROSAMINE, 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANOL IN HUMAN URINE USING MOLECULAR IMPRINTED POLYMER EXTRACTION, HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRIC DETECTION. Brian BAILEY, Covance Laboratories Ltd, Harrogate, North Yorkshire, HG3 1PY, UK

The objective of this project was to develop and validate a highly sensitive and specific analytical procedure for the determination of 4 (methylnitrosamino)-1-(3 pyridyl)-1-butanol (NNAL), a tobacco specific nitrosamine, in human urine. A combination of molecular imprinted polymers (MIPs) solid phase extraction, hydrophilic interaction liquid chromatography (HILIC) and triple quadrupole tandem mass-spectrometric detection was used.

MIPs are engineered cross-linked polymers that can exhibit high affinity and selectivity towards a single compound. MIPs are able to bind specifically to trace level analytes in complex matrices (such as urine) in the presence of large excesses of other compounds that have similar physico-chemical properties.

HILIC uses a polar stationary phase such as silica and a less polar solvent as the mobile phase, typically acetonitrile. Elution is achieved by increasing the aqueous portion. HILIC gives good retention of polar bases such as NNAL.

Triple quadrupole tandem mass spectrometric detection was used with Turbo Ionspray in positive mode. A mass transition of 210.3 to 180.1 Da was monitored.

The glucuronide (NNAL-N-β-D-glucuronide) was determined by liberation of NNAL using enzymatic deconjugation (β-glucuronidase from *Escherichia coli*) and quantified versus an NNAL calibration curve.

A quantitative analytical procedure with sensitivity to 5 pg/mL using a sample volume of 500 μL and linearity to 1000 pg/mL was successfully developed and validated.

6. ANALYSIS OF FREE AMINO ACIDS IN CIGARETTE TOBACCO BY CAPILLARY ELECTROPHORESIS COUPLING WITH NOVEL MULTI-PHOTON EXCITED FLUORESCENCE DETECTION. Sheng CHEN, Dan Li, Wei Zhu, Long Huang, Tonglin Zhao, Xin Liu, Technology Center of Wuhan Tobacco Company, Shisheng Road, Hanyang District, Wuhan City, China, 430051

Analysis methods of amino acids are in the ascendant for lots of research field. Capillary electrophoresis (CE) with continuous- wave-based on multiphoton-excited fluorescence (MPEF) detection, as a novel analysis method, has been introduced into investigating five different types of amino acids in our previously work. In this paper, key parameters of the Micellar Electrokinetic Chromatography (MEKC) separation mode were modulated, and 21 different FITC-labeled amino acids were separated completely with high consistency. Furthermore, amino acids in cigarette tobacco were successfully determined from analyzing MPEF-CE experimental data.

7. A RAPID METHOD FOR THE DETERMINATION OF MALEIC HYDRAZIDE IN TOBACCO. B.J. Rajesh, S.V. Dhalwadekar and S.K.MEHTA, ITC R&D Centre, Bangalore, India

Maleic Hydrazide (MH), a plant succericide, used to control auxiliary bud growth in tobacco is a known genotoxin at high doses. It is currently determined by ISO 4876:1980 method, which involves distillation and colorimetry. This method has lower sensitivity and is less time consuming. The titled method using HPLC with Photo-diode Array (PDA) detector involves hydrolysis of tobacco with 3N Hydrochloric acid and adjusting the pH to 5.6 with 12N Sodium Hydroxide solution and subsequent chromatographic separation on RP Zorbax 300 SB- C18 4.6 x 250mm x 5 micron with Caffeine as internal standard. A clear resolution is obtained between MH peak (RT: 6.34 min) and Caffeine peak (RT : 17.56 min) using a Gradient program with Mobile phases A (Sodium sulfate and Sodium dihydrogen orthophosphate dihydrate) pH 3.6 and B (Methanol) at a flow rate of 0.7 ml/min.

The absorbance is monitored at 290nm. Various parameters i.e. mobile phase, sample preparation, flow rate, resolution of MH and IS from tobacco impurities were optimized to yield reliable results. The method has been validated by standard validation protocols. Recoveries of (93 - 101%) was obtained with linear regression coefficient of 0.9999 for the range of 2.5 - 100mg/kg MH. Tobacco samples of various grades were analyzed simultaneously by both methods. The maximum deviation between the results obtained by two methods was 4 ppm.

The titled method offers several advantages over the current ISO method such as Sensitivity, Precision, time and sample preparations. The method is suitable for rapid determination of MH and hence useful for quick MH determination in large number of tobacco samples.

8. FLAVOR PROFILING OF TOBACCO BY HEADSPACE TECHNIQUE USING GC-MS. S.K. MEHTA, Nalini Dilip Sangli, Raghu H.S., S.V. Dhalewadikar, ITC R&D Centre, Bangalore, India

Flavor is perhaps the most important attribute desired by the cigarette consumer. Flavor depends on various specific marker compounds present in the smoke and in turn on their respective precursors present in cured leaf. To understand the flavor, one must understand

the marker compounds present in the substrate of interest. The flavor profiling of tobaccos on the basis of these marker compounds would help in identifying the qualitative gaps between different tobaccos. Various sample preparation techniques such as SD, SDE, HCD, HS & SPME have been reported for the analysis of volatiles/flavors in tobacco. The work on headspace of tobacco for flavors/volatiles done till date uses techniques such as purge and trap followed by cryofocussing, extraction with solvents followed by concentration, closed loop stripping analysis etc. Some of the disadvantages of these methods are highly laborious sample preparation resulting in longer analytical times, use of hazardous solvents, less recovery of volatile compounds due to various conditions during the analysis and finally lower sensitivity to analysis. In this regard, an alternate method based on headspace technique followed by GC MS was developed to achieve results at par with the existing methods. The new Headspace GC MS method for flavor profiling is simple, less time consuming, needs no solvents and can use minimal sample size. The sample preparation step involves mainly heating of the tobacco sample in a vial and injecting the hot gaseous sample vapors from the static headspace in the vial into to the column for GCMS analysis by means of a heated syringe. The analysis was carried out in duplicate for Flue cured, Air cured and Oriental tobacco grades. The results obtained by the new HS/GCMS method were found to be at par with other existing methods.

9. A RAPID METHOD FOR QUANTIFICATION OF DICAMBA, 2,4-D AND 2,4,5-T IN TOBACCO BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. H.S.Raghu, A. Devaraj, T.K.Dinesh., N. PALANI and S.V. Dhalewadikar. Organic Chemistry Lab, ITC R&D Centre, Peenya, Bangalore-560058, India

The acidic herbicides, Dicamba, 2,4-D and 2,4,5-T, are widely used for controlling annual grasses and broad leaf weeds in a large nunber of agricultural crops. Due to their persistent nature the herbicide residues are frequently found in the soil, water and crop.

Many methods are available in the literature to quantify herbicides in soil and water. It was reported recently that the above herbicide residues could be quantified in tobacco using nonaqueous capillary electrophoresis technique.

The present work describes a more practical method for residue analysis of Dicamba, 2,4-D and 2,4,5-T in tobacco leaves, using reverse phase high performance liquid chromatography. The abovementioned residues were extracted from tobacco with acetonitrile and filtered. Acetonitrile was evaporated to dryness using turbo vap and residue was dissolved in brine solution, extracted with 75:25 mixture of hexane and ether. The aqueous layer was collected in a volumetric flask and acidified using 6N H_2SO_4 and the solution was made up using methanol. The sample was injected in HPLC and all three residues were quantified using PDA. All the residues were well separated and the limit of detection range was 0.2-0.4 ppm for all the above residues. The overall recovery ranged from 75 - 110%. The above method does not involve any sample clean up procedures and is very fast and simple.

10. SINGLE PHOTON IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY: QUANTIFICATION OF SELECTED HOFFMANN COMPOUNDS USING THE BORGWALDT LM2X-PHOTON-TOFMS. Ralf ZIMMERMANN, Helmholtz Zentrum Muenchen, Neuherberg, Germany, Stefan Mitschke, University of Augsburg, Germany, Nils Rose, Borgwaldt KC, Hamburg, Germany

Mass spectrometry (MS) with soft ionisation (i.e. non-fragmenting ionisation) such as photo ionisation (PI) methods are particularly well suited for fast online tobacco analysis. In the past few years several important technical milestones have been set which allow the highly-sophisticated instruments to be operated in common tobacco R&D labs. In cooperation with Borgwaldt-KC GmbH, Hamburg, Germany, a user-friendly prototype system (LM2-Photon-TOFMS) was designed, which is equipped with an ultra compact time-of-flight mass spectrometry system (TOFWERK AG, Thun, Switzerland) and a novel vacuum-ultraviolet (VUV)-lamp system (Coherent, Germany). In principle, the use of photons proves very useful in terms of quantification as each component inhibits distinctive interactions with photons of specific wavelengths. In a mixture of various different compounds quantification is possible knowing only one exact concentration and the specific relative cross sections of the other compounds to this substance. The photon cross sections of more than 50 tobacco and tobacco smoke with suspected hazards to health were determined for two different wavelengths of VUV-light sources (Ar- (9.8 eV) and Kr-EBEL (8.3 eV). Application of cross section based quantification was demonstrated on a set of research cigarettes using the Borgwaldt LM2X-Photon-TOFMS.

11. A NEW METHOD FOR MEASURING THE APPARENT DENSITY OF SHRED AND VOID FRACTION IN A TOBACCO COLUMN. In-Hyeog OH, Han-Joo Chung, Byong-Kwon Jeh, Do-Young Ra, Dae-Keun Kwak, Byeoung-Ku Kim, Si-Hyung Jo and Moon-Soo Rhee, KT&G Central Research Institute, 302 Shinseong-Dong, Yuseong-Gu, Daejeon, 305-805 South Korea

The physical properties of tobacco materials such as void fraction and apparent density are the important factors affecting the quality of products, cigarette combustion, smoke formation as well as tobacco manufacturing process. However, so far there is no method able to determine them easily, safely with low cost and less time. In this study, a new method able to measure easily such physical properties was established by mathematical equations derived from the Ergun equation, and validated its potential possibility and convenience through applying some basic data from Muramatsu et al. (1979). Especially, the method by using a computer image analyzer was suitable to measure the surface area and volume of single shred in a tobacco column. As results by this new method, the void fractions in tobacco columns of commercial tobacco shreds of flue-cured, burley, the expanded stem and the reconstituted were 0.668, 0.739, 0.758 and 0.679, respectively. And the apparent densities of them were 1.121, 0.903, 1.088 and 1.193 g/cm^3, respectively. The porosity of each tobacco was the expanded stem, burley, the reconstituted, then flue-cured in a descending order. In this presentation, the establishment of this new method and its application for measurement of physical properties of several tobacco shreds will be discussed in detail.

12. DESIGN OF TOBACCO FLAVOR QUALITY CONTROLLING SOFTWARE. Xiong Guoxi, WANG Na, Si Hui, ZhuWei, Xiong Bin, LI Dan Technology Center of China Tobacco Hubei Industrial Corporation; No. 22, Road Shisheng, District Hanyang, Wuhan City, P.R. China, 430051

To ensure different batch of same tobacco flavor has same product quality, the quality and quantity of 62 kinds of tobacco flavors used in 17 brands cigarettes were analyzed. Combined with tasting results, "Quantity Controlling Index of Characteristic Compounds (QCc Index)" and "Quantity Controlling Index of Total Compounds (QCT Index)" were

designed to control tobacco flavor quality. On these bases, the tobacco flavor quality controlling method was built and further developed tobacco flavor quality controlling software. The software has functions of searching and auto-calculation of indexes. In this way, it can automatically determine whether tobacco flavor quality is eligible by logical judgment procedure. The compatibility of other chromatograph workstation is great and can be used conveniently. The method and software reasonably stabilize tobacco flavor quality and powerfully support cigarette quality.

13. AN IMPROVED UV METHOD TO DETERMINE FILTRATION EFFICIENCY OF CELLULOSE ACETATE FILTERS. V. S. WILLIAMS, A. S. Watts and L. W. Renfro, Eastman Chemical Company, Kingsport, TN 37662

Filtration efficiency is an important attribute of cellulose acetate tow filters and a reliable method for measuring filtration efficiency is required for assessing the performance of filter cigarettes. Due to the difficulty of directly measuring dry smoke condensates on cigarette filters, gravimetric efficiency measurements require additional smoke testing or modification of the cigarette filter system prior to smoking. Spectrophotometric efficiency measurements can be fast and efficient, but traditionally require separate smoke testing in addition to that needed for determining smoke yield. In this work, an improved analytical method employing ultraviolet (UV) spectrophotometry to determine filtration efficiency has been developed. The method uses the same solvent extracts prepared for gas chromatographic analysis of typical smoke analyses. Absorbance measurements of the extracts relate to the quantity of smoke condensate deposited on the pads and filters during smoke testing. The method can be used with unaltered ventilated filter cigarettes. A good correlation between UV efficiency and traditional gravimetric efficiency was demonstrated for a broad selection of cigarette brands and styles smoked with both ISO and aggressive conditions. Consideration of a range of wavelengths permitted selection of an optimum wavelength for UV absorbance measurements. The influence of potential interferences, such as triacetin, was investigated. Reduced sample preparation and testing, reduced analysis time, and improved precision make the UV efficiency method an excellent choice for the determination of filtration efficiency.

14. COMPARISON OF LOOSE SNUS AND POUCHED SNUS CONSUMPTION BEHAVIOUR IN SWEDEN. Helena DIGARD, Delcio Sandi and Rudi Hartmann, Group R&D, British American Tobacco, Southampton, SO15 8TL, UK

A telephone survey was conducted in March and April 2007 of approximately 3,000 Swedish snus users to investigate snus consumption behaviour. The survey addressed a range of topics from consumption per day to usage factors.

In Sweden snus is predominately sold in two forms; loose tobacco and portioned (with the tobacco in a porous pouch), 58.8% of the participants were portioned snus users, 37.7% loose and the remainder used both. Comparing the consumption in terms of the average amount in grams per day, the portioned users use on average the least (mean 11.2g, median 10g), the loose users on average use the most, between 2.5 and 3 times more per day (mean 29.5g, median 25g) and the users of both on average were similar to but lower than the loose users (mean 25.7g, median 24g).

A number of aspects of snus usage were investigated, including position of placement in the mouth, whether the product is moved during use and how the product was disposed of. The majority of both loose and portioned snus users (98.7% of loose users and 96% for portioned) place the product under their upper lip. During use 82.4% of loose users and 62.4% of portioned users stated they never move the product around in their mouth and 99.5% of loose snus users and 79.3% of portioned snus users do not swallow the product at the end of use.

15. PROPOSAL FOR A STANDARDIZED METHOD FOR WATERPIPE SMOKING. Juergen HAHN, Chemisches und Veterinäruntersuchungsamt Sigmaringen, Sigmaringen Germany, Nils Rose, Borgwaldt KC GmbH, Hamburg, Germany

Waterpipe smoking is a fast growing market segment of tobacco consumption for the past two years. Europe especially seen the smoking of waterpipes becomes very popular amongst young adults and is often their first contact to smoking. There is a perception among young adults, that waterpipe smoking is less harmful compared to cigarette smoking.

Worldwide a lot of discussions and activities are in progress to measure and categorize waterpipe smoking. Much of the testing being completed is in the absence of a standard testing method and equipment, so any published results should be considered with this in mind.

The topic of this poster is the development of a standardized procedure in combination with the technical difficulties relating to such a standardised smoking method. A description of the proposed procedure, as well as first results of smoke components.

16. CHARACTERIZATION OF THE MAINSTREAM AND THE SIDESTREAM CIGARETTE SMOKE SIMULTANEOUSLY GENERATED USING AUTOMATED CIGARETTE SMOKE EXPOSURE SYSTEM. Vladimir MIKHEEV, Alec Hitchman, Ronald Rhoads, Tessa Oxford, K Monica Lee, Bruce Westerberg, Battelle Toxicology Northwest, Richland, WA 99354 USA

Mainstream (MS) and sidestream (SS) smoke of 3R4F reference cigarettes was simultaneously generated using a modified JB-2070i smoking machine and directed to the two different exposure carousels. Three target exposure concentrations within the range of commonly used in smoke toxicity studies were selected per WTPM (wet total particulate matter): MS smoke (500, 250, and 60 μg/L) ; SS smoke (130, 95, and 55 μg/L). The exposure duration was 1 hr for all exposure runs. CO (carbon monoxide), WTPM, nicotine, aldehydes (formaldehyde, acetaldehyde, acrolein, propionaldehyde, and crotonaldehyde), butt lengths, particle size (using cascade impactor and scanning mobility particle sizer), and environmental parameters (temperature and relative humidity) at the animal exposure nose-ports were measured. All data demonstrated high stability of the exposure system for the both MS and SS smoke with relative standard deviation ≤~10%. Most of constituent concentrations of the MS and SS linearly increased with the increase of the target WTPM exposure concentrations. SS smoke demonstrated significantly higher aldehyde content than MS smoke. MS particle size was stable for all three exposure concentrations, whereas SS particle size, significantly smaller than the MS smoke, tended to decrease with decreasing WTPM concentration. This may explain the increasing nicotine to WTPM ratio of the SS

smoke with decreasing WTPM concentration. In summary, the study demonstrates stable and automated generation and monitoring of simultaneous exposure of MS and SS smoke up to 72 animals per each smoke type.

Acknowledgement: This work was supported in part by NIH/NIEHS Grant No. 1 U54 ES016015 and Battelle Internal Funds.

17. TRAP MARKER STUDY OF THE CHARACTERISTIC AROMA OF ORIENTAL TOBACCO-LIKE IN N.TABACUM L. REN Min, Wang Rixin, Jia Xinghua, Fen Quanfu, Wang Shaomei, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao

Aroma plays an important role in evaluating the quality of tobacco leaves. The purpose of this study was to screen genetic marker which linked with the gene of characteristic aroma of oriental tobacco-like. First of all, to evaluate the aroma phenotype of the materials in this study, sensory evaluation was adopted on 16 tobacco varieties including that special aroma flue-cured tobacco, oriental tobacco, sun/air-cured tobacco and common flue-cured tobacco and 5 F1 cross. The results showed two groups existed, the former 3 kinds varieties all possessed a characteristic aroma of oriental tobacco-like (Group A) while common flue-cured tobacco didn't (Group B). The phenotype results lay a foundation to the further study. Of all the genetic markers, TRAP markers are usually selected in several fields. While, difficulties including complex development of the fixed markers and long period existed in the development of TRAP markers in former studies, therefore a new approach that taking the primers of EST-SSR as the fixation primers of TRAP was adopted in this study. The results showed ideal amplification effect based on the new approach of TRAP marker. Of all the markers screened, we found a co-dominant heredity TRAP marker which amplified a characteristic band about 1.8kb. So the results showed that it may link with the gene of characteristic aroma of oriental tobacco-like through screening of Flue-cured tobacco, oriental tobacco and sun/air-cured tobacco varieties. In a word, this characteristic TRAP marker can be used in marker assisted selection (MAS) directly or transformed to SCAR marker. It also lays a strong foundation on the isolation and identification of novel aroma genes.

17a. FACTORS AFFECTING THE EFFECTIVENESS OF SMOKE TRAPPING BY MEANS OF AN ELECTROSTATIC TRAP. Tim MASON and Ian Tindall, Cerulean, Milton Keynes, MK14 6LY United Kingdom

The use of electrostatic traps or precipitators is long established in the field of smoke science research. Recently such devices have been utilised for the determination of heavy metals in the smoke stream. Electrostatic traps employ the Cottrell principle and were originally applied to large scale industrial processes. In a smoke machine EP traps act by placing a high voltage on an electrode within a smoke stream, charging the smoke particles and which are then attracted to glass tube surrounded by a grounded electrode. The smoke condensate remains on the glass surface and is subsequently removed for analysis.

Commercially available traps are not 100% efficient and some smoke will escape the trap. In this paper the authors describe the effect on trapping efficiency of varying the precipitation voltage and polarity, the influence of puff volume and interval on trapping

efficiency (Intensive regimes vs ISO regimes) and the influence of deliberately inducing turbulence within the smoke trap.

Recommendations are made for a design that optimises collecting efficiencies of such electrostatic traps.

17b. INFLUENCE OF CURING PRACTICES ON TSNA PRODUCTION IN DARK FIRE-CURED TOBACCO. Andy BAILEY, University of Kentucky, Princeton, KY 42445, Bill Pitt and Barry Sims, University of Tennessee, Springfield, TN

Experiments were conducted to evaluate effects of conditioning method, takedown method, and time of stripping on TSNA in a standard double crop curing system for dark fire-cured tobacco. Two crops of KY 171 were transplanted and harvested approximately 5 weeks apart in each year. Six small curing barns were used with three barns having overhead misting systems installed while steamers were used for ordering in the other three barns. First cures were fired aggressively over a five-week period and misted or steamed until adequately in order. For both cures, half of the tobacco from each barn was then taken down onto scaffold wagons while the other half was bulked down onto flatbed wagons. For first cures only, half of the tobacco from each conditioning and takedown method was stripped after one week while the other half was stripped approximately three weeks following takedown. Second cures were stripped one week following takedown. Each cure was delivered to a receiving station at the same time. Replicated leaf samples from each barn and cure were taken at takedown to compare conditioning methods, at stripping to compare conditioning and takedown methods, and at delivery to compare conditioning, takedown, and time of stripping effects for first cures; and conditioning and takedown method for second cures. TSNA levels were higher for first cures throughout the entire process. TSNA levels were also significantly higher at takedown for first cure tobacco that was steamed. Trends in TSNA levels also indicated the potential for higher TSNA in first cure tobacco that was bulked at takedown, as well as in first cure tobacco that was stripped early and remained in storage for approximately 3 weeks prior to delivery. There were no significant effects of any factor on TSNA levels in second cure tobacco.

MONDAY AFTERNOON, SEPTEMBER 22, 2008

SESSION A ***Session Chair: Balazs Siminszky***

2:00 PM MONDAY

18. BREEDING FOR REDUCED TSNA IN BURLEY TOBACCO. R.D. MILLER, University of Kentucky and University of Tennessee, Lexington, KY 40546-0312

A study was conducted in 2004 and 2005 at four locations in Kentucky and Tennessee to determine the impact of reduced nicotine to nornicotine conversion on levels of tobacco specific nitrosamines (TSNA) in burley tobacco. Three replications of 15 cultivars were grown at each of four locations, with data analyzed as a split-plot design with locations as whole plots and cultivars as subplots. Highly significant differences were detected among locations and among cultivars for nicotine conversion and nitroso-nornicotine (NNN) and total TSNA levels. As expected, a highly significant correlation was found between percent conversion and NNN (r^2=0.75, Pr>|r| = 0.001), and between percent conversion and TSNA (r^2=0.58, Pr>|r| = 0.02). However, significant differences for NNN were also detected among cultivars that were not significantly different for nicotine conversion. The cultivars having low levels of NNN also had significantly lower levels of nitroso-anatabine (NAT) and nitroso-nicotine (NNK). KT 204LC, the variety having the lowest level of total TSNA, had means of 3.88% nicotine conversion, 0.64ppm NNN, 0.89ppm NAT, and 0.07ppm NNK. In comparison, ms KY 14XL8 had means of 3.78% conversion, 1.87ppm NNN, 4.56ppm NAT, and 0.59ppm NNK. The three cultivars having the lowest levels of TSNA were hybrid varieties that had a common parent, suggesting that genetic differences may account for the variation in TSNA observed. The results from the study demonstrate that while reducing nicotine to nornicotine conversion is effective in minimizing NNN, additional reduction in total TSNA may also be possible by selective breeding efforts.

2:20 PM MONDAY

19. TOWARD DEVELOPMENT OF LOW NORNICOTINE DARK TOBACCO LINES THROUGH MUTAGENESIS. Yanxin SHEN, Dongmei Xu, David Norman, and Mark Nielsen, USSTC, Winchester, KY 40391

Two genes have been identified as having a role in nornicotine formation in tobacco. Blocking these two genes could result in lowering nornicotine in tobacco plants, which could lead to lower *N*'-nitrosonornicotine (NNN), a TSNA formed by nitrosation during tobacco curing. We created a mutant population of dark tobacco NL Madole using Ethyl ethyl methanesulfonate (EMS) treatment. Here we report the identification of new multiple mutants in dark tobacco to eliminate the enzymatic activities of major and minor nicotine demethylase genes. The first generation mutant population was grown under field conditions and the second generation was grown under both field and green house conditions. Two nonsense mutations for the major gene and two nonsense mutations for the minor gene were identified and tested. The nornicotine content in the mutated lines will be reported. Identification of both nonsense mutants on major and minor nicotine demethylase genes and their combination could lead to stable and even lower nornicotine tobacco varieties than the low converter varieties currently available.

2:40 PM MONDAY

20. TOBACCO NICOTINE DEMETHYLASE GENE DYSFUNCTION IS AN EFFECTIVE AND PRACTICAL MEANS OF REDUCING NORNICOTINE LEVELS IN TOBACCO. Dongmei XU, Yanxin Shen, David Norman, Marcos Lusso, Greg Davis, Frank Hart, Mingwu Cui and Mark Nielsen, USSTC, Winchester, KY 40391

Blocking nornicotine formation in tobacco plants could lead to lower *N'*-nitrosonornicotine (NNN), a TSNA formed by nitrosation during tobacco curing. In earlier studies, we reported the discovery of a nicotine demethylase gene and used GMO (RNAi) and non-GMO (mutagenesis) strategies to down-regulate the nicotine demethylase (ND) activity. Here we report on the effectiveness and stability of this down-regulation in advanced and breeding lines. We also describe the breeding process and progress to successfully transfer RNAi and mutation traits into different varieties. Experimental lines were grown under field conditions in multiple trials. Included in the tests were four generations of RNAi transgenic lines and the male sterile counterparts of the R2 generation. The lines were generated in both burley tobacco (TN 90) and dark tobacco (NL Madole) backgrounds. Also tested were four generations of mutant lines carrying a ND nonsense mutation in TN 90 and mutant breeding lines that were back-crossed four times in two dark tobacco backgrounds, NL Madole and KY171 and a burley variety (TN 90). Conversion levels (% nornicotine/(% nicotine + % nornicotine)) in the transgenic lines were as low as 0.1% under field conditions and were stable throughout the four generations. Reversion from low nicotine conversion was not seen in either RNAi transgenic or mutant lines. Lines developed through EMS mutagenesis as well as through the use of transgenes can be used as low converter varieties for commercialization.

3:00 PM MONDAY

21. THE VIRULENCE, BIOVARS AND PATHOTYPES OF RALSTONIA SOLANACEARUM IN TOBACCO PLANT IN YUNNAN PROVINCE, CHINA. Wang Min[1], LIU Yong[1], Li Meiyuan[1], Ji Guanhai[2], Li Yongping[1], [1]Yunnan Institute of Tobacco Science, China Tobacco Breeding Research Southern Center, Yuxi 653100, China; [2]College of Plant Protection, Yunnan Agriculture University, Kunming 650202, China

In order to understand the diversity of Ralstonia solanacearum strains in tobacco plant, The strains isolated from disease tobacco samples collected from 6 counties of Yunnan provinces were evaluated by virulence, biovars and pathotypes test. The virulence test of detached leaves of tobacco variety K326 show that 165 strains of R. solanacearum could classified into three groups of strong, middle and weak virulence strains in the percentage of 49.5%, 43.9% and 6.6% respectively, while strong and middle virulence groups are dominant. The physiological and biochemical characteristics test of 81 representative strains show that among these strains, 43 (or 53.1%) strains belong to biotype III, 32 (or 39.5%) strains belong to biotype III-1, 6 (or 7.4%) strains belong to biotype III-2. The pathotypes test show that among 55 tested strains which were chosen in six county of Yunnan province, Type I is dominant, followed by Type II, Type III in the percentage of 63.6%, 27.3% and 9.1% respectively. The distribution of pathotypes of strains was geographically diversified.

3:20 PM ***Break***

3:50 PM MONDAY

30. REDUCING TSNAs IN AIR-CURED TOBACCO – BY WHAT MEASURE? Anne JACK, Neil Fannin, Xiaolong Li and Lowell Bush, University of Kentucky, Lexington, KY 40546 USA

TSNAs in air-cured tobacco can be lowered by reducing the specific alkaloid precursor, by manipulating curing conditions, or by reducing total alkaloids. The most effective TSNA reduction strategy to date has been to reduce nornicotine, the precursor of the major burley TSNA, NNN (N'nitrosonornicotine), by seed screening. Curing conditions are known to have a major impact on TSNA accumulation, but manipulation of curing has not been a successful strategy because of the difficulty of maintaining acceptable leaf quality. Many studies have shown that TSNA accumulation can be reduced by any practice which lowers total alkaloids, such as lower nitrogen fertilizer rates. However, if the TSNAs decrease at the same rate as the alkaloids, or at a lower rate, the benefit is questionable. The objective of this study was to establish whether reducing alkaloids results in reduced TSNAs per unit of alkaloid as well as in reduced absolute amounts of TSNA. In a two-year study, high and low converter lines were grown with standard and zero nitrogen rates. Cured leaf was analyzed for alkaloids and TSNAs. TSNAs increased exponentially as total alkaloids increased, on both a weight basis and on an equivalent basis. TSNAs were reduced in the zero nitrogen treatment, whether they were expressed as an absolute amount or per unit of alkaloid. This suggests that when alkaloids are reduced by lowering nitrogen rates, TSNAs are reduced to a greater extent. TSNAs relative to total alkaloids were reduced to a greater extent by the low converter line than by the zero nitrogen treatment.

4:10 PM MONDAY

22. EFFECTS OF CONTINUOUS MONO-CROPPING OF FLUE-CURED TOBACCO ON NITROGEN RELATIONS IN THE SOIL AND PLANT. PAN Wenjie, Tang Yuanju, Wang Maoshen, Cheng Yi, Xue Xiaoping, Guizhou Tobacco Research Institute, Guiyang, China

In order to understand the effects of continuous mono-cropping on the forms of soil nitrogen and their effects on plant nutrition, we analyzed soil nitrogen, various soil microorganisms and the nature of nitrogen accumulation in the tobacco leaf in a potting experiment using different soil types. We found that total N in the soil, alkali-hydrolysable N, nitrate N and ammonium N increased with continuous mono-cropping but nitrifying bacteria, ammonium oxygenation bacteria and nitrification decreased. Continuous mono-cropping led to decreased chlorophyll and nitrate reductase levels and increased proline content of the leaf. Mono-cropping also led to a gradual increase in total N, nicotine and protein in the leaf and their decline in the stem and the root. These changes were affected by soil type. A quaternary yellow soil suitable for tobacco had lower amounts of nitrate, nitrifying bacteria, ammonium oxygenation bacteria and nitrification than a less suitable yellow lime soil. Improved crop performance under continuous mono-cropping could be achieved by reducing the soil N content to induce higher levels of beneficial microorganisms.

4:30 PM MONDAY

23. TRANSCRIPT REGULATION RELATED TO POTASSIUM UPTAKE GENES IN TOBACCO ROOTS. Zhaokui GUO, Xiuqing Wan, Peiqiang Yan, Heilongjiang Tobacco Institute, China

Three genes, including K+ transporter AtKup1, Na+/H+ anti-porter AtNHX1 and inorganic pyrophosphatase AVP2 were cloned from Arabidopsis and transformed into tobacco. The transcription patterns of five tobacco internal genes were evaluated using qRT-PCR methods. The genes encoding K+ channel, K+ transporter, vacuole H+-PPase, plasma membrane H+-ATPase and vacuole H+-ATPase were analyzed in transgenic and wild type tobacco plants under potassium starvation, and sodium and ammonia stress conditions. The results demonstrated that the transcript of the NtHAK1 gene was reduced under all treatments, and NHA1 was increased in the roots of AtKup1 transformants. The NtHAK1 gene transcript was also downregulated in the roots of AtNHX1 transformants, but the NHA1 and VAG1 transcripts encoding H+-ATPase were significantly upregulated. The NVP1 gene, encoding vacuolar H+-PPase increased slightly. VAG1 encoding H+-ATPase and NVP1 encoding vacuolar H+-PPase transcripts were downregulated. The NtHAK1 and NKT1 transcripts were slightly increased in the AVP2 gene overexpressed in tobacco roots. In addition, transcripts of five tobacco internal genes were analyzed under different nutrition and salt stress conditions. The study demonstrated that the transcription of NtHAK1 and NHA1 were significantly stimulated, while the expression of NVP1 decreased in response to an external K+ starvation solution treatment. The results also confirmed that the K+ transporter gene NtHAK1 transcript was induced by excessive Na+ stress, but the transcription of the gene was inhibited when the tobacco plants were in a 5 mmol/L NH4+ solution. NKT1 transcript levels exhibited no response to potassium starvation and sodium or ammonium stress treatments, suggesting that NKT1 is constitutively expressed.

4:50 PM MONDAY

24. CLONING AND SEQUENCING OF HELPER COMPONENT PROTEINASE GENE OF A NEW ISOLATE OF POTATO VIRUS Y. WANG Yanying[1], Huang Yingchun[2], Wang Fenglong[1], Chen Wansheng[1], Shen Lili[1], Gong Daping[1], [1]Tobacco Research Institute, Chinese Academy of Agricultural Science, Qingdao 2600101, China; [2]College of Biochemistry Engineering, Beijing United University, Beijing 1000023, China

The potato virus Y helper component proteinase (HC-Pro) plays important roles in the transmission of the virus Y by the aphid (*Myzus persicae*). It will be established a new method for tobacco breeding by transmission mechanism of HC-Pro gene of potato virus Y. The helper component proteinase gene of an potato virus Y isolate from Qingzhou, Shangdong province was cloned by RT-PCR and DNA splicing, and the complete nucleotide sequence of the gene was determined. The results showed that the 1 389 bp gene , encoded a protein of 463 amino acids and shared more than 96% and 92% homology with HC-Pro genes of other PVY strains at the nucleotide and predicted amino acid level, respectively. It is suggested that the HC-Pro should belong to a new isolate of potato virus Y. SDS-PAGE showed that the HC-Pro gene was successfully expressed in *E. coli.*

5:10 PM MONDAY

25. PHYLOGENETIC ANALYSIS OF MICROORGANISMS IN FLUE-CURED TOBACCO AND ITS RELATIONSHIP WITH BIO-ENZYMES. DUAN Yanqing[1], Wu Yi[1], Yang Jinkui[2], Li Qinghua[1], Zhang Kejin[2], [1]Hongyun Tobacco(Group) Co., Ltd.; [2]Laboratory for Conservation and Utilization of Bio-resources, Yunnan University

Microorganism and bio-enzymes were isolated and detected, and microorganism varieties and their phylogenetic relationship were analyzed at molecular level, based on the Yunnan K326 flue-cured tobacco from different aging stages and grades, the relationship between microorganism varieties and bio-enzymes were also discussed. The results showed that: 1. Bacteria on flue-cured tobacco are composed of two groups: *Bacillus* spp. and *Enterobacte* spp. *Bacillus* spp. is primary and *Paenibacillus* spp. is the secondary group on tobacco leaves. Moreover, some bacteria showed different 16S rDNA from the model species. These bacteria maybe the uniquely microorganisms and influence on the aging process of flue-cured tobacco. Fungi are mainly found on the briefly-aged tobacco samples, but not on the long-time-aged samples. 2. There are positive correlations between the quantities of microorganisms and bio-enzyme activities. Meanwhile, bio-enzyme activities of middle (C3F) are higher than that in upper (B3F) and lower (X3F) tobacco leaves. Microorganisms in flue-cured tobacco were analyzed using phylogenetic method for the first time in this study. Our results provide new ideas and methods for systematically analyzing the microorganisms' diversities in the flue-cured tobacco. Moreover, our results also provide theoretical basis.

5:30 PM ADJOURN

MONDAY AFTERNOON, SEPTEMBER 22, 2008

SESSION B ***Session Chair: Randy Hudson***

2:00 PM MONDAY

26. EVALUATION OF HPLC MOBILE PHASES AND EXTRACTING SOLUTIONS FOR THE DETERMINATION OF GLYCYRRHIZIC ACID IN LICORICE AND TOBACCO. Charles H. RISNER, R.J. Reynolds Tobacco Company, Winston-Salem, NC

An improved high performance liquid chromatography (HPLC) analysis of glycyrrhizic acid (GA) in licorice and tobacco to which licorice was applied was developed. An acetate buffer was used in the mobile phase which resulted in more constant retention times for GA. Nine extractants were found to remove GA from licorice, but only six were capable of removing GA from both licorice and tobacco. Precision was very good with less than 4% relative standard deviation for licorice and tobacco and percent recoveries were at least 92%. Aqueous solutions of 1,4-dioxane, ethanol, tetrahyrofuran, 2-butoxyethanol, 2-methoxyethanol and 1,3-dioxolane can be used to extract GA from both licorice and tobacco. Glycyrrhetinic acid and various compounds found in tobacco do not interfere with the analysis.

2:20 PM MONDAY

27. DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE DETERMINATION AND QUANTITATION OF HETEROCYCLIC AROMATIC AMINES (HAAs) IN MAINSTREAM SMOKE USING A SIMPLE EXTRACTION AND SAMPLE PREPARATION. Anthony GERARDI, R.J. Reynolds Tobacco Company, Winston-Salem, NC

Specific heterocyclic aromatic amines (HAAs) are on the full Hoffmann analytes list and are considered highly mutagenic. These compounds are pyrolytic products of amino acids or proteins and have been reported in cooked meat, diesel exhaust and tobacco smoke condensate. Reported here is a simple procedure applicable to the quantitative determination of several HAAs, including AαC, MeAαC, IQ, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, and PhIP, as well as MeIQ and MeIQx. A liquid chromatography-triple-quadrupole tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the quantitative determination of HAAs in mainstream smoke condensate. Unlike previously reported methodology, this procedure does not employ solid phase extraction (SPE) clean-up and pre-concentration, thereby decreasing cost while increasing sample throughput. A longer HPLC analysis time was used to provide better chromatographic separation of the HAAs on the LC column with the minimized sample preparation. The HAAs were extracted with methanol from a 44 mm Cambridge filter pad containing mainstream cigarette smoke condensate from 2-5 cigarettes, depending on smoking regime intensity. The extract was filtered then diluted 12 fold with more methanol. This diluted sample was then injected onto the HPLC coupled with a triple-quadrupole tandem mass spectrometer equipped with electrospray ionization (ESI). A C_{18} (ODS) column was used for the separation. HAAs were characterized using product ions selected during direct infusion of individual stock standard solutions into the mass spectrometer LC mobile phase flow. Quantitative analysis was performed using 4,8-DiMeIQx as internal standard. The accuracy of this procedure

was determined by standard addition experiments, which showed average recoveries of most HAAs between 98 - 119%, except PhIP at 89%.

2:40 PM MONDAY

28. DEVELOPMENT OF A METHOD FOR QUANTITATIVE ANALYSIS OF HYDROGEN PEROXIDE GENERATED FROM AQUEOUS EXTRACT OF CIGARETTE SMOKE. Yuichiro TAKANAMI, Takako Moriyama and Yasutaka Kosaka, Japan Tobacco Inc. 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan

The aqueous extract of cigarette smoke generates reactive oxygen species such as hydrogen peroxide. Several methods for analysis of hydrogen peroxide from cigarette smoke have been reported. However, the results obtained by these methods can be affected by other smoke constituents. In this study, a method for analysis of hydrogen peroxide from cigarette smoke using a HPLC electrochemical detector (ECD) has been developed, which is thought to be a highly selective technique. Samples are prepared by collecting the particulate phase of cigarette smoke on a glass-fiber filter and extracting it with a phosphate buffer. The obtained solution is purified by a cation ion exchange cartridge, Waters Oasis MCX, and then analyzed by the HPLC-ECD system with a mixed-mode resin column, Shodex KS-801. The recovery ratio of hydrogen peroxide using smoke as a matrix was more than 80% and the variation of data was less than 5%, which provides validation of the method. The quantitative results depend on the extracting time, the concentration of tar, and the pH of the solution used for extraction. These conditions must be controlled for evaluation of hydrogen peroxide generated from the aqueous extract of cigarette smoke.

3:00 PM MONDAY

29. PRODUCTIVITY IMPROVEMENTS THROUGH CHROMATOGRAPHY AND AUTOMATION FOR GC/TEA TSNA ANALYSIS OF TOBACCO LEAF. W. Eric HARRIS, Stephen Gibson, Jamie N. Finch, Frank Hart, Daniel Heltsley, Jennifer Johnson, Cecil Ray, and Thomas Thorburn, U. S. Smokeless Tobacco Manufacturing Company, Nashville, TN 37203 USA

To improve productivity of TSNA analysis in the laboratory by GC/TEA, an automated sample handling/extraction system coupled with micro-bore chromatography was developed and implemented. The goal of the development system was to be able to analyze 80+ samples/instrument/day with a TSNA analysis MQL (total TSNA, dry weight) at or below 1.0 ppm, with no increase in analysis cost per sample.

The implemented system incorporates a CTC "Prep and Load" automation system for sample preparation and utilizes a micro-column, "Fast-GC" chromatographic approach. This system has proven robust and effective. Our presentation will discuss in detail the chromatographic parameters, automation system modifications and method development, our current validation data, and the necessary system maintenance plan.

3:20 PM ***Break***

3:50 PM MONDAY

30. Moved to Session A at 3:50 PM on Monday

31. SEPARATION AND DETERMINATION OF P-CRESOL AND M-CRESOL IN MAINSTREAM CIGARETTE SMOKE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC USING β-CYCLODEXTRIN AS MOBILE PHASE ADDITIVE.
Qian-Rong PENG[1], Jie Zhang[2], Min Yang[2], Jian-Ling Xie[2], Zhong-Xiang Liu[1], Yuan-Qing Cai, [1]Technology Center of China Tobacco Guizhou Industry Company, Guiyang 550003, China; [2]Chemical Engineering Department, Guizhou University, Guiyang 550003, China

A method by reversed-phase high performance liquid chromatography, using β-cyclodextrin (β-CD) as mobile phase additive, has been developed for separation of the structural isomers of p-cresol and m-cresol in mainstream cigarette smoke. The two compounds can be isolated with high resolution on a C18 reversed-phase column with the addition of β-CD in the mobile phase. The separation mechanism of the isomers was discussed. It was assumed that the separation of the isomers might have been resulted from different inclusion forces of complexes with β-CD. The effects of β-CD concentration were investigated. It was found that the resolution of the isomers increased with the increase of β-CD concentration. When the mobile phase consisted of 6g/L β-CD , the variation coefficient of the method was between 1.27% ~ 3.94% and the recoveries of phenols were from 92.5% to 106.2%. This method was successfully used to determine the p, m-cresol in cigarette smoke.

4:10 PM MONDAY

32. DETERMINATION OF ALKALOID IN TOBACCO BY UPLC.
LU Sheming, Ni Caoming, Yang Liu, Li Zhongchang, Wang Di, Miao Mingming, R&D Center of Hong Ta Tobacco Group Co., Ltd., Yuxi 653100, China

A method based on ultrasonic extraction combined with ultra high performance liquid chromatography for simultaneously determining alkaloid in tobacco was developed. The alkaloid in tobacco was extracted with 0.5%sodium hydroxide and ethanol under ultrasonic for 30 minutes. The extract was filtered with 0.22 μm filter membrane then separated and detected by UPLC. The contents of alkaloid in 10 tobacco samples and 10 cigarette samples were determined by the method. The results indicated that: 1)the recoveries of the method were from 96.5% to 100.3%with RSD of 2.06%-3.49% and the limit of detection of 6.33 to 11.9 ng/g, the analyzing took 4 minutes; 2)The stalk positions of leaves in order of alkaloid content were upper>middle> lower; and 3) In tobacco of the same type, the contents of different alkaloids were in the order of nicotine>myosmine> nornicotine >cotinine >anabasine. The method is suitable for fast analysis of alkaloids in Tobacco samples.

4:30 PM MONDAY

33. TRANSFER OF SOME FATTY ACID FLAVORS IN CIGARETTE. CAI Junlan, Zhang Xiaobing, Zhao Xiaodong, Xie Jianping, and Liu Kejian, Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China

Ten fatty acid flavors of C5 to C14 chain length were injected into cigarette and analyzed with simultaneous distillation and extraction, GC, and GC/MS. The distribution of these flavors in the cigarette and their transfer behavior to mainstream smoke and filter tip were determined. The results showed that: 1) for fatty acids homologous compounds, the larger molecular weight and higher boiling point were, the higher the transfer ratio to mainstream smoke and the immigrated ratio to filter tip would be, however, the retention ratio and the loss ratio of cut tobacco and the absorption ratio of filter tip changed irregularly; 2) two pairs of fatty acid isomers had similar transfer behavior, for fatty acids of the same molecular weight, the lower boiling point generally associated with lower retention ratio of cut tobacco, and relatively high loss ratio, transfer ratio to mainstream smoke, immigrated ratio and absorption ratio of filter tip. Moreover, the transfer ratio to mainstream smoke, the immigrated ratio and absorption ratio of filter tip significantly differed between the two isomers.

Our objective was to study the transfer behavior of some important fatty acid flavors added into cigarette and to obtain some data which maybe helpful to using the flavors and developing low tar cigarettes.

4:50 PM ADJOURN

TUESDAY MORNING, SEPTEMBER 23, 2008

SESSION A ***Session Chair: James Strickland***

9:00 AM TUESDAY

34. APPLICATION OF THE DIRECT SILYLATION GC-MS SCAN TECHNIQUE TO REFERENCE SMOKELESS TOBACCO PRODUCTS. John H. LAUTERBACH, Lauterbach & Associates, LLC, Macon, GA, USA and Deborah A. Grimm, Tulane University Coordinated Instrumentation Facility, New Orleans, LA

Considerable attention has been focused recently on the toxicological properties of smokeless tobacco products. However, compared with cigarette tobacco, relatively little is available in the public domain on the detailed chemistry of such products. Consequently, we used the direct silylation GC-MS scan technique, which is known to provide identifications and semi-quantitative data, on acids, humectants, sugars, and certain other compounds (Moldoveanu *et al.*, 46th TCRC, Paper #28) for the partial characterization of the three types of reference smokeless tobacco products [loose-leaf chewing tobacco (1S1 and 2S1), dry snuff (1S2), moist snuff (1S3 and 2S3)]. Analyses were carried out on an Agilent 6890 gas chromatograph coupled with an Agilent 5972 mass spectrometer. The version of the direct silylation technique developed for bench-top mass spectrometers was used (Lauterbach, 42nd TCRC, Paper #28). Before analyzing the smokeless tobacco products, the performance of the system was checked with tobacco taken from a commercial US blend cigarettes and ground before analysis. The resulting total ion chromatogram and mass spectra were comparable with those reported previously. Once system performance was verified, the reference smokeless tobacco samples were analyzed beginning with the dry snuff, followed by both versions loose-leaf chewing tobacco, and then both versions of the moist snuff. In general, the total ion chromatograms and mass spectra were reflective of published data on these reference products and the underlying tobacco chemistry. However, there were unexpected compounds found and tentative identifications have been made. These compounds may have originated as a result of product age and storage conditions.

9:20 AM TUESDAY

35. DETERMINATION OF ACRYLAMIDE IN TOBACCO SMOKE AND SMOKELESS TOBACCO PRODUCTS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY. Jingcun WU, Peter Joza, Bill Rickert, Labstat International ULC, Kitchener ON Canada

Acrylamide forms when carbohydrate-containing foods are fried, baked, or roasted at high temperatures and may cause cancer in laboratory animals at high doses. Although LC/MS methods have been developed for the analysis of acrylamide in food, there is little information on tobacco-related applications. In this project, a sensitive, selective and robust LC-MS/MS method was developed and validated for the determination of acrylamide in tobacco related matrices. Due to the complexity of the sample matrix and low expected levels of analyte, extensive sample clean-up steps were found to be necessary. Two grams of the ground sample were extracted with 20mL water and filtered. An aliquot of the filtrate was then washed with dichloromethane (DCM) and the aqueous portion subjected to two

solid phase extractions (SPE); Bond Elut AccuCAT (mixed-mode strong cation and strong anion exchange resin) followed by Lichrolut-EN (polymeric resin). The final eluate was analyzed by LC-MS/MS with positive ESI under MRM mode. Quantification of acrylamide was accomplished using three MS/MS ion transitions (72/55, 72/54, and 72/44) to enhance the method's selectivity. The limit of quantification (LOQ) using the signal-to-noise ratio (S/N =10) of processed tobacco samples was 50 ng/g. Method precision, based on the relative standard deviation (RSD) of 12 replicates for KR2R4F, was less than 15%. The method accuracy was evaluated using 3 levels of fortified sample performed in triplicate and resulted in recoveries ranging from 92 to 107 %. Since the acrylamide content of cigarette smoke is considerably higher, the method is also applicable to diluted cigarette smoke extracts without further clean up.

9:40 AM TUESDAY

36. MARKET SURVEY OF CHEMICAL CHARACTERISTICS OF SMOKELESS TOBACCO PRODUCTS SOLD IN CANADA. Bill Rickert, Peter Joza, Wendy WAGSTAFF, Labstat International ULC., Kitchener ON Canada

This study was undertaken in order to characterize the chemical properties of smokeless tobacco products (STPs) for sale in Canada. A comprehensive marketplace survey was carried out in 2007 and resulted in the collection of 32 brands. The sample set consisted of 15 brands described as either Long or Fine Cut, 7 Snuff, 6 Pouch, 2 Chewing and 2 brands of Plug tobacco. Each of these was analyzed, in triplicate, for nitrate, tobacco specific nitrosamines, ammonia, sodium propionate, sorbic acid, triacetin, humectants, benzo[a] pyrene, Ni, Pb, Cd, Cr, As, Se and Hg using the mandated Health Canada methods. Nicotine, pH, moisture and 'free' nicotine were determined as described in the Federal Register Vol 64, No. 55 (1999). Average moisture content (%) was Long/Fine Cut, 53.3; Pouch, 43.2; Plug, 19.3; Snuff, 16.4 and Chewing, 6.5. Results for nicotine and (calculated 'free' nicotine) expressed as mg/g were: Plug, 10,1 (0.03); Chewing, 3.13 (1.96); Long/Fine Cut, 12.5 (2.64); Pouch, 12.9 (3.69); Snuff, 6.69 (6.41). Results for most other constituents fell into two groups; one designated as 'high' (H) made up of Pouch and Long/Fine Cut products and the second as 'low' (L) consisting of Chewing, Plug and Snuff tobaccos. Average moisture-corrected values (H vs L) were: cadmium (ng/g), 951 vs. 353; nitrate (mg/g), 28.9 vs. 5.71; benzo[a]pyrene (ng/g), 53.5 vs. 15.9; ammonia (µg/g), 8332 vs. 594; NNN (ng/g), 4871 vs. 1303. By way of contrast, the average NNN content of Canadian cigarette filler was 612 ng/gm. In summary, STPs for sale in Canada are rather heterogeneous with wide range of constituent levels. 279,415 kg of STPs were sold in Canada in 2007.

10:00 AM TUESDAY

37. QUANTIFICATION OF DMNA IN SNUS BY LC-MS/MS. Jasper VAN HEEMST, British American Tobacco, Group R&D, Southampton, SO15 8TL, UK

Dimethylnitrosamine (DMNA) is a compound that is present in only minor amounts in most snus tobacco. In line with emerging ESTOC standards on snus chemistry, the amounts of DMNA are routinely monitored by BAT. Therefore, a sensitive and reliable method was required to quantify these relatively low amounts of DMNA. The analysis of DMNA is

particularly challenging because it is a very small molecule, which is also quite volatile and does not produce any unique fragments when analyzed by mass spectrometry.

In the extraction method advantage has been taken of the fact that DMNA is both soluble in diethyl ether as well as in water. Snus tobacco was first extracted with diethyl ether and the extract was then partitioned twice with acidified water. Because acidified water is used, DMNA becomes protonated and thus has a preference for the water layer. In earlier experiments, it was determined that 80% of the DMNA was present in the water layer. Two partitioning steps would therefore recover >95% of DMNA. No further clean-up was required. The water layers were combined and analyzed by LC-MS/MS, operated in positive APCI mode. Low quantification levels could be achieved: 0.1 ng/ml (or 0.45 ng/g on wet weight basis), although reporting limits were typically set to 0.25 ng/ml (or 1.125 ng/g on wet weight basis). An internal standard (d_6-DMNA) was used throughout the method to compensate for any matrix effects.

10:20 AM ***Break***

10:50 AM **TUESDAY**

38. IN VITRO MICRONUCLEUS ASSAY FOR CIGARETTE SMOKE USING A WHOLE SMOKE EXPOSURE SYSTEM. Kosuke OKUWA, Yasuo Fukano and Tomoki Nishino, Japan Tobacco Inc. Tobacco Science Research Center, Yokohama, Kanagawa 227-8512, Japan

Previous studies on the biological assessment of cigarette smoke mainly focused on the total particulate matter (TPM) collected with a Cambridge filter or gas vapor phase (GVP) bubbled through phosphate buffered saline (PBS). However, these extracted fractions of cigarette smoke may not completely reflect the biological effects of the actual aerosol. To research the effects of native cigarette smoke *in vitro*, direct exposure methods have been developed recently. Meanwhile, *in vitro* micronucleus (MN) assays have been reported to evaluate the mutagenicity of cigarette smoke.

The objective of this research was to investigate the MN-inducing activity of whole smoke (WS) and GVP using a whole smoke exposure system, CULTEX®, which allows direct exposure of cultured cells to native cigarette smoke. Smoke was generated according to International Organization for Standardization (ISO; 35ml puff volume, 2 sec duration, once per minute) or Health Canada Intensive (HCI; 55ml puff volume, 2 sec duration, once per 30 sec, with complete blocking for filter ventilation) conditions and exposed to Chinese hamster lung cells (CHL/IU) cultured on microporous membranes. Dosages were adjusted according to the amount of smoke introduced into the exposure position. The unit of the dosage was indicated as the percentage of cigarette smoke (% of cig.). Under both ISO and HCI smoking conditions, WS and GVP from K2R4F reference cigarettes showed dose-related micronucleus responses and dosages which indicated that the highest micronucleus frequency in WS exposure was about one half that of GVP. The CULTEX® system provides insights into biological effects caused by native cigarette smoke *in vitro*.

11:10 AM TUESDAY

39. CONTRIBUTION OF FIVE NITROGEN-CONTAINING COMPOUNDS TO TPM MUTAGENIC POTENCIES IN SALMONELLA TYPHIMURIUM TA98. Yasunari OTSU, Toshiro Fukushima and Hideki Takahashi, Japan Tobacco Inc. Tobacco Science Research Center, Yokohama, Kanagawa 227-8512, Japan

The objective of this study was to clarify the contribution of individual Nitrogen-containing compounds (NCs) in tobacco leaves to the mutagenic potencies of the TPM.

Protein, a mixture of amino acids, sodium nitrate, ammonium chloride and nicotine, which are the major components of the NCs, were individually pyrolyzed using a pyrolysis apparatus model (100% N2 atmosphere, 16.7ml/sec,.gas flow rate and 800°C maximum temperature). The pyrolyzed products were subjected to the mutagenicity assay using strain TA98 with metabolic activation. The results indicated that the pyrolyzed protein and the mixture of amino acids induced strong mutagenicity, but the other NCs did not. Based on the relationship between the amount of individual components, as measured in several single grade tobacco leaves, and the specific activity of each pyrolyzed product, the contribution of the total NCs to the TPM mutagenicity generated from the leaves was calculated to be 20-50%.

To identify the effects of interactions with the other components in the leaves, experimental cigarettes were made using leaves to which an additional 0.5 and 1.0-fold amount of inherent NCs in Burley and Flue-cured, respectively, had been added. The cigarettes were smoked under ISO conditions and the TPMs gathered were consequently assayed. Although the pyrolyzed product of ammonium salt alone showed no activity, the TPM activity from the Flue-cured that it had been added to increased compared with that of the control cigarette. Moreover, the activity of the Flue-cured with added protein showed more than the theoretical increase. It was suggested that the impact of the NCs on the mutagenic potency of the TPM was enhanced by pyrolyzing with other components in the Flue-cured leaf.

11:30 AM TUESDAY

40. CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF BASE FRACTIONS DERIVED FROM CIGARETTE SMOKE CONDENSATE (CSC). Lijia YANG, Larry T. Taylor, Virginia Tech, Blacksburg, VA 24061-0212 and Michael F. Borgerding, William M. Coleman III, Betsy R. Bombick, Jeremy B. Mabe, Kathy P. Putnam, R.J. Reynolds Tobacco Company, Inc., Winston Salem, NC 27102-1487

Chemical characterization of mainstream cigarette smoke to identify possible sources of biological activity has been of interest for several decades. While the base fraction of cigarette smoke condensate (CSC) is thought to be a principal contributor to observed CSC biological activities, the relationship between its chemical composition and biological activity is not clear. In this research, base fractions were extracted from the CSC of 2R4F reference cigarettes by using four solvents: methyl t-butyl ether (MTBE), diethyl ether, dichloromethane, and ethyl acetate. Chemical composition of the base fractions were analyzed by GC-MS using three capillary columns of varying polarity. Retention time

comparison and mass spectral match with known compounds confirmed the identification. An Ames assay (preincubation modification) was employed for testing the mutagenic activity of the base fractions by using *Salmonella typhimurium* (TA98, TA100) with and without addition of a rat liver fraction (S9) mix. A Neutral Red Uptake (NRU) assay (CHO cells, -S9) was used to measure the cytotoxicity, from which EC50 of each fraction was calculated. The MTBE and diethyl ether fractions showed the highest mutagenic and cytotoxic activities in both Ames and NRU assays. About 90 compounds were identified in the base fractions, of which 11 compounds were previously reported to yield a positive Ames test for TA98 or TA100, and 21 compounds were previously reported to be negative in the Ames test. The biological activity of the MTBE fraction was also compared with the activity of the base fraction extracted via diethyl ether that was stabilized by butylated hydroxytoluene (BHT). BHT had little, if any, effect on observed biological response.

11:50 AM TUESDAY

41. EFFECTS OF PARTICLE SIZE DISTRIBUTION ON CIGARETTE PHYSICAL QUALITY. SHEN Xiaofeng[1], Du Jinsong[1], LuoDengshan[1], Li Yuefeng[2], Li Huajie[2], [1]Zhengzhou Tobacco Research Institute of CNTC, [2]Fujian Branch of China Tobacco Industry Co.

Cut tobacco size distribution (CTSD) is a key factor influencing cigarette physical quality. In this paper, a batch of cut tobacco was passed through a Protos maker for six times to generate six different CTSD. The relationship between CTSD and cigarette physical quality was established by grey incidence. The results showed that: 1) There existed a critical size value. Those portions, which were above the size value, affected the physical quality of cigarette positively, while those portions below the value affected negatively. In the said test conditions, the size value was 2.80mm. 2) There existed a maximum incidence coefficient between cut tobacco size and cigarette physical quality. The closer to this marginal size, the greater the incidence coefficient. The marginal size was different for different cigarette physical quality parameters. In the said test conditions, it was 2.80mm for individual cigarette weight, tobacco rod density and draw resistance, 0.71mm and 1.00mm for the ends fallout and loose ends respectively. 3) The effects of CTSD on individual cigarette weight, tobacco rod density draw resistance, the standard deviation of draw resistance, ends fallout and loose ends were contrary to its effects on the standard deviation of individual cigarette weight, tobacco rod density and hardness, however, the ends fallout and loose ends was more affected by shorter strands, therefore, to manufacture a cigarette of good physical quality demands the majority of cut tobacco to fall in a given size range and minimize the proportion of shorter strands. In the said test conditions, the most probable size of the cut tobacco was considered to fall in the range of 2.00~4.75mm.

12:10 PM LUNCH

TUESDAY MORNING, SEPTEMBER 23, 2008

SESSION B ***Session Chair: Victor Little***

9:00 AM TUESDAY

42. SIMULTANEOUS DETERMINATION OF 1,3-BUTADIENE, ETHYLENE OXIDE, VINYL CHLORIDE, PROPYLENE OXIDE, ACRYLONITRILE, BENZENE AND ISOPRENE IN MAINSTREAM VAPOR PHASE CIGARETTE SMOKE. Ji-Zhou DONG, R.J. Reynolds Tobacco, Winston-Salem, NC 27105 USA

This presentation is a continuation from previous TSRC conferences in which a simple, rapid and accurate procedure was described for the quantitative determination of 1,3-butadiene, ethylene oxide, vinyl chloride and propylene oxide in mainstream cigarette smoke. In this presentation, three additional analytes (acrylonitrile, isoprene and benzene) are added to that method. A procedure is also described for in-house preparation of calibration gas standards for all seven analytes. This calibration procedure reduces costs and eliminates uncertainties of purchased premixed gas standards. The vapor phase smoke is collected in a 10L Tedlar gas bag and analyzed by gas chromatography and mass spectrometry operated in the SIM mode. Five isotopically labeled compounds are used as internal standards for quantitation and are n-butane-d10, propylene oxide-d6, acrylonitrile-d3, isoprene-d8 and benzene-d6. This method was validated by comparing 2R4F results obtained under two smoking regimens with those found in the literature. Accuracy of the procedure was determined via standard addition experiments where recoveries were all within100±10%.

9:20 AM TUESDAY

43. SELECTIVE DETECTION AND CLASSIFICATION OF COMPOUNDS IN TOBACCO SMOKE BY GCXGC-TOFMS. Donald C. HILTON, LECO Corporation, Fort Myers, FL and Jean-Marie D. Dimandja, Spelman College, Atlanta, GA

Chromatographic analysis of tobacco or tobacco smoke results in the identification of many compounds present in the samples. The use of high resolution chromatographic techniques such as GCxGC analysis can produce lists of tens of thousands of compounds present in a single sample, giving rise to the problem of extracting useful information from all the data.

With mass spectral data, compounds can be identified according to compound classes. Some classes of compounds, such as fatty acid methyl esters, are easily identified by the presence of specific masses in the spectrum and the lack of significant abundance of other molecular fragments. Other compounds, such as chlorinated compounds are readily identified by the characteristic isotope cluster shown for the molecular ion, once the molecular ion is located in the spectrum. In other cases, such filters may lack specificity, but the structure of a GCxGC chromatogram tends to localize compounds by class, so the use of automatic spectral identification may be used with selection by location in the chromatographic plane to select the compounds of interest.

This presentation shows the application of such automatic identification to locate compounds of classes in which identities might be desired or in which only summary information is needed. Chromatograms of smoke are automatically filtered for chlorine-containing compounds and results for individual compounds are provided. Summary data is obtained from smoke samples, giving estimates of total concentration of compounds by classes such as fatty acid methyl esters, methyl ketones, benzene aromatics and other compounds, which can be used in the comparison of one type of cigarette against another.

9:40 AM TUESDAY

44. DEVELOPMENT AND VALIDATION OF A METHOD FOR THE DETERMINATION OF 17 POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN MAINSTREAM TOBACCO SMOKE. J. Evan TARRANT, Ken Mills, Cynthia Williard, Lorillard Tobacco Company, Greensboro, NC 27420

Polycyclic aromatic hydrocarbons (PAHs) are compounds formed as a result of incomplete combustion of organic fuels or materials (e.g. gas, coal, wood, or tobacco). In the past, the analytical determination of PAHs in mainstream tobacco smoke has presented challenges in method precision, accuracy, potential matrix interferences, and long analysis run times. The gas chromatograph/mass selective detector (GC/MSD) method presented in this paper resulted in shorter run times, better analyte recovery, and quantitation of six additional PAH analytes compared to the current in-house methodology that was presented at the 2007 TSRC. The method also improved accuracy with an average analyte recovery of 97%, versus 86-92% reported in the literature. Lastly, capillary column technology, a Restek RXi-17 15m x 0.25mm x 0.25mm, eliminated matrix interferences and decreased run times by approximately 25%, while it maintained excellent repeatability and precision compared to in-house and published methods.

10:00 AM TUESDAY

45. THE STUDY ON THE GENERATION OF 17 POLYCYCLIC AROMATIC HYDROCARBONS DURING PUFFING. Shinya YOSHIDA and Yoji Uwano, Japan Tobacco Inc., Yokohama, Kanagawa, Japan

In past studies, the amounts of polycyclic aromatic hydrocarbons (PAHs) in cigarette mainstream smoke have been usually reported as the yields from one cigarette. But these results include the effects of tobacco column filtration, the number of puffs, and the ventilation of the cigarette paper. To consider the PAHs generated during puffing, it is necessary to omit these effects. The aim of this study was to investigate the relationship between the cigarette design parameters and the yields of PAHs, particularly during puffing.

In order to decrease the effects of tobacco column filtration and paper ventilation, the total particulate matter (TPM) was collected on a Cambridge filter pad from a single puff of short cigarettes whose butt length was set at 10 mm. The pad was extracted with *i*-propanol. Seventeen kinds of PAHs in the TPM were determined using GC/MS after double solid-phase extraction treatments of the *i*-propanol extracts. The difference in tobacco type (flue-cured and burley), the ratio of expanded tobacco, and the puffing volume were examined.

The following results were found: 1) The yields of five PAHs from short cigarettes made of flue-cured were slightly higher than those made of burley. Six other PAHs were found at a similar level for flue-cured and burley. 2) Expanded tobacco had little effect on the yields of PAHs in the range from 20% to 70% of the blended tobacco. 3) The yields of PAHs during puffing correlated almost linearly with puff volumes between 10.5 and 55 mL/2s.

10:20 AM ***Break***

10:50 AM TUESDAY

46. FACTORS AFFECTING REPEATABILITY AND YIELD WHEN SMOKING BIDI CIGARETTES. Ian TINDALL and Tim Mason, Cerulean, Milton Keynes, MK14 6LY United Kingdom

Indian traditional cigarettes, known as Bidis, are now becoming available world wide and the machine smoking of these products is of interest to health professionals and regulators alike.

The repeatability of smoking experiments when applied to Bidis can be poor and is often attributed to the "hand made" nature of these products. The variability with which the product is sealed in a smoking machine, together with the natural variability of manufacture is explored through the use of specialised test equipment establishing the sealing efficiency for various rods.

The influence of the variability of three physical factors, weight, inserted PD and length upon yield is examined through the use of full factorial experiments. The yields of CO and TPM are considered for three popular brands of bidi and comparisons made with machine made conventional cigarettes. Recommendations upon selection of parameters to reduce yield variability of bidis to that achieved from machine made cigarettes are presented.

Further conclusions are drawn as to the factors that should be selected if yield maxima are to be achieved. These conclusions are tested and confirmed on a separate bidi brand.

47. Moved to poster session #17a

11:10 AM TUESDAY

48. IS THERE PENTOBARBITAL IN TOBACCO? Brian E. Rood, Department of Chemistry, Mercer University, Macon, GA, and John H. LAUTERBACH, Lauterbach & Associates, LLC, Macon, GA USA

In 2006, Jabloński and his colleagues at the Medical University Bialystok in Poland reported finding pentobarbital in tobacco and mainstream cigarette smoke [Jabloński *et al.*, Food Chem. Toxicol. 2006 Nov;44(11):1948-51]. They reported pentobarbital levels of 3 to 6 μg/cigarette [about 4 to 8 ppm based on about 700 mg tobacco (on a dry weight basis) per cigarette] in tobacco taken from cigarettes and 2 to 4 μg/cigarette in mainstream smoke obtained under nonstandard conditions. Those authors also claimed finding pentobarbital in raw, unprocessed tobacco at about the same level as found in cigarette tobacco. There are

apparently no other reports in the peer-reviewed literature to support or deny Jabloński's findings. Therefore, the purpose of this work was to duplicate as closely as possible Jabloński's technique and apply it to both tobacco taken from cigarettes as well as from smokeless tobacco products. We used a Shimadzu QP-5050 GC-MS system with a DB-5 type capillary column. To date, we have analyzed both the tobacco from a commercial US blend cigarette and the 1S2 reference dry snuff have been analyzed for pentobarbital. So far, we have not been able to duplicate the findings reported by Jabloński and his colleagues.

11:30 AM TUESDAY

49. STUDY ON THE ANALYSIS OF TOBACCO COMPONENTS BY USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY/TIME-OF-FLIGHT MASS SPECTROMETER. ZHANG Jianxun, Zhengzhou Tobacco Research Institute, No.2, Fengyang Street, Zhengzhou Hi-Tech Industrial Development Zone, 450001, P.R. China

In this study, the tobacco extractives has been investigated by using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometer (GCxGC/ TOFMS). The different column systems were tested and compared, and the proper GCxGC/TOFMS conditions were suggested. Auto data processing of peak table provided by TOFMS software combined with manual identification was used to assign the components. About 1093 compounds with S/N≥100 were identified by using the developed method. Comparative analyses of the extractives from four commercially available tobacco grades (One is from Zimbabwe, three are from China) were performed, quantitative differences of 83 compounds (from the four grades) were found to be significant.

11:50 AM LUNCH

TUESDAY AFTERNOON, SEPTEMBER 23, 2008

SESSION A ***Session Chair: John Lauterbach***

1:40 PM TUESDAY

50. CURRENT AND HISTORICAL TRENDS IN YIELDS OF FREE-BASE NICOTINE BY CANADIAN CIGARETTES IN RELATION TO SMOKE PH AND CIGARETTE DESIGN PROPERTIES. Bill Rickert Peter Joza, Mingliang BAO, Labstat International ULC., Kitchener ON Canada, N2C 1L3

The amount of free-base nicotine (FBN) in tobacco smoke and its relationship to smoke pH and cigarette design continues to be controversial. This study was undertaken to provide both current and historical data in this regard. The sample set consisted of 14 tobacco products including plain, filter-tipped, fine-cut, 120 mm cigarettes and cigarette-sized cigars, plus 7 brands of 'historical' Canadian cigarettes (plain and filter-tipped) purchased at five points in time between 1969 and 2007. Data for paper porosity, tip ventilation pressure drop were obtained using ISO methodologies. Smoke pH was determined using Health Canada method T- 113. Yields of FBN were determined using headspace solid-phase micro-extraction (HS-SPME), combined with gas chromatography/mass spectrometry. With respect to 'current' cigarettes, under ISO smoking conditions (60/30/2 – puff volume, interval, duration) yields of FBN averaged 28.7 μg/cig, (range: 9.46 to 49.1) and were positively correlated with smoke pH (R^2 = 0.54). When expressed as a percentage of total nicotine, values ranged from 0.7 to 7.4% (average, 3.4%) and were inversely proportional to both tar yield (R^2=0.79) and moisture content of TPM (R^2=0.64) but positively correlated with tip ventilation (R^2 = 0.88). Under intensive smoking (55/30/2 – 100% vent blocking), yields ranged from 15.7 to 34.1 μg/cig (average 21.2 μg/cig). In this case, the percentage of FBN averaged 0.9% and was independent of tar yield, moisture content and smoke pH. Considering the historical sample set, yields of FBN under ISO smoking conditions averaged 10.6 μg/cig between 1969 and 1993, (range: 5.27 to 18.1 μg/cig) and 22.8 μg/cig between 1995 and 2007 (range: 11.1 to 32.0 μg/cig). As with current products, yields of FBN were highly correlated with smoke pH.

2:00 PM TUESDAY

51. DETERMINATION OF SOLANESOL IN EXHALED SMOKE FROM THREE CIGARETTES WITH DIFFERENT HUMECTANT LEVELS. Serban MOLDOVEANU and William Coleman III, R.J. Reynolds Tobacco Co., Winston-Salem NC 27105

In this study, the level of solanesol in exhaled cigarette smoke from a commercial cigarette with no additives (Cigarette A) and two experimental cigarettes was measured. Both experimental cigarettes were made with the same specifications as the commercial cigarette except for 3% added propylene glycol (PG) (Cigarette B), and 2.3% added glycerin (Cigarette C). The number of smokers from whom the exhaled smoke was collected was 22 smokers for Cigarette A, 10 for Cigarette B and 12 for Cigarette C. Exhaled smoke was collected using a vacuum assisted procedure that avoids strain in exhaling, and solanesol was analyzed using an HPLC technique. The cigarette butts from the smokers were collected and also analyzed for solanesol. The results obtained for the cigarette butts from the smokers were

used to calculate the level of solanesol in the smoke delivered to the human subject, based on calibration curves. The amount of solanesol retained by the smokers was, on average, 312 µg/cig for Cigarette A with 17.2% relative standard deviation (RSD), 348 µg/cig with 20.4% RSD for Cigarette B, and 303 µg/cig with 20.3% RSD for Cigarette C. The retention % of solanesol from Cigarette A averaged 70.9% with 10.5% RSD, 70.8% with 14.5% RSD for Cigarette B, and 69.4% with 10.5% RSD for Cigarette C. ANOVA single factor analysis indicated, as expected, no significant differences in the retention of solanesol for the three cigarettes tested.

2:20 PM TUESDAY

52. RESULTS FROM A NATIONAL SURVEY OF YIELD IN USE. Paul R. NELSON and Thomas J. Steichen, R.J. Reynolds, Winston-Salem, NC 27102-1487

A study was completed to determine yield in use (YIU) from a wide variety of cigarettes. Smokers of 26 different brand-styles were recruited to collect used filters from all cigarettes smoked during the course of a single day. The cigarette selection covered a wide range of FTC "tar" deliveries (0.5 - 17.9 mg "tar"/cigarette) and included king-size and 100 mm products in addition to menthol and non-menthol products. A subset of brand-styles had multiple panels or visits to evaluate reproducibility and repeatability of the YIU method. A total of 1330 smokers, recruited nationally, participated in the study.

In general, YIU increased with increasing FTC yield. There is substantial inter-individual variability of yield within each of the brand-styles examined. As has been reported previously, average YIU for each brand-style was higher than its FTC yield. Each brand-style was also smoked using Massachusetts (MA) and Canadian Intense (CI) regimes. The MA regime predicted YIU well for the 1 mg "tar" cigarettes, but increasingly tended to overestimate YIU as FTC yield increased. The CI regime consistently predicted yields at or above the 95th percentiles of those measured for human smokers. There was no statistically significant difference in YIU trends based on cigarette length or menthol inclusion.

The reproducibility/repeatability tests allowed prediction of the number of smokers in each group that would be needed to see statistically significant differences among products in both paired and unpaired test designs.

2:40 PM TUESDAY

53. SIMULTANEOUS DETERMINATION OF NICOTINE AND UV ABSORBANCE OF TIP AND PAD EXTRACTS USING A CIRCULAR DICHROISM / UV SPECTROMETER. P. CLAYTON, British American Tobacco, Group R&D Centre, Southampton, SO15 8TL UK, A.F. Drake and R.J. Fielding, Applied Photophysics Limited, Leatherhead, KT22 7PB UK

The estimation of human smoke exposure through the analysis of cigarette filters collected from subjects and consumers has historically relied on the measurement of nicotine in filter extracts using GC with flame ionisation detection and the measurement of tar by UV absorbance at 310nm[1]. An investigation has been conducted to assess whether circular dichroism (CD) spectroscopy confers any advantages compared with the established methodology.

Nicotine satisfies both prerequisites for CD activity: it absorbs in an accessible region of the spectrum and it is chiral. Since nicotine is the major tobacco alkaloid, and its S-enantiomer predominates, the CD signal at 270nm is overwhelmingly attributed to nicotine[2]. This may be used to quantify nicotine in both Cambridge filter pad and filter tip extracts (IPA and methanol respectively) without sample clean-up or chromatographic separation, only requiring dilution in methanol. The Chirascan CD instrument (Applied Photophysics Limited, UK) is able to quantify nicotine down to 0.5μg/mL in matrix (based on signal-to-noise ratio) and the method was linear to at least 500μg/mL.

Whilst quantifying nicotine by CD at 270nm, the Chirascan instrument can measure simultaneously the UV absorbance of sample extracts at 310nm. Absorbance (310nm) down to 0.05 absorbance units could be measured. The method is both economic and rapid since addition of internal standard is not required and instrument calibration is only necessary infrequently.

[1]C.J. Shepperd et al., (2006) Beitrage zur Tabaksforschung, 22, 176
[2]B.Liu et al., (2008) J.Chromatogr. B 865 13-17

3:00 PM ***Break***

3:30 PM **TUESDAY**

54. QUANTIFICATION OF METABOLITES OF ARYLAMINES, ACROLEIN and CROTONALDEHYDE IN URINE SAMPLES. Mehran SHARIFI, Peter Joza, Bill Rickert, Labstat International ULC, Kitchener Ontario, Canada, N2C 1L3

The tandem mass spectrometry coupled with chromatography has been widely used for the analysis of urinary metabolites of compounds found in tobacco smoke. The purpose of this project was to simplify sample preparation procedures and, at the same time, to increase sample throughput without sacrificing method sensitivity. For arylamines, the improved method resulted in the isolation and quantification of 2 additional compounds (i.e. 2-aminonaphthalene and 3-aminobiphenyl) as compared to the reference method [1]. The automation of liquid phase extraction step allowed an increased sample throughput. The introduction of an additional clean up step after derivatization resulted in cleaner samples and less maintenance of the detection system. Method sensitivity was increased with limits of quantification below 7 pg/mL for all arylamines. The linear calibration range was from 14-2200 pg/mL. Method accuracy, based on recoveries of added arylamines (i.e. laboratory fortified matrix (LFM)), was 100±14 % for amounts ranging from 300 to 1500 pg/mL of urine. With respect to acrolein and crotonaldehyde, an automated single-step reversed-phase SPE procedure was developed for the analysis of urinary mercapturic acid derivatives 3-hydroxypropyl (HPMA) and 3-hydroxy-1-methylpropyl (HMPMA). The limits of quantification were 1.5 ng/mL and 16.2 ng/mL respectively with a coefficient of variation of less than15% for both analytes. Recoveries determined for nonsmoker's urine fortified between 18-460 ng/mL (HPMA) and 304-519 ng/mL (HMPMA) ranged from 85% to 110%.

[1] Riedel et al., Journal of Analytical Toxicology, 30, 187-195, 2006.

3:50 PM TUESDAY

55. OPTIMAL QUANTITATIVE ANALYSIS FOR URINARY BIOMARKERS OF EXPOSURE OF POLYCYCLIC AROMATIC HYDROCARBONS THAT VARY IN RING NUMBER: 1-HYDROXY PYRENE, 3-HYDROXYPHENANTHRENE, AND 2-HYDROXY BENZ[c]PHENANTHRENE. Larry T. TAYLOR, Mehdi Ashraf-Khorassani, Virginia Tech, Blacksburg, VA 24061-0212 and Michael F. Borgerding, William M. Coleman III, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

Tobacco smoke, certain foods, and diesel exhaust are known to contain polycyclic aromatic hydrocarbons (PAHs). Historically, 1-hydroxypyrene (1-OHP), a metabolite of pyrene, has been the most commonly used biomarker of exposure to PAHs. More extensive analytical methods covering multiple urinary PAH metabolites, however, are needed to adequately assess human exposure to a mixture of PAHs. Analysis of replicate single low dose urine samples for many hydroxy PAHs with quantitative recovery and high precision requires effective enzymatic hydrolysis, efficient sample clean up, high resolution chromatography, selective detection, sensitive response, and fast turn-around time. During the past five years, several reports (to different degrees) have appeared that address with varying success these issues. This presentation will focus on the analysis of 1-OHP, 3-hydroxyphenanthrene (3-OHPh), and 2-hydroxybenz[c]phenanthrene (2-OHBcPh) in urine. Optimized parameters for solid phase extraction along with the merits of quantitative analysis using liquid chromatography via fluorescence versus tandem mass spectrometric electrospray detection will be described. Replicate percent recoveries via external and internal standard calibration for each of the three analytes will be reported. The advantage and disadvantage of single versus multiple internal standard calibrations as it relates to multiple urinary PAH metabolite determination will be discussed.

4:10 PM TUESDAY

56. THE USE OF MAGNITUDE ESTIMATION TO ASSESS THE ODOUR AND IRRITATION OF SIDESTREAM SMOKE - (PART 1) WITH THE FABRIC METHOD. Virginie M.E. COTTE, Vanessa Lovell, David A. Saich, Teresa May and Julie Cote and Paul D. Case, Group R & D, Millbrook, Southampton SO15 8TL, UK

Sidestream smoke, which is the smoke that comes from the lit end of the cigarette, is an active area of research and development for cigarette designers. The overall objective of this research was to develop a 'fabric' methodology for the assessment of sidestream smoke odour and irritation. The method involved sidestream smoke deposited onto samples of fabric held in a perspex box. The fabric was then transferred into jars and assessed by panellists. The first stage was to monitor the consistency of sample sets and assess levels of repeatability, by analysing the headspace of samples. The second stage was to identify the optimum amount of smoke to be assessed by building a dose-response relationship between the amount of sidestream smoke and the intensity of the sensory perception (irritation and odour).

To achieve the second stage, a known reference standard (1-butanol) was used to determine the detection threshold under the conditions of the 'fabric in jar' method. This approach was carried out by preparing serial dilutions of 1-butanol. These dilutions were assessed

to measure psychometric functions for the detection of odour and nose irritation. The detection threshold was obtained at the 50% chance-corrected probability point. Panellists were recruited, selected and trained according to the ISO method 8586-1. Panellists were then trained to score intensity ratings using ratio-scaling (magnitude estimation) and a standardized procedure using 1-butanol with a reference.

Subsequently, panellists were trained to rate a range of sidestream smoke concentrations (reference cigarette 3R4F). The optimum sidestream concentration to be assessed was identified half-way between detection and terminal threshold. The fabric method was used to examine a commercial cigarette and a prototype generating known sidestream smoke amounts.

4:30 PM TUESDAY

57. THE USE OF MAGNITUDE ESTIMATION TO ASSESS THE ODOUR AND IRRITATION OF SIDESTREAM SMOKE - (PART 2) WITH THE CUBICLE METHOD.
Virginie M.E. COTTE, Vanessa Lovell, David A. Saich, Teresa May and Julie Cote and Paul D. Case, Group R & D, Southampton SO15 8TL, UK

Sidestream smoke, which is the smoke that comes from the lit end of the cigarette, is an area of active research and development for cigarette designers. The overall objective of this research was to develop a 'cubicle' based methodology for the sensory assessment of sidestream smoke. The volume of the cubicles is approximately 1 m^3. The first stage was to ensure that the cubicles were suitably sealed by monitoring the CO decay rates generated from sidestream smoke. The second stage was to identify the optimum amount of smoke to be assessed by building a dose-response relationship between the amount of sidestream smoke and the intensity of the sensory perception (irritation and odour).

To achieve the second stage, a known reference standard (1-butanol) was used to determine the detection threshold under the conditions of the cubicle method. This approach was carried out by generating aerosols from serial dilutions of 1-butanol. The aerosols were generated using an ultrasonic particle generator (SONAER model 241PG) and their respective particle concentrations were measured using a Condensation Particle Counter (TSI model 3022). The aerosols were assessed to measure psychometric functions for the detection of odour and nose irritation. The detection threshold was obtained at the 50% chance-corrected probability point. Panellists were then trained to score intensity ratings using ratio-scaling (magnitude estimation) and a standardized procedure using 1-butanol with a reference (fixed modulus).

Subsequently, panellists were trained to rate a range of sidestream smoke concentrations (reference cigarette 3R4F). The optimum sidestream smoke concentration to be assessed was identified half-way between detection and terminal threshold. The cubicle method was used to examine a commercial cigarette and a prototype generating known sidestream smoke amounts.

4:50 PM ADJOURN

TUESDAY AFTERNOON, SEPTEMBER 23, 2008

SESSION B ***Session Chair: Joe Wanna***

1:40 PM TUESDAY

58. SYSTEMATIC STUDY: SHOULD DIFFUSIVITY BE CONSIDERED AS A MAIN PARAMETER OF CIGARETTE PAPER? Dietmar VOLGGER, Dieter Möhring, Roland Zitturi, and Irene Rohregger, Papierfabrik Wattens GmbH & Co KG, A-6112-Wattens

The property of cigarette paper is usually defined by parameters like porosity, basis weight, chalk content, concentration of burn additive etc. The influences and interactions of these parameters were topics of several studies performed in the past.

In this study a further cigarette paper parameter has been investigated, which showed to have a significant impact on several key properties and developments of the cigarette industry, especially for CO reduction and LIP. 105 different paper grades have been investigated. The study involved examining the effect of the levels of basis weight, filler content, porosity, burn additive content and type and pulp grade. The statistical evaluation of the results showed significant influences and interactions of several paper parameters on the diffusivity of cigarette paper.

In a second stage the impact of thermal treatment was investigated. The paper samples were treated at elevated temperature for a defined time. It could be shown that relevant effects can be observed only for the heat treated papers.

Finally it could be shown that diffusivity is a fundamental property of cigarette paper for mainstream smoke yields and LIP performance of a cigarette.

2:00 PM TUESDAY

59. DETERMINATION OF ACETATE TOW CAPABILITY RESPONSES ACROSS STANDARD, SLIMS AND SUPER-SLIMS FILTER ROD CIRCUMFERENCES. Francisco RUVALCABA, Celanese Acetate LLC, Narrows VA 24124

Given the growing market of the Slims and Superslims cigarettes in the world, there is an increasing interest on improved and more accurate estimates of tow performance and filter design parameters for these filter dimensions. A study was conducted in Celanese to obtain relationships and predictive equations on capability line Minimum Point, Maximum Point and Slope as a function of total denier and Denier Per Filament. These relationships will serve for a better yield estimation of current tow items working in different circumferences where they were not designed for. A combination of low, mid and high dpf and total denier tow items was selected to cover a wide range of items used in the market. Filter rods were produced in a standard AF2 rodmaker at 24.33, 22.76, 20.88, 18.99, and 16.79 mm circumferences. Charts and relationships found will be presented.

2:20 PM TUESDAY

60. AT LINE MEASUREMENT OF TRIACETIN PLASTICIZER CONTENT IN MONOACETATE FILTERS USING THE MICROWAVE METHOD. James VINCENT and Ian Tindall, Cerulean, Milton Keynes, MK14 6LY, United Kingdom

Plasticizer, commonly triacetin, is a vital addition to cellulose acetate tow to harden cigarette filters and so ensure that they pass cleanly through the assembly process and maintain their designed filter characteristics during smoking. However excess plasticizer causes melt-holes in filters that result in customer complaints and can impact deleteriously on tar yield. Control of plasticizer content is thus crucial but to date there has not been an accurate and effective method of determining plasticizer content in real-time at-line. Wet-dry methodologies are examined and shown to vary greatly and produce inaccuracies in delivery of worse than ±20%. An at-line measurement technique is described that provides quantitative information on both the amount of plasticizer and its distribution within the filter and results of evaluation trials are presented. The accuracy of this method is determined and compared with wet/ dry methods. The method is shown to have accuracy comparable with GC methods with results available instantaneously to the user. The use of this method to provide process control information and indication of set-up issues that can result in melt-holes is discussed. The influence of time to measurement on measurement accuracy is explored and recommendations regarding sampling made.

2:40 PM TUESDAY

61. THE INFLUENCE OF WATER ON THE SELECTIVE FILTRATION OF PHENOL IN THE UPSTREAM VERSUS DOWNSTREAM FILTER SEGMENTS. A. S. WATTS and S. A. Wilson, Eastman Chemical Company, Kingsport, TN 37662

Selective filtration is one of the unique properties of cellulose acetate filters. Previous studies have shown that the amount of moisture in the filter enhances this filtration property. Additionally, increases in the ventilation level of a filter have been shown to reduce the tar delivery and affect a filter's ability to remove phenol. This study explored the influence of water in the upstream and downstream segments of the filter on the selective filtration of phenol. Experimental cigarettes were prepared with ventilation levels ranging from 0-75% with 7% triacetin, and tested under ISO and aggressive smoking conditions. The filters were cut into upstream and downstream segments and analyzed separately. Nicotine, water, triacetin, and phenolics were measured in the Cambridge pads and in both segments of the filters. Filtration efficiency for both segments was assessed by a UV spectrophotometric technique.

Under ISO conditions, the results show that the removal efficiencies for tar, nicotine, and phenol in the upstream segments of the filters increased with increasing ventilation. Although there is a corresponding decrease in water content of the filter with increasing ventilation level, more water is retained in the upstream segment, which also shows greater selectivity for phenol. These results support the role of water in enhancing the selective removal of phenol by cellulose acetate filters.

3:00 PM ***Break***

3:30 PM TUESDAY

62. THE PERFORMANCE OF CARBON FILTERS AT DIFFERENT SMOKING REGIMES. Tony McCORMACK and Mike Taylor, Filtrona Technology Centre, Jarrow, Tyne & Wear NE32 3UP, UK

Numerous papers have been previously been presented examining the characteristics of filter cigarettes containing activated carbons. These cigarettes have generally been smoked under ISO smoking conditions and little information is available on how the performance of carbon filters is affected at more intense smoking regimes. Under these more intense regimes, the contact times between smoke constituents and the carbon are significantly reduced, thereby leading to a reduced efficiency of removal of smoke vapour phase compounds by the filter.

This paper explores the effects of using non-ventilated filter cigarettes containing carbons at different activity levels that have been derived from different precursor materials (coconut and coal) under three different smoking regimes – ISO, Massachusetts and Canadian Intense. The relative retention by the filter of twelve vapour phase compounds – notably carbonyls and hydrocarbons – were measured using a methodology described at previous TSRC conferences. Filters were also tested with two different weights of carbon so that the final experimental matrix could examine the relative influence of carbon weight, carbon activity, carbon precursor material and smoking regime on filter performance. Conclusions concerning the interactions between these various factors will be discussed.

3:50 PM TUESDAY

63. CONSTRUCTION OF A MODEL FOR BENZENE ADSORPTION FOCUSED ON THE DISTRIBUTION OF CHARCOALS IN CIGARETTE FILTERS. Akihiko SUZUKI, Takashi Hasegawa and Yoichiro Yamashita, Japan Tobacco Inc., Yokohama, Kanagawa, Japan

Charcoal is one of the most effective technologies applied to cigarette filters to remove volatile organic compounds (VOCs) from cigarette smoke in large quantities and substantially changes the characteristics of the smoke mainly due to adsorption. And a number of extensive researches have been conducted to optimize the removal potential of charcoal.

The purpose of this study was to evaluate the influence of cross-sectional charcoal distribution in cigarette filters on the adsorption behavior for VOCs in cigarette smoke. Benzene was adopted for evaluation as a typical compound of VOCs, and an adsorption-model of charcoal filters considering the flow of cigarette smoke in filter-tips was constructed by using a fluid analysis software, FLUENT. The experimental adsorption efficiencies of various types of charcoal filters were compared with efficiencies calculated by the adsorption-model assuming that charcoals were homogeneously-distributed in cross-section of filter-tips.

In the case of paper-charcoal-filters (PCF), experimental adsorption efficiency for benzene was approximately equal to calculated ones. This result showed that charcoals were

distributed homogeneously in cross-section of PCF. As for acetate-charcoal-filters (ACF), on the other hand, experimental value was about 20% lower than calculated value. This result indicated heterogeneous distribution of charcoals in cross-section of ACF.

It was found that the cross-sectional distribution of charcoals in the filter-tips has a large impact on the adsorption efficiency for VOCs in cigarette smoke.

4:10 PM ADJOURN

WEDNESDAY MORNING, SEPTEMBER 24, 2008

COMBINED SESSION ***Session Chair: Vlad Hampl***

9:00 AM WEDNESDAY

64. NITROGEN COMPOUNDS ON MAINSTREAM SMOKE AND TOBACCO PRECURSORS. Bernard BREGEON, Micheline Coupé, Valérie Troude, Nabil Bouzaidi-Tiali, Sophie Gadois-Pommereul, Altadis Research Center, Fleury les Aubrais 45404 - France

Among the Hoffmann list components, nitrogenous compounds constitute a wide family and some of them can be related to nitrogen compounds on tobacco. During the two last TSRC congresses, the spiking approach was presented to demonstrate relationships between HCN yields, Ames responses and tobacco precursors.

Our objective presently is to improve the understanding of the formation mechanisms of additional nitrogenous compounds in smoke by analytical determinations on cigarettes made with enriched blends. Added compounds cover representative nitrogen components present in tobacco such as amino-acids and proteins. The precursors studied are added on a US type blend prior the cigarette making. Chemical and biological determinations on smoke, such as volatiles, semi-volatiles, aromatic amines, hetero-cyclic aromatic amines… and Ames test (Strain TA 98 + S9) have been achieved with classical methods or protocols.

After description of the experimental protocol, some figures show the relationships between each added compound and smoke composition. These results validate this spiking approach. This additional step enables us to undergo mechanisms of formation for nitrogenous compounds in smoke. Finally, such tobacco and smoke relationships would allow to control smoke yields depending on residual amounts of precursors in each type of tobacco or blend.

9:20 AM WEDNESDAY

65. A STUDY OF THE REACTION BETWEEN QUINONE AND 2R4F CIGARETTE SMOKE CONDENSATE. W. M. COLEMAN, III, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

A study using atomic emission detection (AED) investigations to explore the fate of quinone added into 2R4F cigarette smoke condensate (CSC) have been performed. Both natural isotope quinone and ^{13}C labeled quinone were used in the study. When coupled with a gas chromatographic separation (GC/AED), the AED provided informative new data on ^{13}C isotope enriched products generated following reactions between 2R4F CSC and the quinone. Two ^{13}C containing species were detected by GC/AED. Matching chromatographic separation using gas chromatography/mass selective detection (GC/MSD) allowed for a confident structural assignment of a relatively minor CSC $^{13}C_6$ quinone reaction product as nitrohydroquinone ($^{13}C_6$NO2HQ). The chemical mechanism accounting for the formation of $^{13}C_6NO_2HQ$ in the CSC was envisioned to be a reaction product between HONO and $^{13}C_6$Quinone ($^{13}C_6Q$) to form $^{13}C_6NO_2Q$, followed by reduction of $^{13}C_6NO_2Q$ to $^{13}C_6NO_2HQ$.

The amount of $^{13}C_6NO_2HQ$ accounted for ~6% of the added $^{13}C_6Q$. Identical trends in reaction chemistries were found for experiments with $^{12}C_6Q$. The major reaction product detected upon addition of $^{13}C_6Q$ to the 2R4F CSC sample was $^{13}C_6HQ$. $^{13}C_6HQ$ accounted for, on average, ~47% of the initial $^{13}C_6Q$ concentration. Identical trends in reaction chemistries were found for experiments with $^{12}C_6Q$. No additional ^{13}C containing species were detected. A ^{13}C AED compound independent calibration (CIC) approach under the operating conditions was not possible. This body of work further expands the knowledge regarding possible reactions of quinone and hydroquinone in CSC.

9:40 AM WEDNESDAY

66. A DIELS-ALDER REACTION AMONG CIGARETTE MAINSTREAM SMOKE COMPONENTS. W. M. COLEMAN, III, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

The presence of a product(s) from a Diels-Alder reaction between cigarette mainstream smoke components has been described. Data from carbon-13 nuclear magnetic resonance (^{13}C NMR), gas chromatography-atomic emission detection (GC-AED), and gas chromatography-mass selective detection (GC-MSD) revealed a Diels-Alder reaction product resulting from the reaction of benzoquinone (Q) , a dienophile, and 1,3-cyclopentadiene, a diene, to yield tricyclo[6.2.1.$0^{2,7}$] undeca-4,9-diene-3,6-dione, more commonly referred to as cyclopentadienebenzoquinone. The reaction between Q and 1,3-cyclopentadiene was observed to have occurred when fresh mainstream vapor phase smoke (VP) from a 2R4F cigarette, captured in acetone, was subsequently treated with Q. Accompanying the Diels-Alder reaction was an additional reaction of Q to form hydroquinone (HQ). These reactions provide additional information on the complexity of cigarette smoke, particularly as it relates to *in situ* reactions involving Q and HQ.

10:00 AM ***Break***

10:30 AM WEDNESDAY

67. ANALYSIS OF ACROLEIN AND ACETONE GENERATED BY BLENDED C13-LABELED GLYCEROL IN A BURNING CIGARETTE VIA HPLC-MS. Larry T. TAYLOR, Shiu-Hang Yip, Mehdi Ashraf-Khorassani, Jianxin Yu,Virginia Tech, Blacksburg, VA 24061-0212 and M. F. Borgerding, W. M. Coleman III, J. A. Bodnar, R.J. Reynolds Tobacco Co., Winston Salem, NC 27102-1487

The extent of blend glycerol degradation in a burning cigarette to form acrolein and acetone has been quantitatively determined by the addition of glycerol-$3C_{13}$ to three styles of a leading commercial cigarette brand. Multiple Cambridge pads soaked with a solution of 2,4-dinitrophenylhydrazine (DNPH) were employed to trap low molecular weight carbonyl compounds in both mainstream and sidestream smoke. High performance liquid chromatography coupled with negative ion mass spectrometry was used to isolate DNPH derivatives of the volatile carbonyl products of combustion and ascertain their concentration. Acrolein, acetone, and propionaldehyde were the principle compounds of interest. The DNPH derivatives of acrolein-$3C_{13}$ and acetone-$3C_{13}$ were independently synthesized, and they served as external standards for absolute quantitation. The cost of fully

labeled propionaldehyde precluded its use in this study. The brand styles selected for study represent the cigarette design features that are most prevalent in the U. S. market today and afford a representative range of FTC "tar" yields (14, 10, and 5 mg/cig, respectively by the FTC method). The brand styles studied are part of a commercial cigarette brand family that does not contain additives to the tobacco blend, including glycerol. Mainstream smoke was generated by automated smoking machine employing two smoking regimens, the standard FTC smoking regimen and a more intense regimen requiring larger, more frequent puffs and 100% vent blocking that is specified for regulatory purposes by the Canadian Federal government. A small fraction of added glycerol (~0.25%-0.30%) was converted to the compounds of interest, with the largest portion generally observed in sidestream smoke. Less than 0.1% of the added glycerol was converted to acrolein in mainstream smoke for all cigarette designs and smoking regimens studied.

10:50 AM WEDNESDAY

68. INVESTIGATION ON THE NON-ISOTHERMAL BEHAVIOR OF TOBACCO STEM RELATED WITH THE CHEMICAL COMPOSITION. Yong Joo SUNG, Young-Lim Han, Yong-Ok Kim, Chung Ryul Kim and Moon-Soo Rhee, KT&G Central Research Institute, 302 Shinseong-Dong, Yuseong-Gu, Daejeon, 305-805, Korea

The role and usability of tobacco stem for cigarette design have been increasing these days, especially for the low tar products. In this study, a relationship between the chemical composition, especially that of the cell wall bio-polymers, and the non-isothermal behavior of the tobacco stems were investigated.

The thermal behavior of tobacco stems showed different patterns depending on decomposition conditions as well as tobacco varieties. In case of flue-cured tobacco stem, the rapid thermal decompositions at around 473°C and 581°C were recorded as the peaks in DTG (Derivative Thermogravimetric) curve under the air atmosphere condition, while the peaks were not shown in the nitrogen atmosphere condition. However, the freeze dried soluble fraction obtained by hot water extraction of flue-cured tobacco stem showed rapid thermal decomposition at around 581°C.

The distinct difference in thermal decomposition pattern between hemicellulose and cellulose were easily observed in the DTG curve obtained in the nitrogen atmosphere. The higher decomposing temperature of bio-polymers found in the flue-cured versus burley stem might be due to the structural difference in biopolymer structure, such as the higher degree of crystallization and/or polymerization in flue-cured tobacco stem than in burley tobacco stem. These results could be applied to in-depth analysis and evaluation of tobacco stem derived from different tobacco varieties and grades.

11:10 AM WEDNESDAY

69. NOVEL SYNTHESIS OF POLYOL ESTERS OF C2-C6 ACIDS AND THEIR FLAVORING IN TOBACCO. Shitong ZENG, Peng Li, Jun Hu, Zhengzhou Tobacco Research Institute of CNTC

In order to develop a new resource of tobacco flavors, polyol esters of lower fatty-acid were synthesized. The polyols and C_2-C_6 acids used were well-applied tobacco flavorants, including acetic acid, butyric acid, isobutyric acid, isovaleric acid, hexylic acid, glycerol, xylitol and glucose. The objective of this research was to synthesize flavor precursors and also evaluate their functions. A novel catalyst, $TiSiW_{12}O_{40}/TiO_2$ was used in this research. The products were analyzed by MS, GC/MS and Py/GC/MS.

The results of MS and GC/MS show that the products were mixed esters with single-, double-, triple- and total substituents and their isomers. The results of Py/GC/MS show that the corresponding lower fatty acid were released at 400°C and 800°C, which is the same as we assumed. Meanwhile, several other flavor compounds from polyol part are formed too.

Sensory evaluation indicates glucose acetate, glucose isobutyrate, glucose isopentyrate, xylitol isopentyrate and xylitol caproate can provide satisfying tobacco flavoring effect, described as mild, smooth, harmonious, fresh, and wealthy. Their proper addition amounts are 10ppm, 20ppm, 20ppm, 5ppm, 5ppm respectively. The advantage of this kind of ester flavors is that they are non-volatile, without odor itself, stable storage, but they can release volatile small molecule flavor compounds as smoking.

11:30 AM WEDNESDAY

70. DETERMINATION OF VOLATILE ORGANIC ACIDS IN FLUE-CURED TOBACCO BY DERIVATIZATION HEADSPACE LIQUID-PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY/MASS SPECTROMETRY. SUN Shihao, Xie Jian-ping, Zong Yongli, Xie Fuwei, Zhengzhou Tobacco Research Institute, China National Tobacco Corporation, 450001, China

A method coupling derivatization headspace liquid-phase microextraction with gas chromatography-mass spectrometry (DH-LPME/GC-MS) was developed for determining volatile organic acids in flue-cured tobacco. The mixture of N,O-bis(trimethylsilyl) trifluoroacetamide and decane was utilized as the solvent for headspace liquid-phase microextraction, and completed microextraction and derivatization simultaneously in one step. The solvent served two purposes. First, it pre-concentrated volatile organic acids in the headspace of tobacco sample. Second, the volatile organic acids extracted were derivatized to form silyl derivatives in the drop of the LPME. The main parameters affecting DH-LPME procedure such as extraction and derivatization solvent, microdrop volume, extraction and derivatization time, preheating temperature and preheating time were optimized. The standard addition approach was essential to obtain accurate measurements by minimizing matrix effects. Good linearity ($R^2 \geq 0.9775$) and good repeatability (RSDs$\leq$16.6%, n=5) for 15 analytes in sample spiked with standard analytes were achieved. The method is simple, fast, effective, sensitive, selective, and provides an overall profile of volatile organic acids in flue-cured tobacco.

11:50 AM ADJOURN

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