2022 CORESTA CONGRESS ONLINE

10 – 28 October 2022

PROGRAMME & ABSTRACTS

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WELCOME MESSAGE

Joseph THOMPSON
President of the CORESTA Board

Dear Colleagues

I am very pleased to welcome you all, on behalf of the CORESTA Board, to the 2022 Congress. This year the Congress will be taking place online over a period of three weeks, with more than 140 presentations across 26 sessions.

The Congress represents an opportunity to promote collaboration on science and technology across the globe and I would like to thank the Secretariat for their hard work in making this virtual event possible, and building on the successes and learnings from previous online CORESTA events.

The Scientific and Reading Committees have put together an excellent technical and scientific programme for us all. So I encourage you to engage with the content of the video presentations, reflect, and take full part in the live question and answer sessions across the duration of the Congress.

Over the past two years, CORESTA has continued to build its scientific expertise and understanding, working collegiately with its membership, despite the challenges of the Coronavirus pandemic that have limited our ability to physically be together. We have continued to shape CORESTA's short and long term strategy, and deliver the science that makes CORESTA a unique and authoritative source of publicly available, credible science and best practices related to tobacco and its derived products.

Wherever you are joining from, welcome to the 2022 CORESTA Congress Online!

Joseph THOMPSON
Imperial Tobacco
Bristol, U.K.

PROGRAMME

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

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 1
MONDAY 10 OCTOBER

SESSION 1 - Production: impact of nutrition, soil and management

Chair: Anthony JACKSON

Moderator: Lea SCOTT

CET Time Zone

13:30-13:45	AP 01	**Optimization of cytoplasmic male sterility based flue-cured tobacco hybrid seed production system** DEERGASI Satish Kumar; VENKATA REDDY T.; VENKATA GANESH R. *Research Department, ITC Limited – Agribusiness Division, Rajamahendravaram 533107 AP, India*
13:45-14:00	AP 02	**The interaction of water source and fertilizer chemistry on greenhouse tobacco transplant production** REED T.D.; IRBY R.S. *Virginia Tech, Southern Piedmont Center, Blackstone, VA 23824, U.S.A.*
14:00-14:15	AP 03	**The impact of starter fertilizer to flue-cured tobacco growth** VANN M.C.(1); DABBS D.W.(2) *(1) Department of Crop & Soil Sciences, North Carolina State University, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) Alamance Cooperative Extension, 209-C N Graham Hopedale Road, Burlington, NC 27217, U.S.A.*
14:15-14:30	AP 04	**Developing nitrogen and potassium fertilizer recommendations for Connecticut broadleaf cigar wrapper tobacco in North Carolina** SHORT M.M.; VANN M.C.; CHEEK J.A.; MACHACEK J.L.; WHITLEY D.S. *North Carolina State University – Crop and Soil Sciences Department, 101 Derieux Street, Raleigh, NC 27695, U.S.A.*
14:30-14:45	AP 05	**Sustainable soil health solutions: evaluation of biofertilizers for use on tobacco in Zimbabwe** CHINAMO D.; CHIBUDU C.; MAVUKA R. *Tobacco Research Board, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:45-15:00	AP 06	**Response of soil bacterial community structure and co-occurrence network topology properties to soil physicochemical properties in long-term continuous cropping farmland** ZENG Weiai(1); YANG Zhaoyue(2); GU Yabing(2); XIE Pengfei(1); CAI Hailin(1); YIN Huaqun(2) *(1) Changsha Tobacco Company of Hunan Province, Changsha, 410011, China* *(2) School of Minerals Processing and Bioengineering, Central South University, Changsha 410083, China*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

15:00-15:15	AP 07	**Optimization of prediction model for tobacco leaf dehydration rate during intensive curing process based on machine learning** DU Haina(1,2); MENG Lingfeng(1); WANG Songfeng(1); ZHANG Binghui(3); HE Dengfeng(4); XUN Xiaohong(5); GAO Jun(6); WANG Aihua(1); LIU Hao(1,2); LI Zengsheng(1,2); SUN Fushan(1) *(1) Institute of Tobacco Research of CAAS, Key Laboratory of Tobacco Biology and Processing, Ministry of Agriculture, Qingdao 266101, China* *(2) Graduate School of Chinese Academy of Agricultural Sciences, Beijing 100081, China* *(3) China Tobacco Corporation FuJian Corporation, FuZhou 350000, China* *(4) China Tobacco Corporation ShanXi Corporation, XiAn 710000, China* *(5) Chongqing Tobacco Science Research Institute, Chongqing 400715, China* *(6) Liangshan Tobacco Company of Sichuan Province, Xichang, Sichuan 615000, China*

*[**AP 08** cancelled]*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 2
TUESDAY 11 OCTOBER

SESSION 1 - Sucker control: management and genetic control

Chair: Lea SCOTT

Moderator: Anthony JACKSON

CET Time Zone

13:30-13:45	AP 09	**Effect of ridging and hilling on dark tobacco standability and sucker control** BAILEY W.A.(1); RODGERS J.C.(1); RICHMOND M.D.(2); ELLIS R.(2) *(1) University of Kentucky, Research & Education Center, 348 University Drive, Princeton, KY, U.S.A.* *(2) University of Tennessee, Highland Rim AgResearch & Education Center, Springfield, TN, U.S.A.*
13:45-14:00	AP 10	**Field performance of fewer sucker variety in production area of Japan** TAGA T.; NAKAMURA T.; NARAGINO T. *Leaf Tobacco Research Center, R&D Group, Japan Tobacco Inc., 1900 Idei, Oyama-shi, Tochigi 323-0808, Japan*
14:00-14:15	AP 11	**Evaluations of modern spray nozzle technology for maleic hydrazide application** VANN M.C.(1); ROUSSOS R.N.J.(1); ELLINGTON G.(2); CAHOON C.W.(1); GANNON T.(1) *(1) Department of Crop & Soil Sciences, North Carolina State University, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) Department of Biological & Agricultural Engineering, North Carolina State University, 3100 Faucette Drive, Raleigh, NC 27695, U.S.A.*
14:15-14:30	AP 12	**Sustaining maleic hydrazide: an alternative application technique for reduced residues in flue-cured tobacco** ROUSSOS R.N.J.(1); VANN M.C.(1); ELLINGTON G.(2); CAHOON C.W.(1); GANNON T.(1) *(1) Department of Crop & Soil Sciences, North Carolina State University, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) Department of Biological & Agricultural Engineering, North Carolina State University, 3100 Faucette Drive, Raleigh, NC 27695, U.S.A.*
14:30-14:45	AP 13	**Impact of harvest timing on yield, leaf quality, alkaloids, and tobacco-specific nitrosamines in Burley tobacco** RICHMOND M.D. *University of Tennessee, 2505 E.J. Chapman Drive, Knoxville, TN 37996, U.S.A.*

DAY 2
TUESDAY 11 OCTOBER

SESSION 2 - Technology application in genetics and physiology

Chair: Susan DIMBI

Moderator: Limeng ZHANG

CET Time Zone

15:15-15:30	AP 14	**DNA barcoding – practical applications in tobacco identification in Zimbabwe** ZVOBGO G. *Tobacco Research Board, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
15:30-15:45	AP 15	**Automatic identification and precise prevention of deep green infection in tobacco leaf using a hand-held DLP-based NIR spectrometer** YANG Shuangyan(1); YANG Tao(1); SHEN Yanwen(1); YANG Zigang(1); ZHANG Jianqiang(2) *(1) Yunnan Tobacco Biological Technology Co., Ltd, Kunming, 65000, China* *(2) Yunnan Police College, Kunming, 65000, China*
15:45-16:00	AP 16	**Application of protoplast technology facilitates the CRISPR-Cas9 mediated gene replacement in *Nicotiana tabacum* and confers resistance against tobacco mosaic virus** YUAN Cheng; HUANG Changjun; LIU Yong; ZENG Jianmin; TONG Zhijun; YU Haiqin; FANG Dunhuang; XIAO Bingguang *Yunnan Academy of Tobacco Agricultural Sciences, No. 33 Yuantong Street, Wuhua District, Kunming, Yunnan Province, 650021, China*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 3
WEDNESDAY 12 OCTOBER

SESSION 1 - Genetics: impact of breeding and biotechnology

Chair: Dongmei XU

Moderator: Colin FISHER

CET Time Zone

13:30-13:45	AP 17	**Breeding for high nicotine tobacco varieties** MALPICA A.; L'HUMEAU J.; BACHET S. *Bergerac Seed & Breeding, La Tour, 24100 Bergerac, France*
13:45-14:00	AP 18	**Use of exotic *Nicotiana tabacum* germplasm for confronting an inverse genetic correlation in flue-cured tobacco** LEWIS R.S. *Department of Crop and Soil Science, North Carolina State University, NC, U.S.A.*
14:00-14:15	AP 19	**Effects of drought stress on changes in morphology and expression of selected genes in tobacco** PRZYBYŚ M. *Institute of Soil Science and Plant Cultivation – State Research Institute, ul. Czartoryskich 8, 24-100 Pulawy, Poland*
14:15-14:30	AP 20	**Integrative analysis of transcriptome and metabolome provides insights into the underlying mechanism of cold stress response and recovery in two tobacco cultivars** HU Zhengrong(1); YAN Weijie(2);YANG Chenkai(2); LI Yangyang(1); YANG Jiameng(1); HU Risheng(1) *(1) Hunan Tobacco Research Institute, Changsha, Hunan 410004, China* *(2) College of Agronomy, Hunan Agricultural University, Changsha, Hunan 410128, China*
14:30-14:45	AP 21	**Loss of susceptibility loci in tobacco for the development of durable resistance to black shank** FREDERICK J.; PRAMOD S.; ADAMS A.; XU D.; LUSSO M. *Altria Client Services Inc., 601 E. Jackson St., Richmond, VA 23219, U.S.A.*
14:45-15:00	AP 22	**Distribution of the MLO gene mutations for powdery mildew resistance in tobacco cultivars** IWAI Y.; ARAI M.; KOMATSU T.; UDAGAWA H.; TAJIMA T.; SATO S. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900 Idei, Oyama, Tochigi 323-0808, Japan*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

15:00-15:15	AP 23	**Functional characterization of transcription factor *NtERF13a* in regulating phenylpropanoids biosynthesis in tobacco** WANG Zhong(1); ZHANG Zhan(2); LUO Zhaopeng(1); LIU Pingping(1); WU Mingzhu(1); LI Zefeng(1); ZHANG Jianfeng(1); XU Xin(1); YANG Yongfeng(2); YANG Jun(1) *(1) Zhengzhou Tobacco Research Institute of CNTC, No. 2 Fengyang Street, Zhengzhou 450001, Henan Province, China* *(2) Technology Center, China Tobacco Henan Industrial Co., Ltd., Zhengzhou 450000, Henan Province, China*
15:15-15:30	AP 24	**Development of a mutant library and a high-quality reference genome for forward genetics in tobacco wild relatives *Nicotiana sylvestris*** TAKEUCHI T.; ARAI M.; UDAGAWA H.; MAGOME H.; TAJIMA T.; TAKAKURA Y. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900, Idei, Oyama, Tochigi 323-0808, Japan*
15:30-15:45	AP 25	**Single-cell transcriptome of *Nicotiana tabacum* leaves reveals developmental trajectories of glandular trichome** CHEN Hongyu; LI Xiaohan; FAN Longjiang *Institute of Crop Science and Institute of Bioinformatics, Zhejiang University, Hangzhou, China*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 4
THURSDAY 13 OCTOBER

SESSION 1 - Pest and disease management

Chair: Colin FISHER

Moderator: Susan DIMBI

CET Time Zone		
13:30-13:45	**AP 26**	**Evaluation the effectiveness of plant ingredients in slug control** HOSSEINI A.; MORADI ROBATI Gh.R.; NAJAFI M.R.; SAJJADI A.; SHAHADATI MOGHADDAM Z.A.; MASODI A.A.; SAFFAR F. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
13:45-14:00	**AP 27**	**Chemical management strategies for angular leaf spot in dark tobacco** KEENEY A.B.; BAILEY W.A.; RODGERS J.C. *University of Kentucky, Research & Education Center, 348 University Drive, Princeton, KY 42445, U.S.A.*
14:00-14:15	**AP 28**	**Correlation between management practices and angular leaf spot of dark tobacco** KEENEY A.B.; BAILEY W.A.; RODGERS J.C. *University of Kentucky, Research & Education Center, 348 University Drive, Princeton, KY 42445, U.S.A.*
14:15-14:30	**AP 29**	**Assessing *Phytophthora nicotianae* race population in Tennessee** MILLER T.; RICHMOND M.D.; HANSEN Z. *University of Tennessee, Knoxville, TN, U.S.A.*
14:30-14:45	**AP 30**	**New progress in breeding and biocontrol dual approaches for tobacco broomrape management** MALPICA A.(1); BACHET S.(1); GATARD L.(3); REIBEL C.(2); GAUTHERON N.(2); EDEL-HERMANN V.(2); STEINBERG C.(2); GIBOT LECLERC S.(2) *(1) Bergerac Seed & Breeding (BSB), La Tour, 24100 Bergerac, France* *(2) Agroécologie, (AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté), 17 rue Sully, 21000 Dijon, France* *(3) Coopérative CT2F, Maison de l'Agriculture, 2, rue de Rome, 67300 Schiltigheim, France*
14:45-15:00	**AP 31**	**Management of whitefly *Bemisia tabaci*, a vector of tobacco leaf curl virus disease in Virginia tobacco** SREEDHAR U.; SAILAJA JAYASEKHARAN B.; VENKATESWARLU V. *ICAR - Central Tobacco Research Institute, Rajahmundry, 533105, India*
15:00-15:15	**AP 32**	**Habitat management for the enhancement of arthropod services in flue-cured Virginia tobacco** SAILAJA JAYASEKHARAN B.; SREEDHAR U.; VENKATESWARLU V. *ICAR - Central Tobacco Research Institute, Division of Crop Protection, Dr. N.C. Gopalachari Road, Bhaskar Nagar, Rajahmundry 533105, Andhra Pradesh, India*
15:15-15:30	**AP 33**	**Study on the molecular mechanism of maize pollen infecting tobacco** ZOU Congming; GU Kaiyuan *Yunnan Academy of Tobacco Agricultural Sciences, No. 33 Yuantong Str., Wuhua District, Kunming, Yunnan Province, 650021, China*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 5
FRIDAY 14 OCTOBER

SESSION 1 - Crop protection agents (CPAs) in tobacco production and residue analysis

Chair: Anthony JACKSON

Moderator: Lea SCOTT

CET Time Zone

13:30-13:45	AP 34	**Axillary bud control and residues from maleic hydrazide applied at different times of day in Burley tobacco** RICHMOND M.D. *University of Tennessee, 2505 E.J. Chapman Drive, Knoxville, TN 37996, U.S.A.*
13:45-14:00	AP 35	**Characterizing 2,4-D and dicamba residue persistence following tobacco flue-curing** VANN M.C.; GANNON T.; MAXWELL P. *Department of Crop & Soil Sciences, North Carolina State University, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
14:00-14:15	AP 36	**Agrochemical residues: recent experiences with cyantraniliprole, flutriafol, flutriafol + azoxystrobin, and S-metolachlor** VANN M.C.; CHEEK J.A.; MACHACEK J.L.; WHITLEY D.S. *Department of Crop & Soil Sciences, North Carolina State University, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
14:15-14:30	AP 37	**Matrix effect on analysis of crop protection agents in tobacco by GC-MS/MS and LC-MS/MS techniques** BECKER J.M.; ALVES M.; GONÇALVES C.; PAPROCKI A. *British American Tobacco Brazil, TSS-AmSSA, Av. Frederico A. Ritter 8000, Cachoeirinha, RS, 94970-470, Brazil*
14:30-14:45	AP 38	**Promoting sustainable farming practices with real time, up-to-date regulatory information on plant protection products** STRAUMANN V. *Lexagri International, 72 rue Georges de Mestral, Technopôle d'Archamps - Bât. Athéna 1, 74166 St Julien en Genevois Cedex, France*
14:45-15:00	AP 39	**Evaluation of fungicide programs and lower leaf removal on wrapper leaf production in Connecticut broadleaf cigar wrapper tobacco** PERKINS C.(1); BAILEY W.A.(1); RODGERS J.C.(2); KEENEY A.B.(2); RICHMOND M.D.(2); ELLIS R.(2) *(1) University of Kentucky, Research & Education Center, 348 University Drive, Princeton, KY, U.S.A.* *(2) University of Tennessee, Highland Rim AgResearch & Education Center, 3181 Experiment Station Road, Springfield, TN, U.S.A.*

DAY 5
FRIDAY 14 OCTOBER

SESSION 2 - Microbial impact on cured leaf

Chair: Limeng ZHANG

Moderator: Anthony JACKSON

CET Time Zone

15:30-15:45	AP 40	**Diversity of bacteria on tobacco leaves during aging and their aroma-enhancing effects** WANG Zhen; WANG Wenting; LIU Chen; ZHU Xianyue; ZHANG Xia; ZANG Zhipeng; SONG Kai *Technology Center, Gansu Tobacco Industry Co., Ltd., Lanzhou 730050, China*
15:45-16:00	AP 41	**Effects of fermentation medium on cigar filler** CAI Wen(1); HU Wanrong(1); ZHANG Qianying(1), LIU Lulu(1); ZHENG Zhaojun(2); LI Pinhe(1); LUO Cheng(1); LI Dongliang(1) *(1) China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610000, China* *(2) School of Food Science and Technology, Jiangnan University, Wuxi 214000, Jiangsu, China*
16:00-16:15	AP 42	**Changes of bacterial community structure and their correlations with contents of free amino acids during cigar processing** HUANG Kuo(1); LI Dong(1); WANG Bin(2); LIU Kejian(1); CHEN Chen(1); MI Qiang(2); WANG Gengdou(2); MA Mingsai(1); SHI Zhandong(1); MENG Qinghua(2); FAN Li(1); YE Changwen(1) *(1) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* *(2) China Tobacco Shandong Industrial Co., Ltd., Jinan 250014, China*

DAY 6
MONDAY 17 OCTOBER

SESSION 1 - Sustainability through integrated programmes and production practices

Chair: Anthony JACKSON

Moderator: Fabienne LALANDE

CET Time Zone

13:30-13:45	AP 43	**The development of ESG programs to help foster social, environmental, and governance initiatives in Altria's global tobacco supply chain** IRVING B.; GEOVANNELLO F. *Altria Client Services LLC, Procurement, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
13:45-14:00	AP 44	**Changes of soil bacterial community structure and its response to soil physicochemical properties after addition of wheat straw and its biochar** WANG Yi(1,2); SONG Wenjing(2); LIU Zhigang(1); DU Chuanyin(1); GUAN Ensen(1); WANG Xianwei(1); ZHENG Xuebo(1); CONG Ping(1); WANG Shuke(1); MENG Qinghong(1) *(1) Weifang Tobacco Co., Ltd., Weifang, Shandong 262200, China* *(2) Ministry of Agriculture, Tobacco Research Institute of Chinese Academy of Agriculture Sciences, Qingdao, Shandong 266101, China*
14:00-14:15	AP 45	**Determination of carbon sequestration in tobacco plants** FRANTZ M.L.(1); MULLER R.L.(1); SCHAEFFER V.P.(1); KÖHLER A.K.(2); SCHNEIDER R.C.S.(2); PEZZINI C.(2) *(1) Premium Brazil Tabacos, Av. Felisberto Bandeira Moraes, 2405, Distrito Industrial, Santa Cruz do Sul, 96835-900, RS, Brazil* *(2) Universidade de Santa Cruz do Sul (UNISC), Av. Independência 2293, Universitário, Santa Cruz do Sul, 96815-900, RS, Brazil*
14:15-14:30	AP 46	**Construction of plant growth-promoting rhizobacteria with bacterial wilt resistance and its biological control mechanism** LIU Yanxia(1); LI Xiang(2); FU Shenghua(2); PENG Yu(2); WANG Feng(1); JIAO Jian(2); WANG Kemin(2); XU Jian(2); DAI Yuanfeng(2); WANG Weiwei(3); LI Han(1); WANG Junfei(2); SUN Guangjun(2) *(1) Guizhou Academy of Tobacco Science (Guizhou Provincial Academician Workstation of Microbiology and Health; Upland Flue-Cured Tobacco Quality & Ecology Key Laboratory of China Tobacco), Guiyang 550000, China* *(2) Guizhou Tobacco Company, CNTC, Guiyang, 550000, China* *(3) Zunyi Company of Guizhou Tobacco Corporation, CNTC, Zunyi, 563000, China*
14:30-14:45	AP 47	**Progress towards the evaluation of bio-control based insecticides and fungicides for use on tobacco in Zimbabwe** JAZI Z.; CHINHEYA C.; DIMBI S. *Plant Health Services Division, Tobacco Research Board, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

14:45-15:00	AP 48	**Biological control of tobacco root-knot nematode (*Meloidogyne incognita*), using *Trichoderma harzianum* in Iran** SHAZDEHAHMADI M.; SAJJADI A.; NAJAFI M.R.; SAHEBANI N.A.; SHAHADATI MOGHADDAM Z.A. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
15:00-15:15	AP 49	**The effect of liquid smoke from tobacco waste on tobacco black shank (*Phytophthora nicotianae*), tobacco aphid (*Myzus nicotianae*) and whitefly (*Trialeurodes vaporarium*) in Northern Iran** SAJJADI A.(1); SHAZDEHAHMADI M.(2); MORADI ROBATI Gh.R.(3); SHAHADATI MOGHADDAM Z.A.(2); SALAVATI M.R.(2); NAJAFI M.R.(1); BARZEGHAR Y.(2); GHARIB O.(3); NAJAFI H.(3) *(1) Plant Pathology Department of Tirtash Research and Education Center, Iranian Tobacco Company, Iran* *(2) Biotechnology Department of Tirtash Research and Education Center, Iranian Tobacco Company, Iran* *(3) Chemistry Department of Tirtash Research and Education Center, Iranian Tobacco Company, Iran*
15:15-15:30	AP 50	**Efficiency of sawdust briquettes as an alternative source of energy for tobacco curing in Tabora Urban District, Tabora Region, Tanzania** MUNA E.I.(1); ABDALLAH J.M.(2); MONELA G.C.(2); ISHENGOMA R.C.(2); DUNDA D.R.(1) *(1) Tobacco Research Institute of Tanzania (TORITA), P.O. Box 431, Tabora, Tanzania* *(2) Sokoine University of Agriculture (SUA), P.O. Box 3011, Morogoro, Tanzania*

DAY 7
TUESDAY 18 OCTOBER

SESSION 1 - Leaf constituents: TSNA and other chemistry

Chair: Colin FISHER

Moderator: Dongmei XU

CET Time Zone

13:30-13:45	AP 51	**Overview of tobacco specific nitrosamines reduction** KUDITHIPUDI C.; LUSSO M.; LION K.; XU D. *Altria Client Services LLC, Product Design and Maintenance, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
13:45-14:00	AP 52	**Influence of harvesting methods on nitrate content and TSNA formation in lamina and midrib of cigar and flue-cured tobaccos** ZHAO Yuanyuan(1); SHI Hongzhi(1); RONG Shibin(1); QIN Yanqing(2); ZHOU Jun(3); ZHANG Jingyun(1); LIU Deshui(3); WANG Jun(4) *(1) College of Tobacco Science of Henan Agricultural University / Tobacco Harm Reduction Research Center of HAU, Zhengzhou 450002, China* *(2) Sichuan Provincial Tobacco Company, Chengdu, China* *(3) Beijing Cigarette Factory of Shanghai Tobacco (Group) Co., Beijing 100024, China* *(4) Deyang Municipal Tobacco Company, Deyang, 618400, China*
14:00-14:15	AP 53	**Widely targeted metabolomics analysis of flue-cured tobacco growing in different areas and association with sensory characteristics** LI Yingxue(1); YU Xinlei(2); HE Jiewang(1) *(1) China Tobacco Hubei Industrial Co., Ltd., Technology Center, Wuhan 430040, China* *(2) Hubei Tobacco Company, Yichang City Branch, Yichang 443000, China*
14:15-14:30	AP 54	**Quality differences of flue-cured tobacco leaf from the southern Yunnan Gold Corridor Tobacco Cultivation Region and its environmental interpretation** WANG Lan(1); GAO Weikai(2); YANG Jun(1); XIE Bing(1); LIN Xin(1); XIAO Jingyi(1); LI Zhouwen(2) *(1) Yunnan Reascend Tobacco Technology (Group) Co., Ltd., Kunming 650106, China* *(2) China Tobacco Guangdong Industrial. Co., Ltd., Guangzhou 510220, China*

DAY 7
TUESDAY 18 OCTOBER

SESSION 2 - Low nicotine tobacco

Chair: Colin FISHER

Moderator: Dongmei XU

CET Time Zone

15:00-15:15	AP 55	**Agronomic performance of ultra-low nicotine Burley tobacco CRISPR lines** FISHER A.M.(1); YANG Shengming(2); PATRA B.(1); FISHER C.R.(1); JI Huihua(1); ZHOU Xuguo(3); KINNEY J.(1) *(1) Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.* *(2) United States Department of Agriculture, 1616 Albrecht Blvd N, Fargo, ND 58102, U.S.A.* *(3) Entomology Department, Ag Science Center North, University of Kentucky, 1100 South Limestone Street, Lexington, KY 40546, U.S.A.*
15:15-15:30	AP 56	**Generating ultra-low nicotine tobaccos by combining knockout mutations in two key steps of the alkaloid biosynthetic pathway** DEWEY R.E.; SMITH W.A.; STEEDE W.T. *North Carolina State University, Dept. of Crop and Soil Sciences, Campus Box 8009, Raleigh, NC 27695, U.S.A.*
15:30-15:45	AP 57	**Modified cultural practices to reduce nicotine accumulation in tobacco leaf and low nicotine variety impact on Italian growers' revenue** FRANCESCHETTI L.(1); MILLI G.(1); BARGIACCHI E.(2); MIELE S.(2) *(1) Trasformatori Tabacco Italia (TTI) & FAT, I-06012 Città di Castello, PG, Italy* *(2) Consortium INSTM, I-50121 Firenze, Italy*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 8
WEDNESDAY 19 OCTOBER

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

Chair: Xavier CAHOURS

Moderator: Kei YOSHINO

CET Time Zone

13:30-13:45	ST 01	**The importance of assessing puffing topography to inform e-cigarette emissions testing** WADKIN R.(1); ALLEN C.(1); FEARON I.M.(2) *(1) Broughton Life Sciences, Oak Tree House, West Craven Drive, Earby, Lancs., BB18 6JZ, U.K.* *(2) whatIF? Consulting Ltd, The Crispin, Burr Street, Harwell, OX11 0DT, U.K.*
13:45-14:00	ST 02	**Puffing topography and mouth level exposure of two closed-system Vuse e-cigarettes** PRASAD K.; GRAY A.; EDWARD L. *British American Tobacco, MRTP Science, Southampton, Hampshire, U.K.*
14:00-14:15	ST 03	**Attitudes, consumption characteristics and motivations of cigarette smoking and electronic cigarette use among university students in Guangzhou, China: a cross-sectional survey** KUN Duan; LIU Chuan; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
14:15-14:30	ST 04	**Actual use study of a heated tobacco product** PRASAD K.(1); SHETTY M.(1); HART R.(1); EVANS E.(2); CAMPBELL C.(2); MAKENA P.(2); FEEHAN M.(4); KANITSCHEIDER C.(3); LYDEN J.(4); BAXTER S.(2) *(1) British American Tobacco, Research and Development, Regents Park Road, Southampton, SO15 8TL, U.K.* *(2) RAI Services Company, 950 Reynolds, Blvd, Winston-Salem, NC 27102, U.S.A.* *(3) Cerner Enviza, Landsberger Straße 284, 80687 Munich, Germany* *(4) Cerner Enviza, 2800 Rock Creek Pkwy, Kansas City, MO 64117, U.S.A.*
14:30-14:45	ST 05	**Risk perception of *IQOS*™ and cigarettes: temporal and cross-country comparisons** AL MOOSAWI S.(1); BAJEC M.(2); MAINY N.(1); KALLISCHNIGG G.(3); ZWISELE B.(3); FISCHER K.(1); MAGNANI P.(1); ROULET S.(1) *(1) PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switz.* *(2) Bajec Senseworks Consulting, Hamilton, L9A 1L5 Ontario, Canada* *(3) ARGUS - Statistics and Information Systems in Environment and Public Health GmbH, Karl-Heinrich-Ulrichs-Straße 20a, DE-10785 Berlin, Germany*
14:45-15:00	ST 06	**Assessing consumer use and behaviour patterns of oral nicotine pouches in a multi-country study** PRASAD K.(1); SHETTY M.(1); KANITSCHEIDER C.(2); SZENTES B.(2); NASSAR R.(2); EDWARD L.(1) *(1) British American Tobacco, MRTP Science, Southampton, Hampshire, U.K.* *(2) Cerner Enviza RWE, Regulatory and Safety, (Diamond (KH) Germany HoldCo GmbH) Munich, Bavaria, Germany*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 8
WEDNESDAY 19 OCTOBER

SESSION 2 - Biomarkers: method improvement and effects of product switching

Chair: Xavier CAHOURS

Moderator: Paul HARP

CET Time Zone

15:30-15:45	ST 07	**Simultaneous analysis of 18 biomarkers of tobacco smoke exposure in urine by multiple heart-cutting two-dimensional liquid chromatography-tandem mass spectrometry** WANG Yangzhong; WANG Tiannan; QI Dawei; CHEN Yanfang; CHEN Min; LI Gang; FEI Ting; WU Da; LIU Baizhan *Shanghai Tobacco Group Co., Ltd., No. 3733, Xiupu Road, Pudong New District, Shanghai 201315, China*
15:45-16:00	ST 08	**Development and validation of a routine method for the determination of 3-hydroxybenzo[a]pyrene in human urine by GC-MS/MS** WILSON A.H.; MARTIN A.M. *Enthalpy Analytical, LLC, 1470 E. Parham Rd, Richmond, VA 23228, U.S.A.*
16:00-16:15	ST 09	**Reduction in urinary and blood biomarkers of tobacco exposure in smokers switched to an electronic nicotine delivery system product** KANOBE M.N.(1); JONES B.A.(2); BROWN B.(2); CHEN P.(1); SCHMIDT E.(1); DARNELL J.(1) *(1) RAI Services Company, 950 Reynolds, Blvd, Winston-Salem, NC 27102 U.S.A.* *(2) Retired employees of RAI Services Company, 950 Reynolds, Blvd, Winston-Salem, NC 27102, U.S.A.*
16:15-16:30	ST 10	**Dynamics of nicotine status in smoking volunteers after switching to oral nicotine products** TROFIMOV A.V.(1); MENSHOV V.A.(1); BERDNIKOVA N.G.(2); YABLONSKAYA O.I.(1) *(1) Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow 119334, Russia* *(2) I.M. Sechenov First Moscow State Medical University, Moscow, 119991, Russia*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 9
THURSDAY 20 OCTOBER

SESSION 1 - E-vapour: product design and chemistry

Chair: Rob STEVENS

Moderator: Jutta PANI

CET Time Zone

13:30-13:45	ST 11	**Determination of transfer efficiency of seven flavour compounds during vaping of electronic cigarette liquid** LI Xinduo; LUO Junjun; LIU Jiakai; HE Yi; LIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co., Ltd., Shenzhen, Guangdong 518000, China*
13:45-14:00	ST 12	**Assessment of potential atomisation agents on capacity of aroma delivery in e-liquid** LIU Shengyi; ZHU Libing; DOU Jinxi; LIU Weijuan *(1) Ruvian Technology Ltd., Kunming, 650106, China* *(2) Yunnan Reascend Tobacco Technology (Group) Co., Ltd, Kunming, 650000, China*
14:00-14:15	ST 13	**Evolution of particle size distribution of electronic cigarette aerosols through a secondary thermal treatment** YOU Rui; HE Yongxiang; ZENG Fan; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
14:15-14:30	ST 14	**Retention of flavour compounds in e-liquid pods: impact from the air flow channel structure of the pods** SHI Dantong; HE Yi; GUO Haohang; ZENG Fan; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
14:30-14:45	ST 15	**Investigating the potential for ketene generation in flavored e-liquids via thermal degradation of ester flavorants** JABLONSKI J.J.; CHEETHAM A.G.; SILL E.D. *Enthalpy Analytical, LLC, 1470 E. Parham Rd, Richmond, VA 23228, U.S.A.*
14:45-15:00	ST 16	**HPHC market map of open pod-based and closed pod-based e-cigarettes from the North American market including a comparison of selected analyte yield in the aerosol to 3R4F cigarette smoke** COCCIARDI T.S.; OZVALD A.M.; JAMESON J.B.; WANG J.; BATES A.L.; ULLAH S.; YANG C.; JEONG L.N.; COOK D.K.; GILLMAN I.G. *JUUL Labs, Inc.,1000 F St NW, 8th Floor, Washington, D.C., 20004, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 9
THURSDAY 20 OCTOBER

SESSION 2 - Nicotine science

Chair: Rob STEVENS

Moderator: Karl WAGNER

CET Time Zone

15:30-15:45	ST 17	**Distinguishing tobacco-derived nicotine from synthetic nicotine in commercial nicotine samples** DULL G.M.(1); MOLDOVEANU S.C.(2); KILBY K.B.(2) *(1) RAI Services Company, Bowman Gray Technical Center, 950 Reynolds Blvd, Winston-Salem, NC 27105, U.S.A.* *(2) R.J. Reynolds Tobacco Company, Bowman Gray Technical Center, 950 Reynolds Blvd, Winston-Salem, NC 27105, U.S.A.*
15:45-16:00	ST 18	**Nicotine characterization in modern oral nicotine pouch products** CHEETHAM A.G.; COFFA B.G.; PLUNKETT S.E. *Enthalpy Analytical, LLC, 1470 E. Parham Rd, Richmond, VA 23228, U.S.A.*
16:00-16:15	ST 19	**Interaction mechanisms between S/R-nicotine and human serum albumin** YANG Ji; LIU Chunbo; ZHU Ruizhi; YIN Zhijiang; LIU Ze; TIAN Ran; TANG Shiyun; XIA Jianjun; SI Xiaoxi;JIANG Wei; LI Zhengjie; LIU Zhihua *Technology Center of China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, China*
16:15-16:30	ST 20	**Removal of nicotine to recycle machine wash water** BUSSEY R.; MOLDOVEANU S.C. *Reynolds American, 950 Reynolds Boulevard, Winston Salem, NC 27102, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 10
FRIDAY 21 OCTOBER

SESSION 1 - Clinical studies: nicotine effects and data analysis

Chair: Paul HARP

Moderator: Xavier CAHOURS

CET Time Zone

13:30-13:45	ST 21	**A randomised cross-over study investigating the nicotine pharmacokinetics of nicotine pouches** McEWAN M.; AZZOPARDI D.; GALE N.; HARDIE G. *B.A.T. (Investments) Limited, Regents Park Road, Millbrook, Southampton SO15 8TL, U.K.*
13:45-14:00	ST 22	**Nicotine delivery and pharmacokinetics of an electronic cigarette compared to conventional cigarettes in Chinese smokers: a randomised open-label crossover clinical study** GUO Yi(1); LI Shoufeng(1); LI Chenmin(1); WANG Zhi(2); ZHONG Guoping(1,2) *(1) Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China* *(2) Clinical Trial Center of Dongguan KangHua Hospital, Dongguan, Guangdong 523000, China*
14:00-14:15	ST 23	**Abuse liability assessments of Vuse Alto Golden Tobacco in adult smokers** HONG K.S. *Reynolds American, 401 North Main Street, Winston Salem, NC 27101, U.S.A.*
14:15-14:30	ST 24	**Examining nicotine exposure from e-cigarettes with menthol- and tobacco-flavours: a meta-analysis** JACOBSON K.; LARROQUE S.; MARTINEZ J. *JT International SA, 8, Rue Kazem Radjavi, Geneva, Switzerland*
14:30-14:45	ST 25	**FDA-CTP and CDISC project to develop tobacco related standards to achieve efficiencies for all stakeholders** MALLA A.(1); CONNOLLY C.(2) *(1) Data Team Supervisor (acting), Office of Science (OS) | Division of Regulatory Science Informatics (DRSI), Center for Tobacco Products (CTP), U.S. Food and Drug Administration, U.S.A.* *(2) Senior Project Manager, CDISC, Austin, Texas, U.S.A.*
14:45-15:00	ST 26	**Application of innovative big data techniques for improving data processing and accelerating risk assessment in new tobacco products** LARROQUE S.; SONNERAT D.; CHARRIÈRE M.; BECERRIL E.; MOLINA J.M.; OLIVA J.; ABDUSAMIEV K. *JT International SA, 8, Rue Kazem Radjavi, Geneva, Switzerland*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 10
FRIDAY 21 OCTOBER

SESSION 2 - Oral tobacco products: product analysis and methods

Chair: Karl WAGNER

Moderator: Xavier CAHOURS

CET Time Zone		
15:30-15:45	ST 27	**Medium/long term variability of moist smokeless tobacco products from the United States marketplace** MORTON M.; OLEGARIO R.; SENA E.; WAGNER K.A. *Altria Client Services LLC, 601 E. Jackson St., Richmond, VA 23219, U.S.A.*
15:45-16:00	ST 28	**Harmful and potentially harmful constituents in two novel nicotine pouch products in comparison with regular smokeless tobacco products and pharmaceutical nicotine replacement therapy products** BACK S; MASSER A; RUTQVIST L; LINDHOLM J; REDEBY J. *Swedish Match AB, Regulatory & Scientific Affairs, Maria Skolgata 83, 118 53, Stockholm, Sweden*
16:00-16:15	ST 29	**Determination of nicotine related impurities in nicotine pouches and tobacco containing products by liquid chromatography tandem mass spectrometry** AVAGYAN R.; SPASOVA M.; LINDHOLM J. *Swedish Match AB, Regulatory & Scientific Affairs, Maria Skolgata 83, 118 53, Stockholm, Sweden*
16:15-16:30	ST 30	**Modified QuEChERS method for the extraction of nicotine from oral traditional and innovative tobacco products using UPLC-MS/MS** LOPEZ V.; ALDEEK F.; MILLER J.H. *Altria Client Services LLC, Research, Development & Regulatory Affairs, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
16:30-16:45	ST 31	**Overcoming the challenges inherent in effective quality assurance for modern oral pouches** TINDALL I.; BOJINOV I. *Cerulean, Rockingham Drive, Milton Keynes, U.K.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 11
MONDAY 24 OCTOBER

SESSION 1 - E-vapour products: product assessment

Chair: Karl WAGNER

Moderator: Rob STEVENS

CET Time Zone

13:30-13:45	ST 32	**Toxicology risk assessment: an approach for controlling the health risk of electronic cigarette** HUANG Yilan; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
13:45-14:00	ST 33	**Chemical analysis of selected harmful and potentially harmful constituents and *in vitro* toxicological evaluation of leading flavoured e-cigarette aerosols in Chinese market** XU Te; LU Rui; NIU Zhenyu; XU Jing; LI Xinduo; LUO Quan; LUO An; HUANG Yilan; JIANG Xingtao; WU Zehong *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
14:00-14:15	ST 34	**Extractables and leachables evaluation for the safety of materials in electronic cigarettes** LI Xinduo; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
14:15-14:30	ST 35	**Non-targeted analysis of ENDS – challenges, considerations and best practices** JEONG L.N.; CROSSWHITE M.R.; JAMESON J.B.; LYNDON M.; COOK D.K.; GILLMAN I.G. *JUUL Labs, Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.*
14:30-14:45	ST 36	**A 12-month stability study on JUUL2 Virginia tobacco and crisp menthol flavoured aerosols using targeted analytical methods** COOK D.K.; JEONG L.N.; WANG J.; BATES A.L.; ULLAH S.; CARTER K.M.; SCHWARTZ V. *JUUL Labs, Inc.,1000 F St NW, 8th Floor, Washington, D.C., 20004, U.S.A.*
14:45-15:00	ST 37	**A 12-month stability study on JUUL menthol flavoured aerosols using two non-targeted analytical methods** CROSSWHITE M.R.; JEONG L.N.; BAILEY P.C.; JAMESON J.B.; LIOUBOMIROV A.; COOK D.K.; YANG C.; OZVALD A.M.; LYNDON M.; GILLMAN I.G. *JUUL Labs, Inc., 1000 F Street NW, Washington, D.C. 20004, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 11
MONDAY 24 OCTOBER

SESSION 2 - E-vapour products: analytical methods

Chair: Rob STEVENS

Moderator: Karl WAGNER

CET Time Zone

15:30-15:45	ST 38	**Solvent-free squeezing extraction method to obtain higher concentrated test article formulation for e-cigarettes and heated tobacco products** ITO H.; SHIGETO A.; FUJITA R.; SEKIGUCHI H.; HASHIZUME T. *Japan Tobacco Inc., Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*
15:45-16:00	ST 39	**An analytical screening method to characterise changes of flavour ingredients in e-liquids** HE Yi; LIU Peixian; LIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co.,Ltd., Shenzhen, Guangdong 518000, China*
16:00-16:15	ST 40	**Non-target analysis of flavour ingredients in aerosols of electronic nicotine delivery systems by cryogenic trapping** HE Yi; LIU Peixian; LIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co.,Ltd., Shenzhen, Guangdong 518000, China*
16:15-16:30	ST 41	**An UPLC-MS/MS method for the determination of 11 amine compounds in electronic cigarette liquids and aerosols** LUO Junjun; LI Xinduo; LIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co., Ltd., Shenzhen, Guangdong 518000, China*
16:30-16:45	ST 42	**An UPLC-MS/MS method for the determination of 11 carbonyl compounds in electronic cigarette liquids and aerosols** LUO Junjun; LI Xinduo; LIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co., Ltd., Shenzhen, Guangdong 518000, China*
16:45-17:00	ST 43	**Sicrit-Q-ToF-MS for on-line monitoring of nicotine and cooling agents in ENDS aerosol** LUO Junjun; LI Xinduo; LIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co., Ltd., Shenzhen, Guangdong 518000, China*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 12
TUESDAY 25 OCTOBER

SESSION 1 - E-vapour products: *in vitro* toxicological assessment

Chair: Kei YOSHINO

Moderator: Paul HARP

CET Time Zone

13:30-13:45	ST 44	**Comparison of the toxicological potential of JUUL VT3 and ME3 ENDS products to reference cigarette 3R4F and filtered air in a 90-day OECD 413 nose-only inhalation toxicity study** WADHWA DESAI R.(1); DEMIR K.(1); TSOLAKOS N.(2); WEIL R.(1); OLDHAM M.(1); LALONDE G.(1) *(1) JUUL Labs, Inc., 1000 F Street NW, Washington, D.C., U.S.A.* *(2) National Centre of Scientific Research, Demokritos, Patriarchou Grigoriou E' & Neapoleos 27 Scientific Park Lefkippos, Bldg 27, 15341, Ag. Paraskevi, Attiki, Greece*
13:45-14:00	ST 45	**Biological and proteomic changes in C57BL/6 mice after 10 weeks of inhalation of tobacco cigarette and electronic cigarette** KUN Duan; LIU Chuan; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
14:00-14:15	ST 46	**Comparison of *in vitro* human alveolar macrophage responses after exposure to cigarette smoke and e-liquids** HUTTER V.(1,2); HOPPER S.(3); SKAMARAUSKAS J.(2); HOFFMAN E.(1) *(1) ImmuONE Ltd, Science Building, College Lane, Hatfield, Herts. AL10 9AB, U.K.* *(2) Centre for Topical Drug Delivery and Toxicology, University of Hertfordshire, College Lane Campus, Hatfield, Herts. AL10 9AB, U.K.* *(3) School of Clinical and Applied Sciences, Leeds Becket University, City Campus, Woodhouse Lane, Leeds, U.K.*
14:15-14:30	ST 47	**A 3D *in vitro* electronic-cigarette flavours testing strategy using cigarette smoke as context** BISHOP E.; EAST N.; BREHENY D.; GACA M.; THORNE D. *B.A.T. (Investments) Limited, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*
14:30-14:45	ST 48	**A weight of evidence review on the potential acute and chronic risks of e-cigarette use** THORNE D.(1); SCOTT K.(2); GOSS C.(1); COONEY S.(1); CAMACHO O.(1); PROCTOR C.(3); MURPHY J.(1) *(1) B.A.T. (Investments) Limited, Regents Park Road, Millbrook, Southampt., SO15 8TL, U.K.* *(2) ToxDesk Ltd, 8 Newry Road, Banbridge, Co. Down, BT32 3HN, U.K.* *(3) DoctorProctorScience Ltd, 157 Cavendish Meads, Ascot, Berkshire, SL5 9TG, U.K.*
14:45-15:00	ST 49	**The use of ToxTracker for the toxicological assessment of tobacco and nicotine delivery products** SMART D.; BOZHILOVA S.; MIAZZI F.; HASWELL L.; GACA M.; THORNE D.; BREHENY D. *B.A.T. (Investments) Limited, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 12
TUESDAY 25 OCTOBER

SESSION 2 - Novel tobacco products: *in vitro* toxicological assessment

Chair: Paul HARP

Moderator: Kei YOSHINO

CET Time Zone

15:30-15:45	ST 50	***In vitro* toxicology assessment of ZYN®, oral nicotine pouches** MOSES S.; DWIVEDI A.; LJUNG T.; LINDHOLM J. *Swedish Match, Regulatory and Scientific Affairs, SE-118 53 Stockholm, Sweden*
15:45-16:00	ST 51	**Comparative study of a 24-well miniaturiseThd Ames test vs the standard Ames test for mutagenicity assessment of tobacco products** TAKAHASHI Y.; ISHII T.; SAKAI Y.; KUMAGAI E.; TSUTSUMI Y.; HASHIZUME T.; FUKUSHIMA T. *Japan Tobacco Inc., Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa, 227-8512, Japan*
16:00-16:15	ST 52	**Comparing the cytotoxic potential between e-cigarette aerosols and cigarette smoke** XU Jing; LIU Qianyun; LU Rui; WU Zehong; XU Te; WANG Weiwei; ZHAO Zhen; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
16:15-16:30	ST 54	**Comparison of heated tobacco product aerosol to cigarette smoke in human bronchial epithelial tissues using high content screening** TRELLES STICKEN E.(1); WIECZOREK R.(1); POUR S.J.(1); CHAPMAN F.(2); SIMMS L.(2); STEVENSON M.(2) *(1) Reemtsma Cigarettenfabriken GmbH, Albert-Einstein-Ring-7, D-22761, Hamburg, Germany* *(2) Imperial Brands PLC, 121 Winterstoke Road, BS3 2LL, Bristol U.K.*

[ST 53 withdrawn]

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 13
WEDNESDAY 26 OCTOBER

SESSION 1 - Heated tobacco products: product design, modeling and testing

Chair: Bernhard EITZINGER

Moderator: Bin HU

CET Time Zone

13:30-13:45	ST 55	**Bridging fundamental cigarette combustion science to drive innovative heated tobacco products** LIU Chuan *Pinevale Ltd, Pine Way, Southampton SO16 7HF, U.K.*
13:45-14:00	ST 56	**A strategy to bridge between THP product variants using exemplar data** MIAZZI F.; HADLEY S.; THORNE D.; JAUNKY T.; GACA M.; BREHENY D.; GOODALL S. *B.A.T. (Investments) Limited, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*
14:00-14:15	ST 57	**Simulation study on the effect of perforation on mainstream smoke temperature in an heated tobacco product stick** JUNG Yongmi *KT&G Research Institute, Daejeon 305-805, South Korea*
14:15-14:30	ST 58	**Numerical simulation of heat transfer processes and releases of key components in electrically heated tobacco products** GAO Yihan(1); HUANG Jiejie(2); JIANG Xu(1); WU Jinlu(2); GAO Jie(1); GAO Naiping(2) *(1) Shanghai New Tobacco Product Research Institute, Shanghai 201315, China* *(2) Tongji University, Shanghai 200092, China*
14:30-14:45	ST 59	**Research on preparation technology of heated tobacco core material based on powder forming process** LI Zhongren; ZHANG Wenjun; WU Jianlin; CHEN Yuchao; ZHOU Jun; LIU Jing; FENG Tao; ZHANG Mengyuan; JIN Quanrong *China Tobacco Schweitzer (Yunnan) Reconstituted Tobacco Co., Ltd., Yunnan Yuxi 653100, China*
14:45-15:00	ST 60	**Application of "cooling and low retention" filter rods made of cellulose acetate microspheres for heated tobacco products** SONG Xiaomei; CAO Jianguo; SU Kai; ZHANG Li; YANG Guangmei; YANG Zhanping *Technology Center, Nantong Zhuhai Kunming Cellulose Fibers Co., Ltd., Nantong 226008, Jiangsu, China*
15:00-15:15	ST 61	**A comparative study on delivery of nicotine, humectants and endogenous aroma constituents from reconstituted tobacco materials in granule and sheet form under heat-not-burn condition** LIU Shengyi; ZHU Libing; DOU Jinxi; LIU Weijuan *(1) Ruvian Technology Ltd., Kunming, 650106, China* *(2) Yunnan Reascend Tobacco Technology (Group) Co., Ltd, Kunming, 650000, China*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 13
WEDNESDAY 26 OCTOBER

SESSION 2 - Heated tobacco products: analytical methods

Chair: Bernhard EITZINGER

Moderator: Jutta PANI

CET Time Zone		
15:45-16:00	ST 62	**Studies on analytical method and transfer rate for minor alkaloids in tobacco filler and aerosol of heated tobacco products by GC-MS** KIM Hye-Won; SONG Hye-Young; JIN Sun-Jin; LEE Jeong-Min *KT&G Research Institute, Daejeon 305-805, South Korea*
16:00-16:15	ST 63	**Development of an analytical method for carbonyls in aerosol of aHTP** LEE S.B.; DO E.Y.; HAN E.J.; LEE J.M. *KT&G Research Institute, Daejeon 305-805, South Korea*
16:15-16:30	ST 64	**Development and validation of a GC-MS method for the analysis of PAHs in heated tobacco product aerosols** HAMMOND D.; McGUIGAN S.; THOMAS J.; GIBBONS P. *Hall Analytical Laboratories Ltd, Waterside Court, 1 Crewe Road, Wythenshawe, Manchester, M23 9BE, U.K.*
16:30-16:45	ST 65	**Application of TD-GC×GC-TOFMS for the comparison of the emissions profiles of non-tobacco based substrates in tobacco heating systems** PINTO M.I.; GUSSMAN S.; HERMES N.; DALTON D.D.; WRIGHT C. *B.A.T (Investments) Limited, R&D, Regents Park Rd, Southampton SO15 8TL, U.K.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 14
THURSDAY 27 OCTOBER

SESSION 1 - Tobacco and cigarette smoke: analytical methods

Chair: Jutta PANI

Moderator: Bin HU

CET Time Zone

13:30-13:45	ST 66	**Rapid determination of nicotine content of reconstituted tobacco based on near-infrared spectroscopy technology** YANG Shuangyan(1); YANG Tao(1); SHEN Yanwen(1); YANG Zigang(1); ZHANG Jianqiang(2) *(1) Yunnan Tobacco Biological Technology Co., Ltd, Kunming, 65000, China* *(2) Yunnan Police College, Kunming, 65000, China*
13:45-14:00	ST 67	**Ion-exclusion chromatography coupled to tandem mass spectrometry for quantification of multiple organic acids in tobacco and reduced-risk products** WINDISCH P.; ZIERLINGER M.; EILENBERGER G. *JTI - ÖKOLAB Gesellschaft für Umweltanalytik GmbH, Hasnerstrasse 127, A-1160 Vienna, Austria*
14:00-14:15	ST 68	**Development of an analytical method for nitrogen compounds in mainstream cigarette smoke using GC-MS** JU Soyoung; LEE Hye-Jeong; MIN Hye-Jeong *KT&G Research Institute, Daejeon 305-805, South Korea*
14:15-14:30	ST 69	**An improved method for the determination of carbonyls in cigarette smoke and butyraldehyde co-elution** JIN X.C.; BALLENTINE R.M.; LI W.; MILLER J.H. *Altria Client Services LLC, Center for Research and Technology, 600 E. Jackson St, Richmond, VA 23219, U.S.A.*
14:30-14:45	ST 70	**Evaluation on accuracy improvement of GC-MS determination of seven minor alkaloids in mainstream cigarette smoke by novel analyte protectants** WANG Xiaoyu(1); GUO Qiong(1); Li Xianyi(2); QIN Yaqiong(1); MAO Deshou(2); PAN Lining(1); XIE Fuwei(1); LIU Shaofeng(1); LIAO Tougen(2); LIU Huimin(1) *(1) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China* *(2) R&D Center, China Tobacco Yunnan Industrial Co., Kunming 650231, China*
14:45-15:00	ST 71	**Analysis of six aromatic amines in the mainstream smoke of tobacco products** JI Huihua; JIN Zhenyu *Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 14
THURSDAY 27 OCTOBER

SESSION 2 - Flavours and water pipes: analytical methods

Chair: Karl WAGNER

Moderator: Jutta PANI

CET Time Zone		
15:30-15:45	ST 72	**Analytical approach to replacing a number of sensory panel based tests** FERREIRA T.; CASTRO L.; MARCELO M.; KLERING A.; NASCIMENTO J.; BAZANELLA D.; COLOMBO V.; JOBIM T.; MEIRELLES M.; ASSIS C.; SCHAEFER F. *British American Tobacco Brazil, TSS-AmSSA, Av. Frederico A. Ritter 8000, Cachoeirinha, RS, 94970-470, Brazil*
15:45-16:00	ST 73	**Quantitative determination of 23 flavour compounds related to potential additives in tobacco products and regulatory reporting requirements** RODRIGUEZ-LAFUENTE A.; BIERNACKA P.; JOZA P. *Labstat international Inc., 262 Manitou Dr., Kitchener, Ontario N2C 1L3, Canada*
16:00-16:15	ST 74	**Quantitative determination of 28 flavour compounds (lactones and pyrazines) related to potential additives in tobacco products** RODRIGUEZ-LAFUENTE A.; DONISA C.; JOZA P. *Labstat international Inc., 262 Manitou Dr., Kitchener, Ontario N2C 1L3, Canada*
16:15-16:30	ST 75	**Waterpipe bowls and heaters: does the ISO standard reflect what is available to consumers of waterpipe tobaccos?** LAUTERBACH J.H. *Lauterbach & Associates, LLC, 211 Old Club Court, Macon, GA 31210-4708, U.S.A.*
16:30-16:45	ST 76	**Do residues after use provide more information on in-use product chemistry than do emissions? A study with waterpipe tobaccos** LAUTERBACH J.H. *Lauterbach & Associates, LLC, 211 Old Club Court, Macon, GA 31210-4708, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 15
FRIDAY 28 OCTOBER

SESSION 1 - Cigarette component testing and design

Chair: Kei YOSHINO

Moderator: Bernhard EITZINGER

CET Time Zone

13:30-13:45	ST 77	**Effect of filter ventilation hole characteristic on cigarette ventilation rate** DENG Nan(1,2); ZHANG Qi(2); YANG Zhongpan(3); LI Xiqiang(4); WANG Le(2); LI Hui(2); WU Lianlian(3); WANG Xiaofeng(5); WANG Bing(2); Li Bin(2) *(1) Instrumental Analysis Center, Xi'an Jiaotong University, Xi'an, Shaanxi, China* *(2) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan, China* *(3) China Tobacco Gansu Industrial Co., Ltd, Lanzhou, Gansu, China* *(4) Hongta Liaoning Tobacco Co., Ltd., Shenyang, Liaoning China* *(5) China Tobacco Anhui Industrial Co., Ltd, Hefei, Anhui, China*
13:45-14:00	ST 78	**Microscopic analyses of filtering media** CHERKAS O.; BLIN T.; RAVERDY-LAMBERT D. *SWM International, c/o LTR, Usine Le Mans, 72702 Allonnes, France*
14:00-14:15	ST 79	**Development of pack tactile consumer usage test method** REJIMON S.; RAVI C.; ANINDYA K.; AJIT KUMAR P.C.; NAIK P.V.; MUKHERJEE S. *ITC Life Science and Technology Center, ITC Limited, 1st Phase, Peenya Industrial Area, Peenya, Bangalore 560064, India*
14:15-14:30	ST 80	**Analysis of absorption hysteresis of different cigarette blends based on adsorption thermodynamics method** QIU Changgui(1,2); LIU Ze(3); WEI Qing(1); HE Banghua(3); YANG Qianxu(3); LIU Jing(1,2); LI Siyuan(4); WANG Zeyu(4); ZUO Wen(4); YANG Lan(4); GUO Miaomiao(3) *(1) Yunnan Reascend Tobacco Technology (Group) Co., Ltd., Kunming 650106, China* *(2) Yunnan Comtestor Co., Ltd., Kunming 650106, China* *(3) China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, China* *(4) Hongyun Honghe Tobacco (Group) Co., Ltd., Kunming 650231, China*
14:30-14:45	ST 81	**Influence of leaflet shape and size on processing properties of reconstituted tobacco** LIU Jing; LI Zhongren; ZHU Ting; LU Jin; MAO Junjie; QIN Qi; CHEN Hua; ZHANG Lizhong; LI Junchao *China Tob. Schweitzer (Yunnan) Reconstituted Tob. Co., Ltd., Yunnan Yuxi 653100, China*
14:45-15:00	ST 82	**Establishment of an algorithm model for internal sensory quality and key process parameters of paper-making reconstituted tobacco** LI Zhongren; FENG Tao; WANG Zhipeng; LI Hailong; ZHANG Wenjun; ZHOU Jun; YUAN Jue; WU Jianlin; CHEN Yuchao *China Tob. Schweitzer (Yunnan) Reconstituted Tob. Co., Ltd., Yunnan Yuxi 653100, China*
15:00-15:15	ST 83	**Preparation of flavour metal complexes with adjustable pyrolysis temperature by metal ion selection** ZOU Peng; HE Zengyang; ZHANG Jin; SHU Junsheng; WANG Wenbin; NING Yong; SHAO Ning *China Tobacco Anhui Industrial Co., Ltd., Hefei 230088, China*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 15
FRIDAY 28 OCTOBER

SESSION 2 - E-vapour and e-liquid modeling

Chair: Bin HU

Moderator: Bernhard EITZINGER

CET Time Zone

15:45-16:00	ST 84	**Population balance - Monte Carlo simulation for aerosol evolution of glycerol** SHI Dantong; ZENG Fan; LIU Chuang; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
16:00-16:15	ST 85	**Capillary evaporation model of binary mixed solution of propylene glycol and glycerin in e-cigarette atomizer** CHEN Jingbo(1,2); WANG Zhiguo(1); PAN Zhenhai(3); CAO Jun(1); SUN Zhiwei(1,2); LIU Wei(1); KONG Bo(1); DU Wen(1) *(1) Technology Center, China Tobacco Hunan Industrial Co., Ltd., Changsha 410007, China* *(2) College of Mechanical and Vehicle Engineering, Hunan University, Changsha 410082, China* *(3) College of Mechanical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China*
16:15-16:30	ST 86	**Numerical simulation of aerosol evolution for e-cigarette** SHI Dantong; ZENG Fan; LIU Chuang; JIANG Xingtao *Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China*
16:30-16:45	ST 87	**Brand verification and counterfeit identification of e-liquid based on electronic nose technology** SONG Zheng; LIU Peixian; JIANG Jingjing; JIANG Xingtao *Shenzhen Matrix Technical Service Co., Ltd., Shenzhen, Guangdong 518000, China*

ABSTRACTS

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

AP 01

Optimization of cytoplasmic male sterility based flue-cured tobacco hybrid seed production system

DEERGASI Satish Kumar; VENKATA REDDY T.; VENKATA GANESH R.

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Tobacco is one of the commercial crops grown in India in an area of 0.45 M ha with 800 M kg production. India stands third in tobacco production and exports in the world. Flue-cured tobacco has a major share in exports. Seed is the most important determinant of agricultural production potential. A sustained increase in production and productivity is dependent, to a large extent, on development of new and improved varieties and hybrids and an efficient system for timely supply of quality seeds to farmers. Tobacco is an often cross-pollinated crop and hybrid seed production is practicable using male sterility systems. As leaf is the economic part in tobacco cytoplasmic male sterility (CMS), it is used in hybrid seed production. ITC ABD R&D is engaged in production of hybrid seed to cater for the seed requirements of different tobacco growing zones. During the initial years of hybrid seed production, the productivity is low. Higher seed productivity ranging from 30-50 % could be achieved by improving certain practices such as 1) Synchronisation of flowering in male and female parents by staggered sowing 2) Modification of planting ratio of male and female parental lines, 3) Optimisation of practices and training of manpower in manual hand pollination. Anthers were collected and dry pollen was extracted and stored for future use for pollination. Hand pollination was carried out after flower initiation in the female parent. The seed collected from the capsules was dried to reach a designated moisture level for storage. Hybrid seed thus produced meets the seed requirements for producing flue-cured tobacco in Northern Light Soils and Mysore.

AP 02

The interaction of water source and fertilizer chemistry on greenhouse tobacco transplant production

REED T.D.; IRBY R.S.

Virginia Tech, Southern Piedmont Center, Blackstone, VA 23824, U.S.A.

A float greenhouse transplant study was conducted at the Virginia Tech Southern Piedmont Center (Blackstone, Virginia, United States) to evaluate the interaction of water source and fertilizer analysis on tobacco transplant production. One water source (municipal) had a relatively low buffering capacity with a neutral pH and a relatively low total alkalinity. The second water had a high pH and alkalinity level plus a conductivity level twice that of the first water. Data were collected of bay water and soilless media pH, electrical conductivity, and nutrient content throughout the season. Samples for tissue analysis were collected just prior to first clipping and when seedlings were ready for transplanting. Both bay water and soilless medium pH were higher from all four fertilizers with the well water source. Trends in soilless medium pH corresponded to the acid/base reaction of the fertilizer treatments. Tissue analysis showed differences in a few nutrient elements but not of much consequence and no deficiency or toxicity symptoms were observed. Results of this study illustrate the impact of water source on greenhouse transplant production factors as well as the need to match fertilizer chemistry to water source chemistry.

AP 03

The impact of starter fertilizer to flue-cured tobacco growth

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An increasing number of flue-cured tobacco farmers are including water soluble fertilizer products in transplant water applications. The goal of these starter fertilizer programs is to supply a small quantity of phosphorus, which may stimulate early-season plant vigor particularly in cold, damp soil conditions.

Commonly, some of these fertilizer products have not been evaluated by independent researchers and their impact is sometimes questionable across a wide range of growing conditions. Our objective was to evaluate a small number of commercially available transplant water fertilizer products (Ultrasol 9-45-15, 11-37-0 ammonium polyphosphate, 6-20-0 Black Label Zinc, 6-24-6 Advance RTU, and 8-31-4 Exceed), each providing 5.6 (1×) and 11.2 kg P_2O_5 ha^{-1} (2×), in order to quantify their impact on vegetative growth up to six weeks after transplanting. Two non-treated controls were included for comparison, transplant water only and no transplant water. Data are reported from two locations in 2021. In the first data analysis (Fertilizer Product × Application Rate), Ultrasol treatments were more vigorous than all other products and had a higher shoot dry mass at two and four weeks after transplanting. The 1× application rate was also superior to the 2× rate, regardless of fertilizer product, thus indicating that excessive starter fertilizer may be more harmful due to soluble salts injury. Treatment differences were not observed by week six, which suggests that plant recovery from minor injuries is possible. In the second data analysis (Fertilizer Product × water only × no water), those treatments absent of fertilizer or with 1× rates (Ultrasol and Black Label Zinc) had the highest vigor ratings two weeks after transplanting. The Ultrasol 2× treatment produced a similar response. All other treatment combinations resulted in visual vigor ratings that were poorer than the non-treated controls, again, indicating that soluble salts were inhibiting seedling recovery after transplanting. These observations were not documented at four and six weeks after transplanting. Our results suggest that the inclusion of transplant water fertilizer solutions is no more beneficial than water alone – particularly in transplanting seasons characterized by dry soil and air conditions, such as were experienced in 2021.

AP 04

Developing nitrogen and potassium fertilizer recommendations for Connecticut broadleaf cigar wrapper tobacco in North Carolina

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Connecticut broadleaf cigar wrapper tobacco is a new crop to North Carolina. As such research-based fertilizer recommendations are needed to assist farmers with commercial production. Nitrogen (N) and potassium (K) rate trials were conducted in four environments across North Carolina in 2021. The research locations represented Coastal Plain, Piedmont, and Appalachian Mountain production areas of the state. Nitrogen application rates of 0, 90, 179, 269, 359, and 448 kg/ha were tested in each environment. In a separate but co-located trial, rates of 0, 56, 112, 168, 224, 280, and 336 kg K_2O/ha were evaluated. Preliminary data showed that higher N and K_2O rates produced higher N and K concentrations in the leaf tissue, respectively, especially later in the season (6 and 9 weeks after transplanting). Additionally, our limited results indicate that the optimal N rate for maximized leaf yield is roughly 199 kg/ha. The K_2O rates we evaluated did not affect yield; however, this may be due to high initial K levels within those soil systems prior to transplanting (equivalent of 131 to 383 kg K_2O/ha). Results from all environments will be presented.

AP 05

Sustainable soil health solutions: evaluation of biofertilizers for use on tobacco in Zimbabwe

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The increasing number of farmers going into tobacco production in Zimbabwe has meant that land availability has increasingly become scarce and the norm of rotations of 3-4 years with Katambora Rhodes grass is now rarely adhered to. Tobacco has thus been grown continuously on the same piece of land for years to decades and the sandy to sandy loam soils that tobacco is characteristically grown in have become degraded over the years. To sustain tobacco productivity under the limited land resources, there is thus a dire need to develop sustainable soil management systems that improve soil fertility. To this end, a wide range of biofertilisers have been evaluated at Kutsaga for their efficacy to boost soil fertility in soils where tobacco is grown. Biofertilizers are natural fertilizer preparations containing living microbial inoculants of bacteria, algae, plant growth promoting rhizobacteria (PGPRs), endo- and ecto-mycorrhizal fungi, cyanobacteria and other efficient micro-organisms. Most of these preparations are reported to boost the amount of organic matter in the soil, improve soil texture and structure and improve the availability of nutrients to plants thereby enhancing plant growth and plant tolerance to abiotic and biotic stress. This presentation will give a summary of the research done in the last three years to evaluate the efficacy of biofertilizers on tobacco, the outcome of the evaluations and discuss the implications of these results. Generally, as a result of this evaluation three products have so far been found effective and have since been recommended for registration on tobacco, while some still need protocol refinement to enhance their effectiveness.

AP 06

Response of soil bacterial community structure and co-occurrence network topology properties to soil physicochemical properties in long-term continuous cropping farmland

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The objective of this study was to explore the relationship between the soil bacterial community (i.e. structure and molecular ecological network) and environmental evolution in long-term continuous cropping field.

In this study, soil samples were collected from two 12-year continuous cropping fields (GD with continuous cropping disorder and healthy YA) in Liuyang, Hunan Province. The bacterial community were analysed using high-throughput sequencing of 16S rRNA gene amplicons, molecular ecological network was constructed using molecular ecological network analyses (MENA), and both were related to the soil physicochemical properties.

The results showed that the total nitrogen and available phosphorus in GD were significantly higher, while the nitrate nitrogen and available potassium in GD soil were significantly lower than that in YA ($p < 0.05$). The soil bacterial community diversity was higher in GD than YA, and the bacterial community structure showed significant difference between GD and YA ($p < 0.01$). The bacterial community was significantly constrained by the soil pH and available phosphorus. The ecological network was more complex in GD than YA, which was mainly reflected in the modules containing energy metabolism, carbon cycle and nitrogen cycle functions.

In conclusion, continuous cropping caused significant changes in the soil bacterial community diversity and the structure and ecological network, which might affect the deterioration of soil physicochemical properties and soil fertility, and therefore, affect crop growth and development.

AP 07

Optimization of prediction model for tobacco leaf dehydration rate during intensive curing process based on machine learning

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The objective of this study was to quantify the apparent characteristic values of tobacco leaves during the intensive curing process, to grasp the law of changes in water loss, and to achieve precise control of curing process parameters during the curing process.

Using the middle leaves of CB-1 as the material, the image and weight data of tobacco leaves during the curing process were collected, and 10 colour and texture features of tobacco leaves were extracted by image processing technology. The analysis took the optimised tobacco leaf characteristic value as the input variable, and used the grid support vector machine (GS-SVM), the genetic algorithm optimized BP neural network (GA-BP) and the extreme learning machine (ELM) algorithm to establish the tobacco leaf loss during the curing process. Water rate prediction was modelled, and evaluation and analysis of prediction accuracy and model verification and selection was carried out.

According to the feature variable clustering and correlation analysis, two colour features (a*/b*, R) and two texture features (Gradient entropy, gradient unevenness), the test set root mean square error (RMSE) of the established grid SVM and GA-BP neural network and ELM model are 0.0117, 0.0139, 0.0140 respectively, and the test set determination coefficient (R^2) are 0.9973, 0.9962, 0.9961, respectively. The GS-SVM model had the highest accuracy.

The prediction model of the moisture loss rate of tobacco leaves during the intensive curing process established by machine learning meets the needs of real-time detection, and the GS-SVM model has the best prediction effect. The research results can provide a basis for the optimisation of intensive curing technology, and lay a theoretical foundation for the development of an intelligent control system for tobacco curing.

AP 09

Effect of ridging and hilling on dark tobacco standability and sucker control

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Sucker control practices for dark tobacco are done primarily with contact and local systemic agrochemicals. These are applied as labor-intensive, manual stalk-rundown applications with droplines due to the tendency of dark tobacco to have crooked stalks at the time that sucker control applications need to be made. Management practices that keep dark tobacco straighter may make it possible for more efficient spray applications to be made for sucker control. In 2021, two field trials were conducted in Graves Co., KY, and one trial in Springfield, TN, to evaluate the effect of ridging and topping height on standability and sucker control. Tobacco was either transplanted flat as is traditional, or on a ridge in the first Graves Co. trial and in the Springfield trial. In the second Graves Co. trial, tobacco was transplanted flat in the entire trial, and then half of the plots were hilled to form a small ridge after transplanting. Within each ridging, hilling, or flat-transplanted block, topping heights (12 vs. 16 leaves in the first Graves Co. trial or 14 vs. 18 leaves in the second Graves Co. and Springfield trials) and sucker control applications (sprayed broadcast in the first Graves Co. trial or directed 3-nozzle per row in the second Graves Co. and Springfield trials, or droplined) were compared. In the first Graves Co. trial and the Springfield trial where ridging was used, benefits in field drainage were observed, but ridging did not increase standability of the tobacco compared to flat transplanting. In the second Graves Co. trial, hilling after flat transplanting increased standability over flat transplanting but did not increase sucker control or yield. In the ridging trials, yield was higher with flat transplanting and higher topping heights, and sucker control was greater with manual dropline application compared to either spray method.

AP 10

Field performance of fewer sucker variety in production area of Japan

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Sucker control is a key process for tobacco production because it affects yield and quality. In general, sucker growth is suppressed by using a suckercide and the remaining suckers are removed by hand. However, such sucker management is a tough workload on farmers. In addition, the suckercide itself has environmental concerns. At present, some research papers on reducing suckers have been reported but their practical use has not been tested yet. In a previous CORESTA Conference, we already presented the three knock-out mutants (*ls*, *bl*, *rev*) suppressing sucker growth. The objective of this study was to evaluate agronomic traits and the effect of the target gene in a fewer sucker variety in a tobacco production area of Japan. The fewer sucker flue-cured Virginia cultivar with *ls*, F80, was developed using Coker 319 as a recurrent parent. This variety was cultivated and its characteristics measured in a production area of southern Japan. As a result, the secondary suckers of this variety were 20 % less than the check variety, Coker 319, and the tertiary suckers hardly appeared. F80 showed almost the same quality and agronomic traits as Coker319 and a higher yield tendency than Coker 319. It had dissected flowers; however, it was possible to produce 80 % of seed weight compared to Coker 319. F80 showed feasible agronomic traits to contribute to reducing workload and chemical use in Japan. We are planning to start commercial production in the 2023 crop. And breeding of new varieties with *ls* is being considered.

AP 11

Evaluations of modern spray nozzle technology for maleic hydrazide application

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Maleic hydrazide (MH) remains a critical component of sucker control programs in US tobacco production systems. However, MH residues remain a strong critique of tobacco sourced from the region. The purpose of our research was to evaluate medium and high-output nozzles (234 - 468 L ha^{-1}) with ultra-coarse droplet size (> 650 microns) for their impact to sucker control efficacy and cured leaf residues in three stalk positions (cutter, leaf, and tip). Three ultra-coarse nozzles were selected: TurboDrop XL® (TDXL), Air Induction Turbo Twinjet (AITTJ) and Turfjet (TJ) and compared to the grower standard three-nozzle sucker control arrangement (outfitted with three solid cone nozzles) and a single solid cone nozzle. Each nozzle type and configuration was evaluated at 234 and 468 L ha^{-1} solution volumes. A non-treated control was included to establish sucker control parameters but was not included in the data analyses. Sucker control was > 97 % among each treatment combination (nozzle type × output volume); however, the TDXL (468 L ha^{-1}), AITTJ (468 L ha^{-1}), AITTJ-(234 L ha^{-1}), and TJ (234 L ha^{-1}) increased sucker control above the current grower standard. Cured leaf MH residues were similar among nozzle types in the cutter and leaf stalk position, but were lowest in tip leaves when the AITTJ and TJ nozzles were used. Cured leaf MH residues were also greater within the 234 L ha^{-1} solution volume than the 468 L ha^{-1} solution volume across each of the three stalk positions. Our results suggest that growers might utilize new nozzle technology in an attempt to lower MH residues in cured tobacco, but they should not change the recommended solution output volume of 468 L ha^{-1}. Our results also suggest that in situations where growers do not have restrictions with MH residues, they may consider the 234 L ha^{-1} solution volume in an attempt to reduce the number of times they have to fill sprayer tanks.

AP 12

Sustaining maleic hydrazide: an alternative application technique for reduced residues in flue-cured tobacco

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Cured leaf residues of the plant growth regulator maleic hydrazide (MH) remain as a serious concern to the allied tobacco industry in the United States. Current MH application programs rely on foliar, over-the-top delivery systems that saturate the plant with the spray solution. We tested an alternative delivery system in three different growing environments that applied an MH solution to the base of tobacco plants after first harvest. Treatments comprising the alternative delivery system contained three MH concentrations (40 %, 50 %, and 60 % MH v/v), each applied at four different output volumes: 5 mL, 10 mL, 15 mL, and 20 mL plant^{-1}. A treatment containing topped, but not de-suckered plants was included as a negative control. A conventional, over-the-top MH application (3 % v/v at 31.5 mL plant^{-1}) was included as the current grower standard. After the final leaf harvest, sucker growth, yield, visual quality, economic value, and MH residues in three stalk positions were quantified. Relative to the over-the-top application, the alternative stalk applied treatments had similar sucker control, yield, quality, and value. However, MH residues in each alternative treatment were significantly lower in the cutter stalk position. In the leaf and tip position, the 40 % and 50 % MH concentrations applied at 5 mL to 10 mL plant^{-1} most consistently resulted in lower MH residues than the conventional standard. When the conventional standard was excluded from the data analysis, we observed a yield decline in the tip leaf stalk position in treatments containing the 40 % and 60 % MH treatments. In addition, it was noted that MH residues were typically lowest in treatments containing the 40 % or 50 % v/v concentrations that were applied at 5 mL or 10 mL plant^{-1}. Our results suggest that stalk applications of MH at a 50 % v/v concentration applied at 5 mL to 10 mL^{-1} may prove to be a useful residue reduction strategy that does not compromise end of season sucker control or yield.

AP 13

Impact of harvest timing on yield, leaf quality, alkaloids, and tobacco-specific nitrosamines in Burley tobacco

RICHMOND M.D.

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Harvest timing of Burley tobacco is in relation to removal of the flower or terminal bud (topping). Current university recommendations suggest 3 to 5 weeks after topping (WAT) should be targeted with optimal at 4 WAT. The objectives of this study were to: i) confirm recommendations for optimal harvest timing for yield and leaf quality; ii) understand the impact of harvest timing on accumulation of alkaloids and tobacco-specific nitrosamines (TSNA); and iii) determine maleic hydrazide (MH) residues from cured leaf samples. Burley tobacco cultivar TN90LC was grown at the Northeast Tennessee Research and Education Center in 2021 and 2022. This trial utilized a randomized complete block design with five harvest timing treatments and four replications. Treatments included Burley harvested at 2, 3, 4, 5, and 6 WAT. In 2021, tobacco was topped and sprayed with MH and flumetralin on July 21 and harvest occurred between August 4-31. Total yield was significantly reduced when tobacco was harvested at 2 WAT. Burley harvested at 4 and 5 WAT were significantly highest for total yield. Federal Grade Quality Index showed that 5 WAT was significantly highest for leaf quality measurements. As expected, MH residues declined from 2 to 6 WAT which followed rainfall data. Total alkaloids increased from 2 to 6 WAT, however, 3 to 5 WAT were not significantly different. Conversion from nicotine to nornicotine was not impacted by harvest timing. There was a two-fold significant increase in TSNA content at 6 WAT, with 2 to 5 WAT not statistically different. Results from 2022 will be presented.

AP 14

DNA barcoding – practical applications in tobacco identification in Zimbabwe

ZVOBGO G.

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In Zimbabwe, the use of molecular markers, namely Universal Rice Primers (URP) to differentiate tobacco varieties, has been widely used to solve dispute issues, hybrid proving and identification of tobacco genotypes from other countries purported as new varieties. The use of this technique, however, has met some challenges emanating from the close relatedness of the tobacco varieties especially those that share a parent. DNA barcodes are short DNA sequences which can be used to isolate and characterise different plant species. Together with sequencing and bioinformatics information, DNA barcodes offer quick and accurate identification information. Thus, the major aim of this study was to utilise internal transcribed spacer (ITS) DNA barcode for the accurate identification and differentiation of tobacco varieties. A total of 13 tobacco varieties obtained from the Plant Breeding Division at the Tobacco Research Board were employed in the study. Total genomic DNA was extracted and initially amplified using URPs and then ITS primers. ITS sequencing was conducted in order to determine sequence polymorphism within the varieties. A phylogenetic tree was constructed using the ITS sequences with the unweighted pair group method with arithmetic mean, which indicated that the sister taxa variety of tobacco plant is likely to be successfully categorised into one cluster. These results showed that the DNA barcode comparison method can satisfy the need for a rapid, low-cost, frontline differentiation of the Zimbabwean tobacco varieties. Future plans are to conduct SNP-genotyping in order to develop markers that can accurately separate each tobacco variety.

AP 15

Automatic identification and precise prevention of deep green infection in tobacco leaf using a hand-held DLP-based NIR spectrometer

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(2) Yunnan Police College, Kunming, 65000, China

Identification and prevention of deep green infection play an important role in high-quality production of tobacco leaf. However, at present, it is difficult to automatically and accurately evaluate the infection level, and especially prevent the disease at asymptomatic stage. In this study, a novel infection identification and prevention method for deep green tobacco infection severity based on portable near-infrared spectroscopy (NIR) technology and extreme learning machine (ELM) algorithm is proposed. Firstly, NIR data of deep green leaf infection at different levels was scanned with a portable NIR spectrometer directly from the fresh tobacco leaves without defoliation and any sample preparation procedures in the field. Then, the qualitative and quantitative models were both built to identify and prevent deep green tobacco infection by means of using ELM algorithm. The experimental results showed that the qualitative model can automatically identify if the tobacco leaf is infected and the quantitative model can accurately acquire the infection condition at asymptomatic stage. These methods are simple, rapid, precise and are helpful to make appropriate decisions in precisely controlling the disease in the field.

AP 16

Application of protoplast technology facilitates the CRISPR-Cas9 mediated gene replacement in *Nicotiana tabacum* and confers resistance against tobacco mosaic virus

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Tobacco mosaic virus (TMV) is an important virus pathogen that threatens the production of tobacco. In the past 50 years, only the N gene has been deployed for TMV-resistant tobacco breeding, while the TMV-resistant flue-cured tobacco varieties carrying the N gene are difficult to be promoted due to the linkage drag. At the same time, the single-gene dominant resistance is at risk of being overcome by the virus. Therefore, the development and utilisation of new resistance genes is of great significance. The *N'alata* gene from wild tobacco *Nicotiana alata*, which is the *N'* orthologs from cultivated tobacco, is a TMV-resistance gene and shares a high sequence identity with the *N'* gene, which has no resistance to TMV. By performing a series of fragments swap between *N'alata* and *N'* genes, we confirmed that the 265-798th amino acids and the 1130-1233th amino acids of *N'alata* jointly determined its resistance to TMV-U1. Therefore, gene editing can be used to directly modify the corresponding functional region of *N'* in cultivated tobacco, so that the main cultivar can acquire TMV resistance and maintain the original agronomic and economic traits. This study thus combined the gene insertion method reported in rice and tobacco protoplast technology, and preliminarily achieved gene insertion and replacement in tobacco protoplasts. Afterwards, aiming at the biggest difficulty in the protoplast system—protoplast regeneration of plants, we carried out a series of optimisations for protoplast isolation, transfection and regeneration, and obtained a stable and efficient procedure of protoplast regeneration. Finally, the system of protoplast-mediated insertion and replacement of tobacco genes was established. In this study, the established gene knock-in system was used to replace the corresponding segment of the *N'* gene in cultivated tobacco with *N'alata* resistance region. The replacement of the cultivated tobacco gene was achieved and conferred resistance to TMV. The tobacco gene insertion and replacement system established in this study can be widely used in tobacco breeding research, which could promote the development of tobacco genome editing research.

AP 17

Breeding for high nicotine tobacco varieties

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The development of many 'next-generation' tobacco products is directly linked to the increase in demand for varieties with new chemical profiles. In France there is a commercial interest in a domestic supply of nicotine and this context creates a potential new opportunity for growers to maintain a tobacco crop locally. The objective of this project was to support this new demand by developing new varieties with increased nicotine rates, adapted to French growers' field conditions and material and profitability context.

A large amount of accessions (from the Imperial Tobacco collection) and lines from our breeding programme were screened during the last five years for this high nicotine target. We obtained a list of five best candidates and a breeding plan has been developed based on a combination of field screening, use of molecular markers and pathological tests for resistance selection, and nicotine analysis at each generation. Our programme is based on conventional breeding.

One of the key points of our project is to be able to use all stalk positions for nicotine extraction and we finally reached an average rate of 6,5 % (dry weight base) nicotine on the whole plant with our best variety. In parallel and in collaboration with field technicians and growers we developed an adapted crop management guideline for high nicotine variety production. The tobacco produced has been validated for its usability through the industrial process for nicotine extraction.

AP 18

Use of exotic *Nicotiana tabacum* germplasm for confronting an inverse genetic correlation in flue-cured tobacco

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Inverse genetic correlations between cured alkaloid accumulation and cured leaf yields in flue-cured tobacco complicate the development of higher-yielding cultivars while maintaining alkaloids at commercially acceptable levels. Introgression of genomic regions from exotic source material that positively affect alkaloid levels, but with lower corresponding negative influence on yielding ability, could enhance long-term efforts to develop improved commercial hybrids. Germplasm accessions TI 464 and TI 959 were previously identified as being potentially useful for this purpose. In research described here, the relationships between yield and alkaloid accumulation were studied in BC_1F_1 populations derived from crosses with these materials and found to be slightly negative or slightly positive. Derived BC_1F_2 families were used to calculate heritability estimates for alkaloid accumulation on a single plant basis that were low to intermediate in magnitude. The relationships between alkaloid levels of leaves from individual stalk positions and alkaloid levels averaged over all stalk positions were determined. The third stalk position was found to be most predictive of average alkaloid levels. Selection for alkaloid levels using a single leaf from this stalk position could increase breeding efficiency. BC_4F_6 lines carrying low genomic contributions from either TI 464 or TI 959 were generated and evaluated as lines *per se* and in F_1 hybrid combinations with a very high-yielding inbred line. Data indicated that TI 464 and, to a lesser extent TI 959, may be useful for developing higher yielding tobacco cultivars with acceptable alkaloid levels.

AP 19

Effects of drought stress on changes in morphology and expression of selected genes in tobacco

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Global climate change observed in recent decades indicates an alarming trend of increasing extreme weather events, including the emergence of more frequent and longer periods of drought. The increasing extent of drought in areas devoted to tobacco cultivation has been observed for many years. The aim of this study was to recognize the basic mechanisms of tolerance of tobacco plants to drought stress. The effect of drought stress on selected morphological and physiological parameters was determined. Moreover, based on the activity of genes encoding transcription factors involved in plant response to drought conditions, selected genotypes were evaluated for tolerance to drought stress. Genes involved in abscisic acid (ABA)-dependent and ABA-independent control of gene expression were examined. Based on the results obtained, the tolerance of 17 tested tobacco genotypes to drought stress was determined. Studies were conducted under controlled conditions of temperature, photoperiod and relative humidity. Morphological parameters were determined: plant height, leaf area, and number of stomata per mm^2 of leaf blade. Moreover, expression of four genes involved in plant response to drought stress was determined. Regardless of the genotype, all genes showed the highest expression between the 6^{th} and 10^{th} day of the experiment. The largest 3.2-fold increase in gene expression was observed for the *DREB2* gene on day 6 of the experiment in the *BPTN151* genotype. In five genotypes complete growth inhibition was observed already on the 10^{th} day of the experiment. Based on the results obtained, it was shown that the highest tolerance to drought stress among the tested tobacco genotypes was exhibited by *BPTN151* and *VPPG78* genotypes.

AP 20

Integrative analysis of transcriptome and metabolome provides insights into the underlying mechanism of cold stress response and recovery in two tobacco cultivars

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Low temperature is one of major environmental factors limiting the growth, quality and yield of tobacco. However, the molecular mechanism of tobacco cold response remains largely unknown. Here, integrated biochemical, transcriptomic and metabolomic analyses were carried out on tobacco leaves of cold-tolerant cultivar Xiangyan7 and cold-sensitive cultivar Taiyan8 under short- and long-term cold stress and recovery. Physiological and biochemical results showed that Taiyan8 was sensitive while Xiangyan7 was insensitive to cold stress. Integrated transcriptomics and metabolomics analysis revealed several key pathways in tobacco response to cold, including flavonoid biosynthesis, glutathione metabolism, zeatin biosynthesis, phenylpropanoid biosynthesis, starch and sucrose metabolism; and two important pathways in the recovery, namely, glyoxylate and dicarboxylate metabolism, flavonoid biosynthesis. The two cultivars had similar mechanisms in response to long-term cold stress. Whereas, more enriched pathways were identified in Taiyan8 under short-term stress, and the specifically enriched pathways were mainly involved in amino acid metabolism. Through the analysis of metabolites involved in these pathways, 26 key metabolites were screened out. These metabolite contents and biochemical indicator values were used as trait data for correlation analysis with gene expression modules, and five highly correlated modules were found. Within these modules, we identified 12 key candidate genes weighted as module hub genes, which involved ATPases, chlorophyll A-B binding protein, S-adenosine methionine decarboxylase, chalcone and stilbene synthases, UDP-glucosyltransferases, alcohol dehydrogenase, abhydrolase, proteins with ankyrin-repeat domains. The expression profiles of these genes further verified their involvement in tobacco cold response and recovery. These findings provide new insights into the regulatory networks of tobacco response to cold stress.

AP 21

Loss of susceptibility loci in tobacco for the development of durable resistance to black shank

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Black shank root and crown rot disease caused by the soil-borne pathogen, *Phytophthora nicotianae*, is detrimental to commercial tobacco (*Nicotiana tabacum*) production and is responsible for significant economic loss. Numerous naturally occurring alleles for resistance to black shank have been utilized to increase resistance in current commercial varieties. Due to a possible emergence of resistance breaking strains, novel mechanisms of resistance need to be investigated. Loss of susceptibility is a durable mechanism of resistance involving the removal of genes beneficial to the infecting organism, thereby decreasing the susceptibility of the crop. This approach relies on finding proteins in the plant that are bound by certain virulence factors. In this study, an effectoromics approach was used to determine possible susceptibility genes in tobacco. Conserved virulence genes were identified using a combination of genome sequencing and transcriptomics. Twenty-nine reference quality genomes from different isolates of *P. nicotianae* were assembled and used to determine gene expression of virulence factors during infection. A comparative genomic analysis coupled with RNA-Seq identified 50 conserved virulence genes. These were tested for their ability to increase the virulence of *Phytophthora* utilizing a leaf infiltration assay. Eight high-impact targets were then used to capture proteins that interact with these virulence factors as possible susceptibility genes. This analysis yielded several putative susceptibility genes which could be interrogated as targets for novel black shank resistance, independent of any existing genetics.

AP 22

Distribution of the MLO gene mutations for powdery mildew resistance in tobacco cultivars

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Powdery mildew is a fungal disease affecting various crops, causing serious yield losses under environments with high humidity and moderate temperature. The Japanese domestic tobacco (*Nicotiana tabacum* L.) cultivar 'Kokubu' shows high powdery mildew resistance that is controlled by recessive splice-site mutations of two *MILDEW LOCUS O* genes, *NtMLO1* and *NtMLO2*. The objective of this study was to investigate the existence of the *NtMLO1/2* splice mutations in the genomes of tobacco cultivars in Japan and other countries, and to infer how Japanese domestic cultivars inherited the mutations and gained powdery mildew resistance. In total, 84 Japanese domestic cultivars from various tobacco production areas were used for the splice-site mutation analysis. In addition, 30 native tobacco cultivars of Asia, six native tobacco cultivars of the Americas, 35 Oriental tobacco cultivars and 23 cigar tobacco cultivars from various countries were used for the analysis. Fourteen Japanese domestic cultivars, which were mainly distributed in Kagoshima, had splice-site mutations in both *NtMLO1* and *NtMLO2*. In addition, tobacco cultivars containing only the *NtMLO1* splice-site mutation were found in various tobacco production areas in Japan, but no cultivars with only the *NtMLO2* splice-site mutation were detected. Moreover, the *NtMLO1* splice-site mutation was detected in native Asian, Oriental and cigar tobacco cultivars. Consequently, we speculate that these powdery mildew-resistant tobacco cultivars were generated recently in the Kagoshima area of Japan when a spontaneous mutation occurred at the *NtMLO2* splice site in a cultivar already containing the *NtMLO1* splice-site mutation and that the *NtMLO1* splice-site mutation occurred during the early period of tobacco seed dissemination from the Americas to Asia including Japan.

AP 23

Functional characterization of transcription factor *NtERF13a* in regulating phenylpropanoids biosynthesis in tobacco

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Tobacco leaves contain abundant phenylpropanoids compounds, including chlorogenic acid, rutin, scopoletin, and lignin. These phenylpropanoids are important for plant growth and development, and participate in plant resistance to various environmental stresses. Meanwhile, the phenylpropanoids have a great impact on the aroma quality of tobacco leaves. Many transcription factors have been proved to be involved in the regulation of lignin or flavonoids biosynthesis in plants, but the reports about ERF protein regulating the contents of phenylpropanoid compounds are still very limited. In this study, two *NtERF13* genes were identified from *Nicotiana tabacum*. The transcription of *NtERF13a* gene could be detected in all tobacco tissues, and *NtERF13a* protein was located in nucleus. The contents of lignin, chlorogenic acid, and flavonoids were significantly higher in the leaves, flowers, and capsules of three independent *NtERF13-OE* lines than those in the WT plants, indicating *NtERF13a* could promote the biosynthesis of these phenylpropanoids. Transcriptome analysis showed that eight genes related to phenylpropanoids metabolism were differentially expressed between *NtERF13a-OE* and WT plants. Post-translational activation of *NtERF13a* protein significantly induced the expression of *NtHCT*, *NtF3'H*, and *NtANS* genes. Chromatin immunoprecipitation and dual-Luc assays further confirmed that *NtERF13a* could bind to the promoter regions of the above three genes, and then activate their transcription. In addition, *NtERF13a* can also promote the expression of *NtPYL6*, *NtLEA5*, *NtWRKY41*, *NtWRKY70*, *NtCIPK*, *NtRLK*, *NtDREB3*, and *NtDREB1F* genes, indicating that *NtERF13a* might be involved in plant resistance to unfriendly environments. This study reveals the function of *NtERF13a* in regulating phenylpropanoids metabolism of tobacco, and provides a new target gene for manipulating the contents and composition of phenylpropanoids in plants.

AP 24

Development of a mutant library and a high-quality reference genome for forward genetics in tobacco wild relatives *Nicotiana sylvestris*

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Tobacco (*Nicotiana tabacum*) is an allotetraploid species with complex genome structure. This complexity prevents tobacco from forward phenotypic screen of gene-knockout mutations because their effect can be masked by the corresponding homeologous gene. In contrast, *N. sylvestris*, which is the maternal ancestor of tobacco, is a diploid species, enabling to be applied for forward phenotypic screen, and therefore, expected to be useful for *Nicotiana* forward genetics. The objective of this study is to develop a mutant library as a genetic resource for forward genetics in *N. sylvestris* and to construct a high-quality *N. sylvestris* reference genome for identifying a causal gene which connects the *N. sylvestris* forward genetics with *N. tabacum* reverse genetics. An EMS-induced *N. sylvestris* mutant library consisting of 4890 M_2 lines was developed. The average mutation density was estimated to be approximately one per 50 kb. The constructed reference genome was composed of 3518 scaffolds with N_{50} length of 48 Mb, which achieved significant improvement compared to the existing reference genome. As an example, we screened seven low nicotine mutants, which were classified into two groups of causal genes by allelism test. MutMap analysis using the high-quality reference genome successfully identified two causal genes, *aspartate oxidase 2* (*AO2*) and *ethylene responsive factor 199* (*ERF199*), which are orthologous genes involved in nicotine biosynthesis in *N. tabacum*. In summary, our high-density EMS-mutant library and high-quality reference genome are extremely useful for forward genetics in *N. sylvestris*, and also the identified genes would be applicable for reverse genetics in tobacco.

AP 25

Single-cell transcriptome of *Nicotiana tabacum* leaves reveals developmental trajectories of glandular trichome

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Leaf glandular trichomes of *Nicotiana tabacum* represent a biologically active and stress-responsive tissue that contributes to plant defence response against biotic and abiotic stress and also influences leaf aroma and smoke flavour. Although a number of research groups have been focusing on developmental aspects of glandular trichome biology for several decades, the understanding about the development of glandular trichomes is still quite fragmentary. Single-cell RNA sequencing (scRNA-seq) technology is a powerful tool for discovering heterogeneous cells and development or differentiation of specific cell types. To explore the developmental trajectory of glandular trichomes, we extracted single nuclei from young tobacco leaves and successfully captured > 15,000 nuclei for single-nucleus sequencing (snRNA-seq). After cell type annotation using *Arabidopsis* homologous gene, all nuclei were divided into three large groups, mesophyll, epidermis and vasculature. The cells belonging to glandular trichomes were further identified by GO enrichment analysis and expression patterns of glandular trichomes-specific genes and terpenoid biosynthesis-related genes. At the same time, cell trajectory analysis was performed to delineate the developmental trajectory of glandular trichomes. Our results provide a good case for studying development of tobacco glandular trichomes, and the development-related genes identified by this study also provide a valuable resource for tobacco breeding.

AP 26

Evaluation the effectiveness of plant ingredients in slug control

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Problems with the use of synthetic pesticides have led researchers to look for natural plant compounds as alternatives to synthetic chemicals that pose the least risk to the environment. *Agriolimax agrestis* (Linnaeus, 1758) is one of the most important pests of plants that causes relatively large damage to various plants. The aim of this study was to investigate the effect of several plant ingredients in slug control in tobacco seedlings in a completely randomised design with 17 treatments and three replications in traditional tobacco seedbed at the Tirtash Tobacco Research and Education Center. Research treatments included 5, 7.5 and 10 % of garlic extract, 5, 7.5 and 10 % of anise extract, 10, 15, 20, 30 and 40 % of tobacco wood vinegar, 1, 2 and 4 percent of nicotine sulfate, positive control (metaldehyde 6 % (Metalan G) 2.5 g/m^2), negative control (no control) and rice bran. Treatment was done as poisonous bait prepared with rice bran. Six slugs were placed in each treatment and mortality percentage was evaluated after 24, 48, 72, 96 and 120 hours. Research data were analysed using SAS software. Results showed that there was a significant difference among treatments at 1 % probability level. Mean comparison of slug mortality percentage with LSD showed that metaldehyde B6 % (Metalan G) 2.5 g/m^2 with 100 % and 40 % of tobacco wood vinegar with 83.33 % mortality were in group a. 30 % tobacco wood vinegar and 4 % nicotine sulfate were placed after these treatments. Comparison between the rate and speed of mortality showed that positive control (metaldehyde 6 % (Metalan G)) caused 94.4 % and 100 % pest mortality after 24 and 48 hours, respectively. After that, 40 % tobacco wood vinegar caused 44.4, 72.2 and 83.3 % morbidity after 24, 48 and 72 hours, respectively. Other treatments had lower mortality rate and speed than these treatments. Therefore, 40 % tobacco wood vinegar treatment with 83.33 % mortality was in the same statistical group as the metaldehyde treatment, and due to being organic, the lack of adverse effects on health and the environment and efficiency close to the common chemical means it can be used on a large scale.

AP 27

Chemical management strategies for angular leaf spot in dark tobacco

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Pseudomonas syringae pv. *tabaci* is the causal agent of angular leaf spot, a bacterial disease that affects dark tobacco. This bacterial disease has been the most significant foliar disease in U.S. dark tobacco production since 2015. Dark tobacco producers have relied on streptomycin as the only bactericide to control angular leaf spot. Lab studies conducted at the University of Kentucky, Research and Education Center in Princeton, KY, have shown resistance to streptomycin at the 200-ppm field rate. Since 2015, 113 isolates have been screened for resistance to streptomycin and 25 % (28) of those isolates have shown resistance at the 200 ppm field rate. Field spray trials have been ongoing since 2015, at the University of Kentucky, Research and Education Center in Princeton, KY, and at the West Farm of Murray State University in Murray, KY. Spray trials were established at both locations in 2021 to evaluate direct and plant-mediated inhibitory effects of eight different agrochemical products. Antibiotic, biocontrol and/or synthetic bactericide products were evaluated at Princeton, while copper products were evaluated at the Murray location. Products included in both spray trials are either labeled or have the potential to be labeled for dark tobacco. Both trials were inoculated with a streptomycin sensitive strain of *P. syringae* pv. *tabaci* at 10^7 cf/ml at 30 psi at five weeks post-transplant. Plant disease rating were taken three times during the growing season at both locations, disease pressure continued to increase overtime at both locations. There were no significant differences between treatments in yield and grade quality index at either location. At the Murray location, all treatments yielded higher than the untreated control. Streptomycin is still recommended in fields with streptomycin-sensitive isolates, while integration of copper products and surface sterilants are recommended in fields with streptomycin-resistance isolates.

AP 28

Correlation between management practices and angular leaf spot of dark tobacco

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Pseudomonas syringae pv. *tabaci* is the causal agent of angular leaf spot, a bacterial disease that affects dark tobacco. This bacterial disease has been the most significant foliar disease in dark tobacco production since 2015. The antibiotic streptomycin has been the only chemical control recommendation for angular leaf spot. Lab studies conducted at the University of Kentucky, Research and Education Center in Princeton, KY, have verified resistance to streptomycin at the 200-ppm field rate. Since 2015, 113 isolates have been screened for resistance to streptomycin and 25 % (28) of those isolates have shown resistance at the 200 ppm field rate. In 2020, an observational study was established to monitor angular leaf spot incidence in grower fields. The objective of this study was to find correlations between both environmental and non-environmental management factors and presence of angular leaf spot. Included in this study were 30 dark tobacco fields located across seven counties in western Kentucky and Tennessee. Growers were not asked to alter management plans throughout the growing season, but all major management operations were recorded. Fields were visited three times during the growing season. Seventeen different environmental and management variables were observed throughout the growing season. Three plots (two rows wide, by 40 ft. long) were designated in each field to represent the field's topography and general characteristics (elevation, proximity to shaded areas, etc.). In each field, sensors were installed to measure soil temperature and light interception. A rainfall logger was placed in fields to measure rainfall and storm severity throughout the growing season. In 2020 and 2021, 12 of 60 fields had angular leaf spot confirmed and four of these fields contained streptomycin-resistant isolates. Data are currently being analyzed for correlations between environmental and/or management strategies and angular leaf spot incidence.

AP 29

Assessing *Phytophthora nicotianae* race population in Tennessee

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Phytophthora nicotianae, the causal agent of tobacco black shank, is the most important pathogen in Tennessee tobacco production. A simple and effective approach to reducing black shank is selecting cultivars that contain either partial or complete resistance to *P. nicotianae*. Burley, dark, and flue-cured cultivars containing single-gene resistance against race 0 of *P. nicotianae*, from the integration of the *Php* and *Phl* genes, are available to growers. The deployment of varieties with complete resistance to race 0 has shifted the race population to predominantly race 1, which puts emphasis on integrated management that includes cultural, biological, and chemical controls. Proper identification of *P. nicotianae* race types is critical for management of black shank. Three races, 0, 1, and 3, have been identified in neighbouring tobacco producing states. The goal of the research is to assess the race of *P. nicotianae* isolates collected from tobacco in Tennessee. 48 isolates of *P. nicotianae* were collected in 2021 and used to inoculate the following varieties acting as host differentials: male sterile Kentucky 14 × L8LC with level 10/10 resistance to race 0, Hybrid 404LC which is a fully susceptible variety (0/10 resistance to both races 0 and 1), and KT 215LC with level 10/10 resistance to race 0 and level 9/10 resistance to race 1. Data will be presented from the 2021 isolate collection, and the experiment will be repeated during the 2022 growing season.

AP 30

New progress in breeding and biocontrol dual approaches for tobacco broomrape management

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Tobacco broomrapes (*Phelipanche ramosa*, *Phelipanche aegyptiaca* and *Orobanche Cernua*) are widespread parasitic plants from the *Orobanchaceae* family, that cause important damage in tobacco crops. Broomrapes are mainly present in Europe, Middle East and Asia and no satisfactory chemical control strategy is available for growers. A project combining breeding and agronomical efforts was initiated in France in 2016 involving research and technical partners to evaluate and implement an integrated broomrape control strategy. The objectives of this multidisciplinary approach were to improve the understanding of the genetic broomrape tolerances in connection to different crop strategies. After several years of herbicide treatments evaluation bringing no satisfactory results for growers, the key objective of this project was to develop a new approach based on the use of broomrape pathogens as biocontrol agents.

The breeding part of the project, still led by BSB, focused on evaluating identified sources of tolerance to different broomrape populations and species. Testing Wika and two new tolerance sources to populations of broomrapes collected in different countries revealed that the Wika recessive gene brings interesting tolerances to *P. ramosa* and *P. aegyptiaca* populations while none to *O. cernua* populations. New sources seem to bring a potential of tolerance breeding to *O. cernua*.

The biocontrol part of the project, led by Agrosup Dijon, France, focused on hundreds of fungi isolated from symptomatic broomrapes in 2017 and 2018. Based on morphotypes, a coarse visual identification and original plot locations, one hundred of them were evaluated for their pathogenicity to broomrape. This screening revealed a dozen fungi strains as promising mycoherbicide candidates. One candidate has been selected to realise the first trials in field and promising results including a decrease of broomrape pressure was observed in 2021.

Combining breeding efforts and new biocontrol strategies brings tobacco growers some hope of an integrated control solution to broomrape parasitic activity.

AP 31

Management of whitefly *Bemisia tabaci*, a vector of tobacco leaf curl virus disease in Virginia tobacco

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Tobacco whitefly, *Bemisia tabaci* is an important pest of tobacco. It causes damage by sucking the sap and as a vector of tobacco leaf curl virus resulting in considerable loss of yield and quality. In the recent past, the incidence of leaf curl virus disease increased in all types of tobaccos in the country. At present management of tobacco leaf curl virus (TLCV) is possible only through control of its vector, whitefly. Continuous decrease in the insecticides that provide adequate control of the pest due to various reasons incited us to evaluate different management modules for minimizing the loss due to the TLCV vector, *B. tabaci*. Three management modules *viz*., sorghum barrier crop, chemical control, and integrated (chemicals + barrier crop sorghum) modules along with an untreated check were evaluated for two seasons. Observations were recorded periodically on mean whitefly population, per cent leaf curl infected plants, natural enemies and yield parameters. The mean leaf curl infected plants was least (2 %) at the end of the season in integrated module followed by chemical control module (2.4 - 2.6 %) as against 8.6 - 8.8 % in sorghum border module and 12.4 - 12.6 % in check plots. The data revealed that the mean whitefly population/plant was also found to be significantly less in integrated module followed by chemical control module compared to sorghum border module and check plots. The yield parameters *viz.*, cured leaf, bright leaf and grade index were also higher (2655, 1575 kg/ha & 1965) in integrated module and chemical control module (2620, 1545 kg/ha & 1888) compared to sorghum border module (2370, 1410 kg/ha & 1650) and check (2053, 1165 kg/ha & 1490). It is evident that integrated module could effectively protect flue-cured Virginia tobacco from leaf curl virus disease transmitted by whitefly.

AP 32

Habitat management for the enhancement of arthropod services in flue-cured Virginia tobacco

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Arthropods are a phylum of animals with jointed legs, which includes insects. They offer a wide range of ecosystem services, like nurturing the soil health, pollination, seed dispersal, pest management etc. The objective of the research was to study the diversity of arthropods in flue-cured Virginia tobacco ecosystems by employing habitat management aspects. During 2021-22, arthropod samples were taken utilising novel pitfall traps from tobacco crops managed using various pest management modules including habitat management aspects. These were sorghum barrier modules; chickpea and cowpea intercrop modules; integrated pest management (IPM); bio control; chemical control modules and sole crop. Community diversity was analysed in terms of species richness (Shannon-Weiner index), dominance (Simpson index), effective number of species and evenness indices followed by Bray-Curtis similarity. Species composition was ascertained at order, family and morphospecies level for the 1891 arthropods collected *viz*., insects, spiders, harvestmen, millipedes, pseudoscorpions etc. Hymenopterans predominated by 70 % of the total samples, while acari 8.2, and araneae 6.0 %. The data suggests that arthropods were abundant in all of those modules with ecologically managed habitats compared to the chemical management. Species richness was higher in IPM and chickpea (1.7), followed by cowpea intercropping (1.5). Effective number of species (ENS) was the highest in chickpea (6.01) followed by IPM (5.95). IPM and chick pea intercropping further increased the evenness in the crop. Therefore, inclusion of barrier and intercrops besides following IPM, can facilitate natural pest control and hence, stability of the crop ecosystem. It also has implications in reducing the over-reliance on pesticides and consequently curtails the crop protection agent (CPA) residue issues in tobacco.

AP 33

Study on the molecular mechanism of maize pollen infecting tobacco

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The previous investigation of our group found that corn pollen was scattered on the tobacco leaves of adjacent tobacco fields driven by natural wind, which would lead to dense black spots on the leaves, especially near the leaf veins, which seriously affected the quality of tobacco leaves. However, at present, the research on this phenomenon only stays in the phenotypic observation stage, and the mechanism of this phenomenon is not clear. In this study, flue-cured tobacco "HongDa" and maize "JiYuan No. 8" were used as materials to set up three treatments: non-pollen infection of flue-cured tobacco (CK), active corn pollen infection and inactivated corn pollen infection. The results showed that maize pollen infection could reduce the photosynthetic pigment content of tobacco leaves, seriously damage the cell membrane of tobacco leaves, activate the activity of protective enzymes and inhibit the nitrogen use efficiency of tobacco leaves. Transcriptome analysis showed that pollen infection significantly upregulated many differentially expressed genes (DEGs) in phenylpropane metabolic pathway, while photosynthetic antenna proteins and many DEGs in photosynthetic metabolic pathway were significantly down-regulated. Proteome analysis showed that, like inactivated corn pollen, down-regulated proteins accounted for a large proportion in the photosynthetic pathway, while up-regulated proteins were enriched in the biosynthetic pathway of phenylpropane compounds. The results of metabonomic analysis showed that the expression of flavonoid, phenylpropanoid, flavonoid and flavonol metabolites was up-regulated after active pollen infection. The results of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment showed that flavonoid biosynthesis and phenylpropane biosynthesis were two significant pathways for enrichment, and the results of the inactivated maize pollen treatment metabolic group were the same. Multi-group analysis showed that genes, proteins and metabolites were enriched into flavonoid and phenylpropane metabolic pathways under different active pollen infection treatments. To sum up, from a physiological point of view, maize pollen infecting tobacco leaves is similar to the stress process, which not only damages the phenotype of tobacco leaves, but also reduces the content of photosynthetic pigment, damages the cell membrane and activates the activity of protective enzymes in tobacco leaves. From the molecular level, maize pollen infection mainly affects the photosynthesis and protein synthesis of tobacco leaves, while tobacco leaves up-regulate the expression of phenylpropane and flavonoids and activate the corresponding enzymes and proteins by activating the corresponding genes of phenylpropane and flavonoids. Finally, phenylpropane and flavonoids are synthesised to participate in osmotic regulation to resist infection.

AP 34

Axillary bud control and residues from maleic hydrazide applied at different times of day in Burley tobacco

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Control of axillary buds (suckers) is important to maintain yield and leaf quality in Burley tobacco production. There are three types of sucker control products available in the USA including contact, local-systemic, and systemic. Maleic hydrazide (MH) is the only systemic sucker control active ingredient and Burley tobacco producers rely on MH for sucker control after topping. Previous research in flue-cured tobacco has shown variability in MH residues when the same rate is applied in the morning, midday, and evening. The objective of this study was to quantify sucker control efficacy, yield, leaf quality, tobacco-specific nitrosamines (TSNAs), and MH residues in Burley tobacco treated with MH in the morning, midday, and evening. Burley tobacco cultivar NC BH 129 was grown at the Northeast Tennessee Research and Education Center in 2021 and 2022. A tank mixture of MH (2.24 kg a.i./ha) and flumetralin (0.56 kg a.i./ha) was applied in a randomized complete block design in the morning (8 a.m.), midday (1 p.m.), and evening (6 p.m.). Plots were stalk harvested and cured in traditional air-curing barns. After curing, samples were taken for MH residue, alkaloid, and TSNA determination. In 2021, a reduction in sucker control was observed when MH and flumetralin were applied in the morning, however, this did not impact total yield. There were significant reductions in total alkaloids associated with the midday and afternoon MH applications. MH residues were significantly reduced when applied in the morning (22 ppm) compared to the midday (62 ppm) and afternoon (56 ppm), likely due to slight-to-moderate dew at the time of application. There were no significant differences across application timings for TSNAs. Preliminary results from this study will be presented.

AP 35

Characterizing 2,4-D and dicamba residue persistence following tobacco flue-curing

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Genetically modified field crops expressing tolerance to auxin herbicides, such as 2,4-D and dicamba, are commonly grown in fields adjacent to flue-cured tobacco in North Carolina. The potential exists for physical drift of spray solutions containing those herbicides onto tobacco. The persistence of 2,4-D and dicamba residues during the flue-curing process is not currently known. To answer this question, a 12.5 % dose of 2,4-D (133 g a.e. ha^{-1}) and dicamba (71 g a.e. ha^{-1}) was applied to flue-cured tobacco 17, 7, 3, and 0 days before harvest. Treated tobacco was flue-cured in independent curing chambers for 144 hr to prevent cross-contamination. 2,4-D residues were not detected in fresh or cured leaves when treatment occurred 17 days before harvest. Tobacco treated with 2,4-D 0 days before harvest resulted in greater 2,4-D residue in fresh leaves (4.13 mg kg^{-1}) compared to three days before harvest (2.08 mg kg^{-1}). Treatments delivered seven days before harvest resulted in a fresh leaf 2,4-D concentration of 0.02 mg kg^{-1}, which was > 100-fold reduction when compared to 0 and 3 days before harvest. 2,4-D concentrations in cured leaf were 0.19 and 0.08 mg kg^{-1}, when treated 0 and three days before harvest, respectively, and 2,4-D was not detected in cured leaves treated seven days before harvest or in fresh- or cured-leaf treated 17 days before harvest. Dicamba residue was not detected in fresh- or cured-leaves treated 7 or 17 days before harvest. Tobacco treated 0 days before harvest with dicamba resulted in a concentration of 1.40 mg kg^{-1} in fresh leaf which was a two-fold increase compared to tobacco treated three days before harvest (0.69 mg kg^{-1}). Dicamba concentrations in cured leaf were < 0.015 and 0.06 mg kg^{-1} when treated 3 and 0 days before harvest, respectively. Ultimately, cured leaf residues of 2,4-D and dicamba were reduced by > 20-fold within each treatment interval when compared to fresh leaf samples. Our results suggest that cured leaf residues of 2,4-D or dicamba are unlikely to be detected from drift events occurring > 7 days before harvest. In contrast, physical drift events taking place 0 to 3 days prior to harvest could result in cured leaf levels of 2,4-D or dicamba that are more likely to be detected, depending upon the sensitivity of the instrumentation and analytical equipment used in testing laboratories; however, it is unlikely that they will exceed the current CORESTA Guidance Residue Level (GRL) (0.2 mg kg^{-1}).

AP 36

Agrochemical residues: recent experiences with cyantraniliprole, flutriafol, flutriafol + azoxystrobin, and S-metolachlor

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Recent pesticide residue investigations for cyantraniliprole, flutriafol, flutriafol + azoxystrobin, and S-metolachlor applications were conducted in flue-cured tobacco. Field trials were conducted in five North Carolina growing environments from 2019 to 2021. Across all growing environments, cyantraniliprole residues were less than 2.0 ppm but were highest in the uppermost stalk position. The highest mean residue within a single growing environment was 5.21 mg kg^{-1}, which is nearly 3.5 times lower than the CORESTA Guidance Residue Level (GRL) for cyantraniliprole (18 mg kg^{-1}). Conversely, flutriafol residues ranged from 1.25 to 4.57 mg kg^{-1} and were highest in the lowermost stalk position. The highest flutriafol residue was recorded in 2019 in lower-stalk tobacco sampled at the Cunningham Research Station (9.30 mg kg^{-1}). The same trends were documented in the combination treatment of flutriafol + azoxystrobin, although azoxystrobin residues were < 1.0 mg kg^{-1} across all field sites. Residues of S-metolachlor were not detected in any stalk position group. Ultimately, our research implies that repeated applications of cyantraniliprole and azoxystrobin + flutriafol should not result in cured leaf residues of cyantraniliprole or azoxystrobin that would be of concern to cigarette manufacturers. However, we realize that very little is known about flutriafol and how residues might be viewed by those same manufacturers. Given the dire need for additional tobacco fungicides with novel modes of action, flutriafol should be further evaluated for efficacy against foliar leaf spot disease.

AP 37

Matrix effect on analysis of crop protection agents in tobacco by GC-MS/MS and LC-MS/MS techniques

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Tobacco is a complex matrix which contains many classes of compounds, such as fats, carbohydrates, chlorophylls, lipids, sterols, glycosides and triglycerides. In addition to this complexity the chemical profile changes depending on the curing method, growing conditions, geographical region, and tobacco type. The determination of different classes of pesticide residues at low concentrations can be impacted by matrix effects when using GC-MS/MS and LC-MS/MS techniques, typically increasing (enhancement) or decreasing (suppression) the detector response. Co-extracted substances can thus directly affect the accuracy of the analytical method. Clean-up techniques (e.g., QuEChERS) are required to promote extraction, enrichment of analytes of interest and removal of the interferents. In addition, matrix-matched calibration can be applied but this is effort intensive. The objective of this study was to evaluate the matrix effect of the analytical calibration curves prepared in solvent and with matrix-matching for 165 pesticides using GC-MS/MS and LC-MS/MS. The solutions were prepared by extracting each tobacco (flue-cured, air-cured, dark air-cured and Oriental) using QuEChERS methodology, adding an aliquot of the standard solution for each calibration level and comparing analytical data with those for calibration standards in acetonitrile. The study demonstrated considerable differences between the matrix-matched and the solvent calibration curves. Changes in signal response ranged from -918 % (suppression) to 8343 % (enhancement), depending on the analyte and tobacco type analysed, demonstrating significant effects on the accuracy of quantification. Through this study, it was possible to evaluate the impact of the matrix effect on the detector response to pesticide residues and to optimise the application of matrix-matched calibration.

AP 38

Promoting sustainable farming practices with real time, up-to-date regulatory information on Plant Protection Products

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Farming in sustainable ways requires good knowledge and understanding of plant protection products (PPPs). To facilitate this task, we have developed *PhytoScan*, a mobile app that promotes sustainable farming practices by providing up-to-date regulatory information on PPPs. Real time data include: registered crops, pests & diseases, methods/times of application; private standards information (such as *Rain Forest Alliance*) and their respective PPPs authorised in conventional/organic farming schemes; globally harmonised export dataset options for compliance and traceability purposes, among other features. Take for example *Phytophthora*-induced diseases. *PhytoScan* lists PPPs that are allowed with specific details of a particular product application and information concerning private standard and its level of risk concern. The selected information can then be transmitted from the app to required certified documentation, or to a general farm management system. The exported datasets may contain not only national information of selected PPPs but also Internationally harmonized standards with CAS-numbers, European and Mediterranean Plant Protection Organization (EPPO) codes for crops. Data may also be consolidated at international level for multiple suppliers. Finally, by simply scanning PPP barcodes, users can retrieve information and verify suitability of products. Today this is already the case in France.

AP 39

Evaluation of fungicide programs and lower leaf removal on wrapper leaf production in Connecticut broadleaf cigar wrapper tobacco

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Late-season frogeye leaf spot infection that results in 'greenspot' in cured leaf has been the greatest problem in producing Connecticut Broadleaf cigar wrapper tobacco in Kentucky and Tennessee. Field trials were established in 2021 at Princeton, KY, and Springfield, TN to evaluate effects of fungicide programs and lower leaf removal on greenspot incidence. At Princeton, eleven agrochemical programs were evaluated and included nine standard fungicides and two biologicals. In a separate trial, the effects of lower leaf removal and fungicide application were evaluated. Treatments in the lower leaf removal trial were arranged in a 2 × 2 factorial treatment design with four lower leaves removed at topping vs. no leaf removal, and fungicide application vs. no fungicide application. The lower leaf removal trial was topped at 10 leaves prior to lower leaf removal and fungicide application. An additional treatment included no lower leaf removal or fungicide application, but only the top six leaves were harvested. In the fungicide trial, applications began three weeks after planting and lasted until the final week before harvest. Spray regimens and timings followed product labels. In the fungicide trial, highest total yield per acre was from copper octanoate, thiophanate-methyl, and flutriafol treatments, while highest percent wrapper yield was from flutriafol, fluopyram, and pydiflumetofen+difenoconazole treatments. Highest gross revenue per acre was from flutriafol, pydiflumetofen+difenoconazole, thiophanate-methyl, fluopyram, and copper octanoate treatments. In the lower leaf removal trial, lower leaf removal at topping or only harvesting the top six leaves resulted in significant reductions in total yield. Although lower leaf removal or harvesting only the top six leaves resulted in significant increases in percent wrapper leaf production, these increases in percent wrapper did not offset the total yield losses reflected in gross revenue. Differences seen in the lower leaf removal trial data were similar at both locations.

AP 40

Diversity of bacteria on tobacco leaves during aging and their aroma-enhancing effects

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To clarify the diversity of community structure for bacteria on tobacco leaves during aging and their aroma-enhancing effects on tobacco leaf quality, tobacco leaves from six different producing regions were studied, and the bacteria on those leaves were analysed by 16S rDNA sequencing technology. After the isolation, purification and screening of enzyme production capacity of strains, the sensory evaluation and analyses of routine chemical components and polyphenol compounds of fermented cut tobacco samples were conducted. The results showed that 1511 operational taxonomic units (OTUs) were obtained. The dominant phylum of 24 samples was *Proteobacteria*, and the dominant genus was *Bacillus*. In this study, *Bacillus* strain GY99 capable of producing protease, amylase and cellulase simultaneously was screened out. At the same time, three strains which significantly improved tobacco quality were screened out, namely *Bacillus* strain GY96, *Pseudomonas* strain GY72 and *Micrococcus* strain GY67.

AP 41

Effects of fermentation medium on cigar filler

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The addition of medium during industrial fermentation can improve the quality of cigar tobacco leaves after agricultural fermentation. In this study, the cigar filler tobacco "Brazilian Frogstrips YA14" was used as the test material to determine the contents of main chemical components in cigar tobacco leaves after fermentations with the additions of water (control group) and a medium (test group). The changes in the community structure and abundances of bacteria on tobacco leaves during the fermentation process were analysed. The results of the study were as follows: 1) During the fermentation process, the protein content of tobacco leaves fluctuated slightly, basically stabilised at 19 %-20 %. 2) Under the impact of the medium, the total content of main amino acids in tobacco leaves showed a downward trend, and the difference of which between the control group and the test group was the most obvious on the fourth day of fermentation. 3) The change trend of the content of petroleum ether extract in cigar leaves for the control group was not obvious, and the content of petroleum ether extract in the tobacco leaves for the test group decreased by 12.4 % under the impact of the medium. 4) After fermentation, the relative content of saturated fatty acids for the control group and the test group all increased, while the relative content of unsaturated fatty acids all decreased. 5) After the addition of the medium, the diversity of bacteria on tobacco leaves changed significantly, the number of operational taxonomic units (OTUs) in tobacco leaves increased, and the bacterial community structure changed. This research indicates that after adding the medium to ferment cigar filler, the changes in the bacterial community and the dominant bacterial group on tobacco leaves have impacts on the contents of chemical components in tobacco leaves, and fermentation with the addition of medium has a positive effect on improving the quality of tobacco leaves.

AP 42

Changes of bacterial community structure and their correlations with contents of free amino acids during cigar processing

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The special processing conditions in the production process of handmade cigars may cause great changes in the microbial community structure and contents of free amino acids, thereby affecting the quality of cigars. In order to study the changed rules of bacterial community structure during cigar processing and their correlations with contents of free amino acids, cigar samples at five process stages of wrapper wrapping, moisture balancing, cigar freezing, cigar curing and cigar packaging were selected, respectively. Their bacterial community structures were analysed by Illumina MiSeq high-throughput sequencing technology. The contents of 18 free amino acids were determined by ion chromatography-integrated pulsed amperometric detection, and Spearman correlation analyses between bacterial community structure and contents of free amino acids were conducted. The results showed that: during the processing of cigars, 1) the overall richness and diversity of bacterial communities of cigars showed a downward trend that was more affected by the moisture balancing process; 2) the dominant bacterial phyla were *Firmicutes*, *Proteobacteria* and *Actinobacteriota* with the average relative abundances of 51.75 %, 35.80 % and 10.38 % respectively; the dominant bacterial genera were *Acinetobacter*, *Oceanobacillus*, *Bacillus*, *Staphylococcus* and *Klebsiella* with the average relative abundances of 12.40 %, 9.96 %, 8.48 %, 8.27 % and 4.21 % respectively; 3) the total content of free amino acids showed an overall increasing trend, and among which aspartic acid accounted for the highest proportion, and *Sphingobacterium*, *Staphylococcus* and *Paracoccus* were closely related to aspartic acid content. Therefore, the contents of free amino acids during the processing of cigars were closely related to the changes of bacterial community structure.

AP 43

The development of ESG programs to help foster social, environmental, and governance initiatives in Altria's global tobacco supply chain

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Environmental, social, and governance (ESG) programs can have a positive impact within supply chains of labor-intensive crops, including tobacco, that rely on a dependable labor force and responsible use of crop inputs. Additionally, ESG programs may also include processes that help ensure compliance with evolving laws and regulations and supply chain data reporting obligations.

Altria's approach to the evolving ESG space includes incorporating third party programs into our leaf supply chain to ensure regulatory and legal compliance, promote grower sustainability, and to provide data to satisfy our reporting obligations. Our domestic approach transitioned our direct-contracted growers, over a three-year period (2019-2021), to an annual requirement of participating in the tobacco certification program offered by GAP Connections (GAPC). Their certification program focuses on improving crop, environmental, and labor management practices and includes training, third-party audits, and a worker concerns hotline. Globally, all leaf suppliers were required to participate in the Sustainable Tobacco Program (STP), a due diligence risk-based platform developed to drive sustainability within the tobacco supply chain through continuous improvement. In the STP, industry participants collaborate on eight sustainability themes: Water, Human and labor rights, Crop, Soil health, Climate change, Natural habitats, Livelihoods, and Governance.

Data from these two programs, when combined with our internal supplier relationship management framework, permits us to prioritize efforts with the largest positive impacts for our growers, their workers, and the environment. The third-party validated quantitative data is invaluable as it provides the necessary transparency to external audiences, critical to ongoing compliance efforts in anticipation of associated ESG reporting regulations (e.g., SEC). While our leaf supply chain is well positioned for this compliance, the learnings gained from developing this third-party comprehensive approach will inform other supply chain categories for future portfolio expansion of reduced harm products.

AP 44

Changes of soil bacterial community structure and its response to soil physicochemical properties after addition of wheat straw and its biochar

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Ecosystem degradation is a major environmental problem in Shandong tobacco-planting soil. Aiming to rationally utilise wheat straw resources to improve soil biological quality, a two-year field experiment was carried out to investigate the changes of soil bacterial community in the tobacco-planting field based on the high-throughput sequencing technology of 16S rRNA V3~V4 regions. Soil physicochemical indices were used to explore the main control soil environmental factors affecting the change of soil bacterial community. Four treatments were set up as follows: chemical fertiliser only (CK), 6.75 t hm^{-2} wheat straw plus chemical fertilizer (WS), 2.25 t hm^{-2} (FB1) and 4.50 t hm^{-2} (FB2) wheat straw-derived biochar plus chemical fertiliser. After two years, the result showed that the WS and FB2 treatments increased the soil operational taxonomic units (OTU) numbers, alpha diversity indices of soil microbe Chao1, Ace and Shannon compared with CK. Pearson's correlation analysis indicated that soil microbial biomass carbon (MBC) was significant or extremely significantly and positively correlated with Ace, Chao1 and Shannon indices. Stepwise regression analysis showed that the MBC explained 39.7 % of the bacterial Ace index, 75.1 % of the Chao1 index and 32.0 % of the Shannon index, respectively. Treatments of WS and FB2 significantly increased the relative abundance of *Acidobacteria* and *Planctomycetes*. Principal coordinates analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) analysis showed that the addition of straw or biochar changed the structure of soil bacterial community. Redundancy analysis (RDA) analysis showed the soil total organic carbon (TOC) and soil available potassium (AK) were the dominant factors to the changes of soil bacterial community structure. Overall, the diversity and structure of bacterial communities were improved due to wheat straw or its biochar addition. MBC explained the changes of soil microbial diversity. TOC and AK were the dominant environmental factors for soil bacterial community change in tobacco-planting soil.

AP 45

Determination of carbon sequestration in tobacco plants

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Carbon sequestration is the expression used to define the process of removing carbon dioxide from the atmosphere, being carried out by plants during growth through photosynthesis. Therefore, vegetation has a great contribution to carbon capture and storage, however, the impact of agricultural crops on this process is not yet known. The aim of the present study was to determine the amount of carbon sequestered in Virginia-type tobacco plants throughout the plant's growth phase, under environmental conditions in Old Belt in Rio Grande do Sul, Brazil. Twenty-four tobacco plants (Virginia, variety PVH2343 sourced by Profigen do Brasil) were sampled in four different properties in Santa Cruz do Sul e Passo do Sobrado, following the usual cultivation processes (topping, 1st, 2nd, 3rd, 4th harvest) and subsequent removal of the rest of the plant (stem and roots). All samples were individually weighed (fresh matter) and then dried for 6 days at 70 °C in an oven to define the dry matter. All samples were ground separately for further analysis with a CHN Elemental Analyzer to determine the carbon concentration. It was observed that a Virginia-type tobacco plant has an average fresh weight of 1.85±0.44 kg, with an average of 157.53 g of fixed carbon, maintaining 36.88 % of its dry weight in carbon, that means, 8.5±0.94 % of its fresh weight. The highest concentration of carbon is found in the leaves of the last harvest (39.62 % of the dry matter), and the highest average volume in the roots with 54.68 g. With these data it will be possible to estimate the amount of carbon sequestered in tobacco plants/plantations, a fundamental basis for the analysis of the technological life cycle in the tobacco production chain.

AP 46

Construction of plant growth-promoting rhizobacteria with bacterial wilt resistance and its biological control mechanism

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The unstable effect of plant growth promoting rhizobacteria (PGPR) on biological control of bacterial wilt is the current bottleneck problem for the application of beneficial microorganisms in practice. This study focuses on how to construct a stable and highly efficient PGPR flora against bacterial wilt. The eight strains of PGPR and *Ralstonia solanacearum* (Rs) screened in the previous study were used as the research objects. The niche overlap of main carbon sources in tobacco root exudates between PGPR and Rs was investigated. Rod model design and ecological microplate were employed to determine the best PGPR assembles and its utilisation efficiency of carbon sources. The effect of disease resistance and growth promotion were verified in the restricted microsystem and field experiment. (1) PGPR strains LX4, Ba-S and LX7 fully utilised amino acids and carbohydrate in tobacco root exudates to inhibit Rs growth. Strains LX7 and 112 had inhibitory effects on Rs with all acid carbon sources, and the highest inhibitory rates were 40.12 % with lactic acid and 35.15 % with citric acid, respectively. (2) The basal niche breadth (Bsw) of Ps-S, 112 and VC110 were 41.9 %, 41.0 % and 38.1 % higher than that of Rs, respectively. The basal niche overlap index (NOI) of Ba-S was significantly higher than that of any other PGPR strains, which had obvious nutrient competition with Rs. (3) The average disease index was 27.01 % and the average count of Rs in the rhizosphere was 1.2×10^4 copies/g in the combination treatment of four PGPR strains, which was significantly lower than the single- and two-PGPR treatments. All the tobacco plants treated by eight strains of PGPR were not wilt, with the average Rs population of 3.5×10^2 copies/g. (4) The carbon utilisation efficiency of No. 32 community combination (LX4+Ba-S+LX7+VC110) was significantly higher than that of Rs, especially in the utilisation of alcohol (1.62 times of Rs) and carbohydrate (1.41 times of Rs). (5) The field control effect of No. 32 community combination was significantly higher than that of other treatments, which were 27.18 %, 60.05 % and 54.80 % higher than No. 39, 40 and 43, respectively. The yield and output value of No. 32 community combination were the highest among all treatments, and were 67.50 % and 73.53 % higher than that of the control, respectively. This study shows that the nutrient competition and antagonistic characteristics of different PGPR strains against *R. solanacearum* can be fully utilised to construct PGPR flora. The diverse PGPR flora has better capability to resist the invasion of pathogenic bacteria.

AP 47

Progress towards the evaluation of bio-control based insecticides and fungicides for use on tobacco in Zimbabwe

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The global move towards sustainable agriculture has necessitated an increased interest in the use of biopesticides. This is in line with the Sustainable Development Goal 2 that recognises that highly hazardous pesticides cause adverse human health and environmental effects in many countries. Biological pesticides therefore present pest and disease management options that are more agroecologically friendly and allow chemical intervention to be considered only when necessary. The Tobacco Research Board (TRB) in its capacity as the centre for testing and evaluating all crop protection agents for use on tobacco in Zimbabwe has always put emphasis on the selection and use of environmentally friendly crop protection agents including biological control agents. Thus, many biocontrol agents and different formulations developed worldwide have also been evaluated to establish their efficacy under the Zimbabwean environmental conditions. In addition to evaluating commercial products the TRB also undertakes research aimed at exploring for, isolating and testing local biological agents and biopesticides for the management of plant pathogens and pests. This presentation gives an update of the progress that has been made in promoting and registering biocontrol-based insecticides and fungicides in Zimbabwe.

AP 48

Biological control of tobacco root-knot nematode (*Meloidogyne incognita*), using *Trichoderma harzianum* in Iran

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Biocontrol of root-knot nematodes is now a priority in order to reduce the harmful effects of chemicals, including human health and environmental pollution. The purpose of this research, was biological control of tobacco root-knot nematode (*Meloidogyne incognita*), using *Trichoderma harzianum* (BI isolate). In laboratory studies, the effect of different concentrations of *T. harzianum* culture extract on second instar larval mortality and nematode egg hatching was investigated. The results showed that the fungi spore concentration of 10^6 with 78.5 % parasitism was the most effective concentration in second instar larval mortality and nematode egg hatching. In greenhouse studies, the roots of tobacco seedlings of K326 cultivar in 4-5 leaf stage were sporulated with *T. harzianum* with 10^6/ml and 2000 larvae of *M. incognita* nematode per plant grown in pots. A greenhouse study was done in a completely randomised design with six treatments in four replications, including treatments (inoculation with nematode and without fungus, inoculation with fungus and without nematode, inoculation with nematode with fungus only one step simultaneously with transplantation, nematode inoculation with fungus in two stages: simultaneously and 10 days after transplanting, non-autoclaved soil infected to nematode without any inoculation and Velum Prime® (chemical nematicide). Two months after treatment application, tobacco growth indices and nematode pathogenicity indices were measured. The results of variance analysis showed that there was a significant difference between treatments in terms of all the studied traits at the level of 1 % probability. The results of mean comparison showed that the Velum Prime® and then the nematode inoculated with fungal suspension in two stages, with 81 % and 76.5 %, respectively, reduced the contamination and showed the greatest effect in controlling this nematode. Overall, results of this study indicated the high antagonistic potential of *T. harzianum* BI in reducing nematode contamination indices and also improving tobacco growth indices.

AP 49

The effect of liquid smoke from tobacco waste on tobacco black shank (*Phytophthora nicotianae*), tobacco aphid (*Myzus nicotianae*) and whitefly (*Trialeurodes vaporarium*) in Northern Iran

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The black shank disease, caused by *Phytophthora nicotianae*, is one of the most important tobacco diseases in the world and in tobacco fields of Golestan Province in Northern Iran. Aphids and whiteflies are sucking and polyphagous pests with great economic importance and a wide range of host plants that cause quantitative and qualitative damage to most crops. The aim of this study was to investigate the effect of liquid smoke on tobacco soilborne pathogenic causal agent fungi of the tobacco black shank and tobacco aphid (*Myzus nicotianae*) and whitefly (*Trialeurodes vaporarium*) in the field. This study was carried out based on a randomised complete block design with 13 (in the first experiment) and nine (in the second experiment) treatments and three replications at the Tirtash Research and Education Center in 2020. Treatments included liquid smoke obtained from tobacco stem, waste from the tobacco company warehouse, wood waste and commercial liquid smoke at three concentrations of 5, 10 and 15 % (in the first experiment) and two concentrations of 10 % and 20 % (in the second experiment) and the control included spray with water. In the first experiment, the liquid smoke with the above concentrations was used in the float system seedbed after the two leaf stage of the seedling every two weeks and in the field two weeks after transplanting every two weeks and six foliar applications (sprays) were done in total. The field was transplanted with tobacco totem cultivar in a 4 × 5 m plot. Forty-eight hours after treatment, fungal discs of *P. nicotianae* were inoculated at the collar site of five plants per each plot. The evaluations were performed before application treatment and weekly after application treatment, based on the percentage of infection by counting the number of infected plants. In the second experiment under natural pollution conditions two completely separate experiments were carried out, first against *M. nicotianae* and secondly against whitefly. Treatment application was done using foliar spraying. For evaluation, 10 tobacco plants were randomly selected from each plot and cards were installed on them. Mortality counts for each experiment were performed separately at one day before spraying and days 1, 3, 7, 10 and 14 days after spraying. Pest mortality was calculated according to Henderson-Tilton formula. Data were analysed by MSTAT-C software and mean comparison was done by LSD. The results of variance analysis showed that there was a significant difference between all treatments at the level of 1 % probability in both experiments. Liquid smoke of storage waste and tobacco stem treatments with 10 % concentration respectively showed 72 % and 67 % control of black shank, as well as 4578 and 5137 kg/ha yield and 771 and 862 million rials per hectare income and were introduced as the most effective treatments. Also, the liquid smoke treatments from storage waste and tobacco wood waste at 20 % concentrations with 71 % and 65 % control, respectively, on *M. nicotianae* and *T. vaporarium* were introduced as the most effective treatments and can be used to control these pests.

AP 50

Efficiency of sawdust briquettes as an alternative source of energy for tobacco curing in Tabora Urban District, Tabora Region, Tanzania

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The growth of tobacco production in Tanzania has become a threat to the woodlands due to the amount of firewood used for curing the crop. This paper examines the efficiency of using sawdust briquettes in curing tobacco. 4 kg of waste paper was soaked into 80 litres of water for 24 hours. 42 kg of sawdust was mixed with 4 kg of waste paper which was soaked into water. A low-pressure briquetting machine was used for briquetting. Rocket barn was used for testing the efficiency of sawdust briquettes in tobacco curing. Tobacco was reaped the day of loading and brought to each barn, whereby one barn used firewood as a control and the other one used sawdust briquettes. A Bomb Calorimeter was used to determine the calorific value of sawdust briquette and firewood. The grade indices were determined by dividing the value of tobacco in each harvest/reaping per hectare to the weight of dry tobacco per hectare. The grade indices produced by each biomass were compared. The results indicated that the calorific values of sawdust by using t-test to see if they were statistically different briquette and firewood were 3133.10 Cal/g and 4218.11 Cal/g, respectively. The grade indices of tobacco cured by sawdust briquettes and firewood were 1.756 and 2.257, respectively. There was no significant difference at $p > 0.05$ between the quality of tobacco cured by sawdust briquettes and that which was cured by firewood. The heat content produced by sawdust briquette was able to remove the amount of water present in green tobacco leaf and the quality of tobacco cured by sawdust briquettes was the same as that cured by firewood.

AP 51

Overview of tobacco specific nitrosamines reduction

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Tobacco-specific nitrosamines (TSNAs) are a class of toxicants, present in both combustible and smokeless tobacco (ST) products. Of the known TSNAs, nicotine-derived nitrosamine ketone (NNK) and N-nitrosonornicotine (NNN) are carcinogenic and on the FDA's harmful and potentially harmful constituents (HPHC) list. Other TSNAs include N'-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB). In 2017, the FDA proposed a standard limiting NNN levels to ≤ 1.0 ppm Dry Weight Basis (DWB) in finished ST products sold in the U.S. The TSNA values of both tobacco leaves and the finished products show considerable variability, with their formation being influenced by weather conditions, tobacco alkaloids, nitrosating agents, and curing conditions. A combination of genetics, agronomic practices, climatic conditions, leaf curing methods, and storage conditions can determine the chemical composition of the tobacco leaf and finished tobacco products and potential for TSNA formation. Over the years, tobacco farmers, public institutions, and manufacturers have been trying to reduce total TSNAs, particularly NNN levels. The feasibility of meeting this proposed standard is being investigated. Genetic approaches to develop very low TSNA tobacco varieties as well as management of tobacco production practices have been studied for several decades. In this presentation we will provide an overview of the TSNA reduction work carried out over the years by controlling minor alkaloids, nitrosating agents, low converter (LC) screening, agronomic growth and curing conditions.

AP 52

Influence of harvesting methods on nitrate content and TSNA formation in lamina and midrib of cigar and flue-cured tobaccos

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Cigar production has a history of nearly 400 years in Shifang, Sichuan Province. However, the contents of tobacco-specific nitrosamines (TSNA) are generally high in cured tobacco leaves. Field trials were initiated to investigate the impact of harvesting methods (priming, stalk cutting and lamina-midrib splitting) on alkaloid, nitrate and TSNA contents in lamina and midrib of cigar tobacco leaves (cigar wrapper variety Dexue 3 and cigar filler variety Shiyan 1) and flue-cured tobacco Yunyan 87. The results showed that the total alkaloid contents in lamina were higher than that in midrib, while the nitrate contents in midrib were much higher than that in lamina. For three varieties, the alkaloid contents in lamina were all the highest in stalk cutting followed by priming, with lamina-midrib splitting resulting in the lowest contents. The total alkaloid contents of midrib of cigar filler and wrapper tobaccos were the lowest in primed leaves. The nitrate and nitrite contents of lamina were the highest in stalk cut leaves, and lowest in lamina-midrib splitting leaves. Stalk cutting also resulted in highest nitrate and nitrite contents in midrib of all three varieties. TSNA contents of cigar midrib were much higher than that of cigar lamina, while the TSNA content of flue-cured tobacco midrib was lower than that of lamina. Among the three harvesting methods, lamina-midrib splitting resulted in the lowest total TSNA contents in lamina, and stalk cutting had the highest TSNA level. TSNA contents in the midrib of three varieties were also the highest in stalk cutting treatment, and the primed leaves had the lowest TSNA contents for cigar tobaccos. We concluded that different harvesting methods significantly influenced TSNA accumulation for both cigar tobacco and flue-cured tobaccos due to nitrate and alkaloid allocation and leaf properties changes.

AP 53

Widely targeted metabolomics analysis of flue-cured tobacco growing in different areas and association with sensory characteristics

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Natural climate and geography affect the sensory features of flue-cured tobacco significantly. However, their comprehensive associations with chemical composition accumulated in tobacco are still unknown. In this study, we used widely targeted metabolomics to clarify the differences of tobacco metabolic patterns under different weather factors. Tobacco samples from five growing areas, Hubei, Yunnan, Sichuan, Hunan, and Heilongjiang, were detected by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Based on the standards database, a total of 752 metabolites were obtained, and 181 kinds of them were identified as characteristic differential metabolites by combining the variable important in projection (VIP) value in orthogonal partial least squares discriminant analysis (OPLS-DA) with the fold changes. Furthermore, the screened metabolites were enriched to 53 metabolic pathways, covering the glycolytic pathway, amino acid metabolism, linoleic acid metabolism, phenylpropanoid biosynthesis, niacin and nicotinamide metabolism, and other plant metabolic pathways. The results of association analysis showed that the ecological climate conditions in the growing area were the important factors to determine metabolite content and flavour characteristics of flue-cured tobacco under the condition of fixed tobacco varieties and cultivation modes. This reaffirms the important view that "ecology determines characteristics", which has been widely accepted by the tobacco industry. This study provided new insights for the association of chemical compounds with various sensory traits of tobacco and laid a foundation for further quality control.

AP 54

Quality differences of flue-cured tobacco leaf from the southern Yunnan Gold Corridor Tobacco Cultivation Region and its environmental interpretation

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Tobacco planting is especially responsive to the environment mainly due to the impacts of soil fluctuations on quality and quantity. The availability of environment realisations with an adequate accuracy is an essential component of tobacco production. In this paper, physical characteristics and the major chemical components of C3F flue-cured tobacco from five counties at a relatively similar latitude in the southern Yunnan Gold Corridor Tobacco Cultivation Region from 2013 to 2021 were analysed using Canonical Correspondence Analysis. The result shows the significant differences in the contents of organic matter, total nitrogen, total phosphorus, total potassium, and hydrolysed nitrogen as well as pH value of the soils; also in the thickness, stem ratio, leaf density, equilibrium moisture content, filling power and contents of phosphorus of the tobacco leaves from five areas. The findings underscore the importance of environment effects on physical characteristics. The total phosphorus content and pH value were obviously positive while available phosphorus, total nitrogen and total potassium content had a negative effect on the physical characteristics; available phosphorus, hydrolysed nitrogen, total phosphorus, total potassium, total nitrogen and pH value were the soil factors that most contributed to the tobacco chemical characteristics. It would be of benefit for the utilisation of flue-cured tobacco resources effectively with characteristics for different style cigarette products. On the other hand, suggestion of cultivation techniques would be supported for high-quality flue-cured tobacco.

AP 55

Agronomic performance of ultra-low nicotine Burley tobacco CRISPR lines

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With the possibility of nicotine content being regulated, a number of ultra-low nicotine lines have been developed by genetic engineering. Most of these lines have exhibited severe growth deficiencies in the field. In this study, two ultra-low nicotine Burley tobacco CRISPR lines (*Nic1* edited in a *Nic1 nic2* plant) were compared with a commercial check variety and three low alkaloid (LA) lines, grown in the field with normal recommended production practices and also with practices designed to minimize nicotine accumulation. Nicotine levels were unusually high, ranging from 32.8 to 72.0 mg/g in the check, because there was a very severe drought that season. Nicotine was 1.7 to 8.5 mg/g in the LA lines, and 0.42 to 0.62 mg/g in the CRISPR lines. Yields of the CRISPR lines were significantly lower than the check and the LA lines, but these lines are in a Burley 21 background, which is inherently lower yielding than modern commercial varieties. The check had a significantly higher grade index (60 - 65) than all other entries. The CRISPR lines had a lower grade index than the LA lines when the crop was stressed, but in more favourable growing conditions, the CRISPR lines were no worse than the LA lines. However, the grade index of the CRISPR lines ranged from 31 to 38, which is unacceptably low. The CRISPR lines had heavier insect infestation than the other entries. The poor field growth reported in other ultra-low nicotine lines was not observed in these CRISPR lines. They grew vigorously from the seedling stage throughout the season, and the plants were comparable in size to those of commercial varieties.

AP 56

Generating ultra-low nicotine tobaccos by combining knockout mutations in two key steps of the alkaloid biosynthetic pathway

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The development of ultra-low nicotine tobacco varieties that retain acceptable agronomic and quality properties has been a challenge. Naturally occurring genetic mutations, genetic engineering, and genome editing technologies have all been used to lower the nicotine content of the tobacco plant. In the majority of these cases, however, the nicotine levels are not low enough to meet the target threshold proposed by the US Food and Drug Administration and/or the mutations negatively impact plant growth or cured-leaf quality. We have used genome editing to target genes encoding *N*-methylputrescine oxidase (*MPO*) as a strategy for lowering nicotine content. When grown in a greenhouse environment, knocking out *MPO* function reduced nicotine content by 93 - 96 % with little to no effect on normal plant growth and development. Nevertheless, *MPO* inhibition was accompanied by increases in the anatabine content of the leaf that were several-fold higher than is normally present. By combining *MPO* mutations with mutations in the three most highly expressed members of the *Berberine Bridge Enzyme-Like* (*BBL*) gene family, nicotine levels were reduced by > 99 % in greenhouse grown plants. Importantly, these individuals did not display an elevated anatabine phenotype. Plants possessing both *MPO* and *BBL* mutations were grown in the field for the first time in 2022 and the results will be presented here.

AP 57

Modified cultural practices to reduce nicotine accumulation in tobacco leaf and low nicotine variety impact on Italian growers' revenue

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After the publication by the US FDA of an Advance Notice of Proposed Rulemaking (ANPRM) on Tobacco Product Standard for Nicotine Level of Certain Tobacco Products, the interest in low nicotine cultivars and agronomic practices that could reduce nicotine accumulation in tobacco leaf has increased.

This study aims to evaluate the impact on Italian growers' revenue, as indicated by gross saleable production (GSP) of modified cultural practices and/or low nicotine cultivars when low nicotine tobacco leaf is the target.

For two consecutive years, a varietal test was carried out at Trasformatori Tabacco Italia (TTI - Città di Castello [PG], Italy), comparing reference and new low-nicotine cultivars of Virginia Bright tobacco managed according to local best practices (LBP) (ordinary plant density, N fertilisation and topping) and low-nicotine management (LNM) (increased plant density (+ 40 %), reduced N fertilization (- 50 %) and no topping). For every single variety, agronomic operations and related estimated costs of production were recorded. GSP was calculated by multiplying yield by the €/Kg value, based on grading.

Significant differences in yield and GSP were found among the differently managed varieties.

LNM generated an average decrease of 6 % in GSP. However, taking as reference only the low nicotine varieties, an average decrease of 21 % was recorded.

The study indicates that, in order to make economically sustainable the use of the modified cultural practice and/or low nicotine cultivars under the current Italian market conditions, it is necessary to consider a price increase of at least 10 % in the first case and by at least 40 % in the second one to keep growers' revenue.

ST 01

The importance of assessing puffing topography to inform e-cigarette emissions testing

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Analysis of the chemical composition of e-cigarette emissions is an important step in determining whether e-cigarettes support both individual and population-level harm reduction. Commonly, e-cigarette emissions for chemical analyses are collected by using e-cigarettes according to puffing regimens stipulated by standards bodies such as the International Organization for Standardization (ISO) or the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA). Standardised puffing regimens are often recommended by regulatory authorities who require the submission of e-cigarette emissions data to make a decision regarding allowing a product to be commercially marketed, in part because their use ensures uniformity between studies. However, standardised regimens do not necessarily reflect the puffing behaviour of users of a given e-cigarette and this can lead to under- or over-estimating real-world emissions from e-cigarettes. In this presentation, we describe how human puffing behaviour (topography) information can be collected both in the clinical laboratory and in the real world using a variety of different methodologies. By presenting data collected in human studies we will further discuss how topography information can be used to dictate e-cigarette puffing regimens for collecting emissions for chemical analyses and how this may lead both to better predictions of human exposure to e-cigarette aerosol constituents and to more informed risk assessments that better predict e-cigarette tobacco harm reduction potential.

ST 02

Puffing topography and mouth level exposure of two closed-system Vuse e-cigarettes

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E-cigarettes have the potential to reduce the harm caused by cigarette smoking. However, product likeability and product satisfaction are important in encouraging smokers to switch to less harmful products. Actual use studies play a key part in evaluating the reduced risk potential of tobacco and nicotine products. User's puffing behaviour, including puff duration and sensory effects were evaluated for two types of e-cigarette devices: a coil-and-wick 'pen-type' device (Vuse ePen3), and a ceramic block-and-plate 'pod-type' device (Vuse ePod) with 18 mg/ml nicotine e-liquid. Puffing topography was recorded for these devices with two groups (N = 52 each) of adult regular vapers (age 21 - 64 years) following a fixed 10 puffs protocol where subjects vaped through a special holder attached to a puffing analyser. The sensory characteristics of the aerosol were evaluated using a questionnaire. Mean puff volume was significantly greater ($p = 0.0001$) for ePen3 than for ePod (79.8 vs 49.4 ml), while puff duration and puff interval were similar (2.13 vs 2.29 s and 8.9 vs 10.3 s, respectively). Notably, mouth level exposure (MLE) to aerosol and nicotine from ePen3 and ePod were similar (3.89 vs 4.80 mg and 0.06 vs 0.07 mg, respectively) despite the very different designs of the devices. Participants reported similar overall likeability and other sensory scores for ePen3 and ePod. In summary, the puffing topography attributes support the CORESTA Recommended Method No. 81 (CRM81) puffing regime, used for *in vitro* and chemical analysis. The MLE to nicotine per session from both the products were lower than a typical 6 mg cigarette.

ST 03

Attitudes, consumption characteristics and motivations of cigarette smoking and electronic cigarette use among university students in Guangzhou, China: a cross-sectional survey

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The objective of this study was to assess Chinese university students' consumption characteristics and motivations for combustible cigarettes and electronic cigarettes (e-cigarettes) consumption, and their attitudes toward e-cigarettes.

The study involved an online cross-sectional survey conducted in Guangzhou, China. A total of 10,000 university students completed the questionnaire, after the screening, 9361 participants were included in the survey and their responses were analysed statistically. Descriptive statistics were used to assess participants' characteristics, attitudes towards e-cigarettes, and motivations associated with combustible cigarettes and e-cigarettes used; t-tests and $\chi 2$-tests were used to compare the type and brand of nicotine products consumed by university students with different social-economic levels, as well as the consumption of combustible cigarettes and e-cigarettes at different ages.

A total of 41.7 % (n = 5461) of participants were male and 55.9 % (n = 5230) were from urban areas. 29.8 % (n = 2786) of university students have used nicotine products (combustible cigarettes and e-cigarettes). Among the university students who used combustible cigarettes, 65.8 % (n = 642) were interested in e-cigarettes and 45.0 % (n = 439) were likely to use e-cigarettes in the future. With regard to consumption characteristics, the participants with a high standard of living were more likely to use both e-cigarettes and combustible cigarettes ($P < 0.01$), as well as tend to choose well-known brands in China, such as RELX, FLOW and CHUNGHWA. In our survey, stress relief was the most important motivation for university students to use combustible cigarettes (11.7 %) and e-cigarettes (7.2 %).

University students who used combustible cigarettes were more curious about e-cigarettes and more likely to use them. The type and brand of nicotine products (e-cigarettes and combustible cigarettes) used by university students were related to their disposable income. Stress relief was an important motivation for this population's use of combustible cigarettes and e-cigarettes.

ST 04

Actual use study of a heated tobacco product

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The introduction of new tobacco products in the United States, including those that may be lower on the risk continuum than traditional combustible cigarettes (CC), requires premarket authorization by the FDA and information on how the products will affect the tobacco use behaviour of current tobacco consumers. The aim of this actual use study was to evaluate how U.S adult tobacco consumers use a new tobacco product, glo™ Heated Tobacco Product (HTP) over a 6-week actual use period (AUP) in their real-life/naturalistic environment and in the context of typical consumer marketing materials. This was a multi-site, open-label, 8-week, observational study, conducted in an ambulatory setting at study sites geographically dispersed across the US. 1180 participants were enrolled into the study of which 1076 were identified as eligible participants. Participants that were identified as healthy adult smokers participated in an 8-week study in which they recorded their daily tobacco and nicotine product use in a smartphone-based customized electronic diary (eDiary) before and during introduction of the HTP investigation product (IP) including a 1-week Baseline Assessment Period and 6-week AUP. During the study, participants were allowed to use any combination of four HTP IP variants as well as other non-study Tobacco and Nicotine Products. During the actual use period, participants completed interviewer-led questionnaires, confirmed e-dairy compliance, and received additional study IP throughout scheduled site visits. The results from this study will inform the acceptance of the HTP IP and the patterns of CC consumption in the context of the HTP IP availability among current regular cigarette smokers (defined as smoking ≥ 5 cigarettes a day on at least 20 out of the past 30 days). Additionally, results of this study will highlight the feasibility of performing these studies to assess the impact of product introduction on use patterns in real-life settings.

ST 05

Risk perception of *IQOS*™ and cigarettes: temporal and cross-country comparisons

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Risk perception (RP) is central to smokers' decision to switch to smoke-free tobacco and nicotine products (TNP).

This study assessed temporal trends in the health RP of a novel heated tobacco product, *IQOS™*, relative to cigarettes, among current *IQOS™* users.

The analyses included repeated cross-sectional data from online surveys in Germany (n=2536, years 2018–19), Italy (n = 2457, years 2018 - 19), and Japan (n = 5044, years 2016 - 17, 2017 - 18, and 2018 - 19) among a random sample of current adult *IQOS™* users from local registers of *IQOS™* users. The health RP of cigarettes and *IQOS™* were assessed using the ABOUT™–Perceived Risk instrument. The score ranged from zero, indicating no perceived risk, to 100, indicating very high perceived risk. Relative RP of *IQOS™* to cigarettes ($RP_{Cigarettes\text{-}IQOS}$) was computed as the difference in absolute RP scores of cigarettes minus *IQOS™*.

After adjustment for covariates (sex, age group, *IQOS™* use pattern (exclusive vs. dual) and intensity), the relative $RP_{Cigarettes\text{-}IQOS}$ was higher in 2018 than in 2019 (β-coefficient of 0.93; standard error, 0.33; *P*=0.005). This was driven by an increase in the RP of *IQOS™* over time in Italy (2018: mean RP score = 42.6 [95 % CI, 41.6–43.5]; 2019: 44.4 [43.4–45.4]) and Japan (2017: 44.0 [43.1–44.9); 2018: 45.9 [45.2–46.7]; 2019: 48.6 [47.9–49.4]), while the RP of cigarettes remained stable.

The relative RP of *IQOS™* decreased over time, driven by an increase in the RP of *IQOS™*, in agreement with epidemiological studies indicating a temporal reduction in the relative RP of smoke-free TNPs. Continued surveillance of the RP of novel TNPs is warranted to inform effective TNP risk communication and ensure that adult smokers who would otherwise continue to smoke understand the relative risks of novel TNPs.

ST 06

Assessing consumer use and behaviour patterns of oral nicotine pouches in a multi-country study

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Due to the novel nature of oral nicotine pouches, limited studies have been conducted on the usage of these products in real-world settings. Consumption patterns and use behaviour of current nicotine pouch users need to be investigated to monitor the use and support the assessment of reduced risk products. Here we report the findings of an online survey completed by 550 participants across Sweden, Denmark, Germany and Switzerland who were current nicotine pouch users. The key areas of research were oral nicotine use history, mouth hold duration, nicotine strength, average daily consumption (ADC) and flavour preferences. Across all countries, most participants used oral nicotine pouches for 12 months or less. Longer use of nicotine pouches was reported in Sweden. Pouches containing 6 - 15 mg nicotine were used most frequently in all countries, and particularly 11 - 15 mg pouches in Sweden and Denmark. Average ADC across all countries was 1 - 5 pouches, closely followed by 6 - 10 pouches in Sweden and Denmark. Menthol was the most preferred flavour in all countries, followed by fruity and other food flavours. These findings reflect the differing product use patterns across nascent and mature markets as well as the need to investigate how different experience of product types can affect the use of new categories. Further research is required with a larger sample over time to understand product use patterns more clearly, including transition from and into other product categories.

ST 07

Simultaneous analysis of 18 biomarkers of tobacco smoke exposure in urine by multiple heart-cutting two-dimensional liquid chromatography-tandem mass spectrometry

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Metabolites of nicotine, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons and aromatic amines are currently the most representative biomarkers of tobacco smoke exposure which can reflect the smoke exposure of humans and are important research objects for risk assessment of tobacco products. Simultaneous analysis of biomarkers can greatly improve the detection efficiency. However, due to the complex matrixes of biological fluids, the wide variety of biomarkers and the large differences in their contents and chemical properties, there is no method to simultaneously detect multiple types of smoke exposure biomarkers. In this study, a multiple heart-cutting two-dimensional liquid chromatography-tandem mass spectrometry method was established. The “naturally occurring carbon isotopes” were used to solve the problems associated with signal dynamic range caused by simultaneous analysis of compounds with large differences in their contents. The method realised the simultaneous analysis of 18 smoke exposure biomarkers of four classifications in human urine for the first time. The results showed that the correlation coefficients (*r*) were higher than 0.993 for all biomarkers, the spiked recoveries of the samples ranged from 80.9 % to 108.8 %, the intra- and inter-day relative standard deviations ranged from 1.0 % to 5.4 % and from 2.4 % to 6.0 % respectively, the detection limits ranged from 0.8 to 87.0 pg/mL, and the quantitation limits ranged from 2.7 to 290.0 pg/mL. This method has good resolution and sensitivity, and can provide a more efficient and convenient high-throughput analysis technique for the assessment of smoke exposure.

ST 08

Development and validation of a routine method for the determination of 3-hydroxybenzo[a]pyrene in human urine by GC-MS/MS

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Mainstream smoke is a complex mixture containing thousands of unique compounds. Among these are polycyclic aromatic hydrocarbons, which includes benzo[a]pyrene (BaP), a Group 1 carcinogen. 3-Hydroxybenzo[a]pyrene (3OHBaP), a metabolite of BaP, is commonly used as a biomarker of exposure for BaP. Urinary levels of 3OHBaP are expected to be low as this is not the primary route of excretion. Analytical methods developed for the analysis of 3OHBaP must be capable of achieving detection limits appropriate to account for the expected low urinary concentrations.

Here, we report the development and validation of a new method for the analysis of 3-hydroxybenzo[a]pyrene using gas chromatography with triple quadrupole mass spectrometry (GC-MS/MS). During early development, several analytical detection methods were evaluated, including LC-MS/MS and LC-FLR, but these did not achieve the required sensitivity. GC-MS was also evaluated and was sensitive enough for analyte detection, but lacked selectivity from interfering compounds. By using GC-MS/MS, both selectivity and sensitivity were optimized and deemed acceptable for the analysis of 3OHBaP.

Urinary samples were hydrolyzed enzymatically overnight followed by solid phase extraction clean up using a polymeric strong anion exchange column. Sample extracts were derivatized using N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and injected on an Agilent 8890 GC with a 7010 MS triple quadrupole (QQQ) mass spectrometer. This method was fully validated and has a LLOQ of 25 pg/mL which equates to 125 fg/mL in sample. Sample extract concentrations were found to vary between non-detected (ND) and 387 pg/mL with an average concentration of 67 pg/mL. Intermediate precision was 8.8 (%RSD) and the average accuracy ranged from 110 % to 122 % across all fortification levels.

ST 09

Reduction in urinary and blood biomarkers of tobacco exposure in smokers switched to an electronic nicotine delivery system product

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Electronic nicotine delivery system (ENDS) products have the potential to provide nicotine to adult tobacco consumers while reducing exposure to combustion-related toxicants. This study evaluated changes in biomarker(s) of exposure (BoE) to combustion-related toxicants after smokers switched from their usual brand (UB) non-menthol cigarettes to one of three Vuse Original ENDS products (Vuse Vibe, Vuse Ciro, and Vuse Solo) or to abstinence. The BoE evaluated represent constituents for which validated biomarkers exist and that were recommended for evaluation in e-liquids and aerosols by the FDA in the 2016 (Draft) and 2019 (Final) ENDS Premarket Tobacco Product Application guidance documents, and/or constituents identified by FDA as harmful and potentially harmful constituents (HPHCs) in tobacco products or tobacco smoke. Subjects smoked their UB cigarette *ad libitum* for two days, then were randomized to one of three Vuse ENDS products for a 5-day *ad libitum* use or abstinence. Twelve urinary (including nicotine and five metabolites) and one blood BoE were measured at both baseline and day 5. Changes in BoE from baseline to day 5 were determined and the percent change of mean was calculated for each cohort. Results showed an overall decrease in BoE across the three product-switch and abstinence cohorts. Blood carboxyhemoglobin decreased 51-55 % in all four study cohorts. Urinary biomarkers decreased between 40 - 95 % for aromatic amines, mercapturic acids, tobacco-specific nitrosamines, and 3-hydroxy-benzo[a]pyrene in all four study cohorts. However, smaller changes (5 - 30 % decrease) in total nicotine equivalents were observed from baseline to day 5 for each of the three product-switch cohorts versus a > 95 % decrease in the abstinence cohort. Nicotine levels within the product-switch cohorts increased steadily throughout the study. In this study, smokers who were switched to the ENDS products had reductions in combustion-related BoE similar to the reductions observed in the abstinence cohort.

ST 10

Dynamics of nicotine status in smoking volunteers after switching to oral nicotine products

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For the biomedical implications of using oral nicotine products, the dynamics of the body nicotine status is of prime interest. Our objective was to address this issue. We recruited six smoking male volunteers under the age of 40 with the average smoking experience of 16 pack*years. Snus with a nicotine content of 12 mg/portion was used, and the pertinent nicotine levels along with the other parameters were monitored. Two weeks after switching to snus, volunteers showed 20-40 % decrease in the levels of nicotine metabolites in the morning urine. One volunteer gave up continuing the experiment. A month after the beginning, in all five volunteers, the levels of nicotine metabolites remained reduced by 20 - 50 %. Then, another volunteer returned to smoking, but decreased the number of cigarettes by 2 - 3 per day. After two months of the experiment, another volunteer gave up and opted for a tobacco heating system. Two volunteers continued using snus for nine months. One of them experienced an increase in snus consumption, starting at the 4th month, reaching the baseline nicotine level in seven months, then nicotine metabolites began to exceed the threshold set for him during smoking. For the second volunteer, the nicotine level increased from the 5th month from switching to snus. By the 9th month it reached the level characteristic of smoking. We conclude that the initial decrease in the nicotine level was caused by a long adaptation and a discomfort in the palatal part of the oral cavity at the places of fixation of snus sachets. It took up to six months, following intensifying the snus consumption (from 3 - 4 to 5 - 7 sachets) with a simultaneous reduction in the duration of continuous fixation of the sachet on the palate (from 20 - 30 to 10 - 15 minutes), for a comfortable mode of the nicotine intake to be provided.

ST 11

Determination of transfer efficiency of seven flavour compounds during vaping of electronic cigarette liquid

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Flavour compounds are commonly added into electronic cigarette liquids (e-liquids), such as organic acids, sweeteners, and cooling compounds. Characterising the vaping and transfer efficiency during electronic nicotine delivery system (ENDS) vaping can help determine the optimal amount of flavour compounds to add.

In this study, e-liquids of known formulations with one or more components of benzoic acid, N-Ethyl-p-menthane-3-carboxamide (WS-3), 2-Isopropyl-N,2,3-trimethylbutanamide (WS-23), menthol, sucralose, neotame, ethyl maltol were used to represent different classes of flavour compounds, and they were mixed with propylene glycol (PG) and glycerin (VG) (PG:VG ratio at 1:1) and filled in closed-pods. A RELX Phantom device of 6.5 watts was used to produce the aerosol with a vaping machine under 55.0 ± 0.3 mL puff volume, 3.0 ± 0.1 seconds puff duration and 30.0 ± 0.5 seconds puff intervals. The device was equipped with pods of different versions of atomisation cores. Trapped aerosols produced by the ENDS were determined for the concentrations of these flavouring compounds in the aerosol and compared to their added values in the e-liquid. The vaping and transfer efficiency of the flavour compounds were systematically compared.

The transfer efficiencies measured were: benzoic acid between 76.03 % to 104.51 %, WS-3 between 86.25 % to 103.23 %, WS-23 between 76.19 % to 90.34 %, menthol acid between 58.73 % to 71.61 %, ethyl maltol between 74.48 % to 90.02 %, sucralose between 3.76 % to 42.18 %, and neotame between 11.33 % to 26.43 %.

The results indicated that the same substance in different versions of atomisation cores varied greatly, especially amino acid compounds and compounds with carbohydrate ring. Compounds with lower boiling and melting points appeared to have good thermal stability and higher transfer efficiency. The peptide bond of neotame could be thermally unstable during vaping, resulting in low transfer efficiency. Compounds with sugar ring structures and higher boiling points also resulted in low transfer efficiency.

ST 12

Assessment of potential atomisation agents on capacity of aroma delivery in e-liquid

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Propylene glycol (PG) and glycerin (VG) are two commonly used humectants in e-liquid. To determine a possible substitute that performs equally to PG/VG or functions better as an aerosol forming agent and carrier of aroma constitutes, compounds were selected and assessed.

According to the physicochemical property and safety, four agents were chosen: dipropylene glycol, 1.2-pentanediol, triethyl citrate, and isopropyl myristate. PG and VG were tested as references. A formula of 12 commonly used flavour compounds including acids, aldehydes, phenols, esters and ketones in e-liquid blend was designed and added into these four agents. The mixture was filled into e-cigarettes and evaluated by a sensory evaluation panel on the quality of produced aerosols. E-cigarettes filled with the four blended mixtures were puffed by linear smoking machine, and flavour compounds in emissions were collected by Cambridge filter pad and were quantitatively determined by GC.

In sensory evaluation, aerosol from isopropyl myristate containing the flavour blend got the highest score among tested agents for its richness of aromas. In the aerosol analysis, all the 12 flavour compounds were determined in PG, triethyl citrate and isopropyl myristate blended e-liquid emissions, while nine and eight compounds were determined in dipropylene glycol and 1.2-pentanediol generated emission. Determination of the total amount of flavour compounds in emissions showed that isopropyl myristate delivered the highest level (2.71 mg/puff) of flavours while triethyl citrate delivered the lowest level (1.41 mg/puff). Analysis on delivery of flavour types indicated that isopropyl myristate delivered the highest level of all flavour types (equal ~8 times higher), triethyl citrate showed the poorest delivery of acids and ketones while PG and VG mixture delivered the lowest level of aldehydes, phenols and esters.

Our findings provide evidence on the possible substitute of PG and VG as an atomisation humectant in e-liquid blending.

ST 13

Evolution of particle size distribution of electronic cigarette aerosols through a secondary thermal treatment

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Electronic cigarettes (e-cigarettes) are an evolving product category that is designed to deliver nicotine for inhalation via aerosolization of e-liquids. An atomization process consists of a rapid heating, vaporisation, condensation and coagulation of e-liquids, forming aerosols with particle sizes distributed in the range of 10 - 3000 nm. It is generally believed aerosols with appropriate particle sizes are favourable to being inhaled into pulmonary alveoli to achieve a high efficiency of nicotine absorption, rather than being deposited on the respiratory tract or exhaled. The environmental temperature is normally a key effect in the evolution of particle size of an e-liquid droplet since the saturated vapour pressure of its main constituents varies. In this study, we conducted a secondary thermal treatment of e-cigarette aerosols to modulate its particle size distribution without change of chemical components. A mixture of propylene glycol and glycerol (1:1) as a model e-liquid was atomised on RELX infinite pod-type e-cigarette (puff volume: 55 mL; puff duration: 3 s; inter-puff interval: 27 s). The fresh aerosols out of the atomiser passed through a heated PTFE tube and then cooled, and the particle size distribution of the treated and aged aerosols were measured subsequently on a laser diffraction particle size analyser. The mean particle size grew from 341 nm to 461 nm and the size distribution became narrowed as the aerosol flow was heated from room temperature to 140 °C. However, it changed to decrease gradually to 333 nm at 180 °C. This process was simulated by numerical simulation, the results showed that the particle size distribution of aged aerosols represented a unimodal type below 140 °C, and changed to a bimodal distribution when heated to 140-180 °C.

ST 14

Retention of flavour compounds in e-liquid pods: impact from the air flow channel structure of the pods

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The electronic cigarette (e-cigarette) users' sensory experience is greatly impaired by the flavour decay of e-liquids during storage and in use. For an unpacked e-cigarette pod, the escape and evaporation of the volatile flavour compounds in e-Liquid plays a crucial role in its flavour decay post manufacturing. In the present work, both computational simulation and analytical experiments were conducted to study the influence of the air flow channel structure of the pod on the loss of the flavour compounds in e-liquid during storage. The hypothesis was that the flavour compounds escaped from the pod by molecular diffusion and natural convection. The residence time of volatilised flavour compounds in the pod, obtained from computational fluid dynamics (CFD) simulations, was used to characterise and compare different air channel structures. In a simplified model experiment, the concentrations of several highly volatile esters, measured by gas chromatography–mass spectrometry (GC/MS), were observed to reduce more slowly in a glass container with larger residence time. This supported the assumption that air channels offering a large residence time could promote the retention of flavour compounds. Further CFD simulations implied that not only the residence time, but also the total volume of the air channel affected the volatilisation rate of the flavour compounds. Finally, experimental measurements of lab-made pods mimicking commercial pods revealed that by carefully adjusting the air channel structures in between the coil and the air inlet, the retention rate of the target flavour components including ethyl ester, ethyl propanoate, ethyl butyrate and ethyl 2-methylbutyrate increased by 3 - 10 % after 24 hours sitting (25 °C, 50 % RH). This work highlights the importance of studying the interaction between flavour chemicals and the pod structure on sensory performance.

ST 15

Investigating the potential for ketene generation in flavored e-liquids via thermal degradation of ester flavorants

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E-liquids are composed primarily of propylene glycol, glycerin, nicotine, and flavorings, with ester flavorants becoming of recent interest. Modeling studies have suggested that under extreme operating conditions, certain ester compounds have the potential to undergo thermal degradation to produce toxic, ketene gas. This toxicant has been linked to e-cigarette or vaping use-associated lung injury (EVALI), first reported in 2019 and later attributed to thermal breakdown of the phenolic ester, vitamin E acetate.

This work sought to determine if this degradation pathway was possible and under what conditions it would occur. E-liquid samples were prepared containing a single ester flavoring ingredient and vaped under moderate-to-extreme experimental conditions (0.15–1.8 Ω coils, 12–125 W power setting; 55/5/30 puff regime; puff to dryness). The generated aerosol was bubbled through an impinger containing a 4-bromobenzylamine trapping agent and aliquots were analyzed using positive ESI-LCMS. Potential ketene products were identified using the unique isotopic pattern afforded by the bromine atom. The expected masses were observed in collections where the e-liquid contained ethyl butyrate. Additional peaks with the bromine isotopic signature were observed in the ethyl butyrate sample with the initial hypothesis being these products were the result of the fragmentation and rearrangement of the butyryl group. Further experiments have provided evidence that these and additional degradation products observed may be generated from propylene glycol and glycerol, thereby increasing the complexity of the analysis. In conclusion, sample collection under dry puffing conditions can result in the generation of numerous reactive species that readily react with our amine-based trapping agent and may include ketenes. Under non-dry puffing conditions, depending on the tank/coil system, these species did not appear to form.

ST 16

HPHC market map of open pod-based and closed pod-based e-cigarettes from the North American market including a comparison of selected analyte yield in the aerosol to 3R4F cigarette smoke

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A large body of research is available describing North American combustible cigarette (CC) smoke constituents, including harmful and potentially harmful constituents (HPHCs), that has aided the evaluation of CCs by manufacturers, regulators, and other stakeholders. Many of these publications include “market map” style comparisons illustrative of the expected yield of smoke constituents in CC(s) of interest contrasted with an estimate of the respective CC market mean smoke constituent yield. The objective of this study was to create a broad North American e-cigarette market map in the style of the CC market map reflective of the recent market shift towards refillable pod-based and closed pod-based type e-cigarettes. To this end eight closed pod-based e-cigarette devices and four open pod-based e-cigarette devices were selected as test articles and vaped from puff one to end of life according to two puffing regimes. One cigalike e-cigarette device was also vaped for comparison. A total of 29 unique combinations of devices and formulations were tested. Aerosol was analysed for primary constituents, metals, carbonyls, and glycidol. Aerosol yields were normalized to device mass loss (DML) and nicotine for comparison to the “market” mean (derived from the test articles) and for comparison to CC, respectively. Great diversity in analyte yields were observed within and across the categories of closed pod-based, open pod-based, and cigalike e-cigarettes. Mean DML yield ranged from 1.16 to 6.90 mg/puff, 4.10 to 14.88 mg/puff, and 2.48 to 3.13 mg/puff, respectively, for closed pod, open-pod, and cigalike e-cigarette categories (non-intense regime). Mean nicotine yield ranged from 0.054 to 0.277 mg/puff, 0.144 to 0.517 mg/puff, and 0.045 to 0.061 mg/puff, respectively, for closed pod, open-pod, and cigalike e-cigarette categories (non-intense regime). In addition, mean aerosol yields for metals, carbonyls and glycidol will be presented for each e-cigarette category and compared to reference CC.

ST 17

Distinguishing tobacco-derived nicotine from synthetic nicotine in commercial nicotine samples

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Recently, synthetic nicotine (SN) has become commercially available and can be supplied as United States Pharmacopeia (USP) grade (S)-nicotine, as a 50/50 (S)/has mixture, or as a mixture in varying ratios of (has(R) enantiomers. While both tobacco derived nicotine (TDN) and synthetic nicotine (SN) are all regulated by the FDA, distinguishing between these two forms may be critical for tobacco authentication, and to assess manufacturing methodology and nicotine chirality relative to any pharmacological concerns. As tobacco product development continues, the need for robust analytical methods to differentiate tobacco derived nicotine (TDN) from SN will be of significant importance for nicotine and tobacco authentication. The main purpose of this study was to demonstrate the utility of analytical chromatographic methods to distinguish TDN from SN. Nicotine samples were analysed by GC/MS and SPME/GC/MS and by either chiral GC/MS or chiral HPLC/UV. Several TDN samples were found to contain 2,3'-bipyridine, characteristic of TDN. Some SN samples were found to contain the synthetic starting material, ethyl nicotinate and the synthetic impurity, 1-methyl-2-pyrrolidinone. By chiral chromatography, SN was found to contain either a 50:50 mixture of (R)- and (S)-nicotine or a low level of (R)-nicotine (0.1 - 0.2 %) compared to typical content in TDN (0.8 % - 0.9 %). A low level of (R)-nicotine indicated that the nicotine was most likely synthetic. The use of these chromatographic methods provides guidance for distinguishing TDN from SN in commercial nicotine samples.

ST 18

Nicotine characterization in modern oral nicotine pouch products

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Modern oral nicotine products (MONPs) are a new class of product devoid of tobacco plant material that have been introduced as a potential reduced risk alternative nicotine product. Some manufacturers have taken this concept further by using synthetic nicotine (SyNic), rather than tobacco-derived nicotine (TDN), to furnish a completely tobacco-free product. Characterizing the SyNic used in these products is critical to both verify the nicotine's origin and to determine the (*R*)/(S) nicotine enantiomeric ratio. The latter of these is important since there is still much unknown with regards to the pharmacological and toxicological properties of (*R*)-nicotine. We recently published a study into the characterization of the pure SyNic and TDN used in e-liquid formulations. Here we report on the extension of these methods to their characterization in pouched MONPs. A number of commercially available pouched MONPs containing either TDN or SyNic were purchased and analysed. To determine nicotine origin, the pouch products were extracted using liquid-liquid extraction techniques to isolate the nicotine from the other components. The obtained nicotine was then subjected to radiocarbon analysis to distinguish between synthetic and tobacco-derived origins. The (*R*)/(S)-ratio was determined using chiral HPLC-UV, with the products typically extracted using methanol followed by centrifugation and filtration. Radiocarbon analysis results were found to be consistent with the stated nicotine origins (100 percent modern carb–n (pMC) for TDN and 30 - 40 pMC for SyNic), while the (*R*)/(S)-ratios for SyNic-containing MONPs showed a significant use of racemic nicotine (50:50 (*R*)/(S)) rather than enriched (S)-nicotine (> 99 % (S)-nicotine). Some MONP pouches were found to contain interfering components that required either modified chromatography or isolation of the nicotine in order to accurately determine the has/(S)-ratio. In conclusion, the combination of radiocarbon analysis and chiral HPLC-UV is sufficient to characterize the nicotine used in pouched MONPs.

ST 19

Interaction mechanisms between S/R-nicotine and human serum albumin

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Nicotine is an asymmetric molecule with two enantiomers, namely S-nicotine and R-nicotine. Studies have shown that different configurations of nicotine have different physiological activities in the body. Therefore, taking human serum albumin (HSA) as the model protein, this paper focuses on the different interaction mechanisms and recognition mechanisms of the binding of *S/R*-nicotine to HSA. From the comparative analysis of the results of molecular fluorescence spectroscopy, molecular docking and molecular dynamics simulation it can be seen that interaction exist between *S/R*-nicotine and HSA. The dominant force driving the interaction processes between *S/R*-nicotine and HSA was hydrogen bonding and R-nicotine had a slightly stronger effect on HSA than S-nicotine. Synchronous fluorescence and three-dimensional fluorescence spectroscopy experiments showed that *S/R*-nicotine could locate in the active cavity of HSA and this interaction would change the microenvironment around Tyr and Trp residues, slightly affect the α helix structure and loose of the peptide skeleton structure of HSA without affecting the basic skeleton structure of HSA. In has three-dimensional model of *S/R*-nicotine-HSA constructed by molecular docking, the most stable configuration was formed by the binding of *S/R*-nicotine to the subdomain IIA (site I) of HSA, which was confirmed by probe experiments. Theoretical analysis and experimental results were combined to show that *S/R*-nicotine had high similarity in their binding modes, binding sites, acting forces and the effects on the spatial structure of HSA. Therefore, it could be speculated that *S/R*-nicotine might present consistent blood transport behaviour *in vivo*. The investigation on the interactions between *S/R*-nicotine and HSA is important for the clarification of physiological functions of nicotine entering the bloodstream and also provides experimental and theoretical guidance for its safety evaluation.

ST 20

Removal of nicotine to recycle machine wash water

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The tobacco industry is evolving, not just in products but also in processes. What can we do to lower our carbon footprint? One area of interest is in recycling wastewater in manufacturing facilities. After making nicotine products at our facilities, the equipment is washed with water. This water is collected, classified as hazardous waste, and then shipped for disposal due to the possible presence of nicotine. The disposal costs are high because the waste is shipped to incineration facilities outside of North Carolina. The transportation creates a large carbon footprint, and the incineration of the water adds to this footprint.

Onsite experiments using ion exchange resin and activated carbon have shown at least a 99.5 % reduction in the amount of nicotine in wash water and a projected 95 % reduction in water volume sent for hazardous waste disposal. In addition, activated carbon adjusts the pH of the water close to neutral. The goal would be to recycle water in the facility to contribute to BAT's environmental sustainability goals while reducing the carbon footprint by eliminating the need of transportation outside of North Carolina.

ST 21

A randomised cross-over study investigating the nicotine pharmacokinetics of nicotine pouches

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In recent years a new type of oral smokeless, tobacco-free, nicotine product has become available known as nicotine pouches (NPs). Due to their novelty, very little data are available on the nicotine pharmacokinetics (PK) of NP use.

To develop a better understanding of these products a nicotine PK study was conducted comp–ring commercially available NPs (five different brands; 6 - 10 mg nicotine/pouch) to a combustible cigarette.

In this 8-day confinement study, 35 healthy adults who were current dual users of combustible cigarettes and snus were screened and recruited. On each day of the study after overnight abstinence from nicotine use, participants used one product for a defined period and blood samples were taken at specified timepoints b–fore and after product use.

All NPs had a higher T_{max} (60 - 65 minutes) than the cigarette (7 minutes) as well as a higher $AUC_{0\text{-}6h}$. Results also showed that three of the NPs had a higher C_{max} than the cigarette and that nicotine plasma concentration was not associated with the nicotine content of the NPs.

Subjective questionnaires on product liking and intent to use product again scores were also measured; these were lowest for the NP with the lowest nicotine content, while the cigarette had higher values than any of the NPs.

This study demonstrates that nicotine uptake from NPs can replicate that of smoking combustible cigarettes following a switch from conventional cigarettes to these potentially less harmful NP products. It also provides an important understanding of nicotine PK and subjective effects during NP use.

ST 22

Nicotine delivery and pharmacokinetics of an electronic cigarette compared to conventional cigarettes in Chinese smokers: a randomised open-label crossover clinical study

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Nicotine delivery and pharmacokinetics of electronic cigarettes (e-cigarettes) are likely to determine the potential to facilitate smokers switching to e-cigarettes.

The objective of this study was to evaluate the nicotine pharmacokinetics of a commercial e-cigarette (RELX) relative to conventional cigarettes in Chinese adult smokers.

A randomized, open-label, crossover clinical study was conducted on 23 healthy adult Chinese smokers. In two sessions, subjects used either the e-cigarettes with 30 mg/g nicotine in e-liquid or conventional cigarettes of a given brand, at one puff every 30 s for a total of 10 puffs. Blood samples were collected at specified time points for 4 h after the first puff. Subjective effects on desire-to-smoke, and physiological parameters such as heart rate and oxyhemoglobin saturation levels were also examined before and after using the two products.

The baseline-adjusted maximum nicotine concentration (C_{max}-BL), time-to-peak nicotine concentration (T_{max}), and nicotine absorption rate (C_{max}-BL divided by T_{max}) were found to be similar for the e-cigarette compared to those of the conventional cigarettes ($P > 0.05$). Total nicotine exposure measured as the area-under-curve ($AUC_{0\text{-}t\text{-}BL}$) was significantly lower for the e-cigarette relative to that of the conventional cigarettes. In addition, the subjects found that e-cigarettes were well tolerated under the controlled puffing conditions.

The test e-cigarettes achieved similar nicotine delivery and pharmacokinetic profiles to those of the comparator cigarettes, indicating that this e-cigarette could be a potential alternative to conventional cigarettes for those adult smokers.

ST 23

Abuse liability assessments of Vuse Alto Golden Tobacco in adult smokers

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The assessment of abuse liability (AL) of electronic nicotine delivery systems (ENDS) in human subjects is critical to determining the appropriateness for the protection of public health in the FDA's premarket tobacco product application process. We conducted a randomized, open label, crossover study to assess AL for Vuse Alto ENDS including e-liquids at 2.4 % (27.4 mg/ml) and 5 % (57.8 mg/ml) nicotine levels. Fifty subjects (27 male/23 female) were enrolled in this 9-day confinement study. Combustible cigarette (CC) and nicotine gum were included as high- and low-AL comparators, respectively. Subjects participated in four test sessions in confinement (each following a consecutive 1.5-day product acclimation and 12-hour nicotine abstinence period). During each test session, subjects used the assigned product (based on a Williams design) *ad libitum* for either 10 minutes (cigarette and ENDS) or 30 minutes (nicotine gum). Blood samples, subjective measures, vital signs, and adverse events were collected over four hours prior to, during, and following product use. Measured endpoints following the start of product use included: product liking, overall intent to use again [OIUA], product effects, urge to smoke [UTS], overall product liking, nicotine pharmacokinetics [PK], and physiological effects. ENDS at both nicotine levels had lower scores for product liking, OIUA, product effects, overall product liking; and baseline-adjusted nicotine PK were less than CC, but greater than nicotine gum. ENDS had similar UTS scores compared to CC. These results are consistent with previous AL studies of Vuse products and provide evidence that Vuse Alto has an AL between nicotine replacement therapies and CC, thus, supporting that Vuse Alto provides sufficient levels of nicotine to enable some adult cigarette smokers to transition to the use of Vuse Alto.

ST 24

Examining nicotine exposure from e-cigarettes with menthol- and tobacco-flavours: a meta-analysis

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Many smokers choose to use electronic cigarettes as alternatives to combustible cigarettes. E-cigarettes are offered in various flavours, and menthol is among the most commonly used. Until recently, menthol cigarettes have also been available in many countries and preferred by a considerable proportion of the smoking population. Menthol smokers interested in trying alternative products, which do not involve exposure to smoke and deliver similar nicotine levels, might choose menthol-flavoured e-cigarettes as a first option. As previously reported, nicotine delivery from e-cigarettes is influenced by different factors, including device characteristics and user behaviour. The purpose of this study was to examine whether there is a difference in nicotine exposure from e-cigarettes with tobacco-flavour versus menthol-flavour. We conducted a meta-analysis including nicotine pharmacokinetic data from published research as well as internal studies. In addition to the results, we will discuss the limitations of the included data sets. The analysis indicated that there is no difference in nicotine exposure between the tobacco-flavoured and menthol-flavoured e-cigarettes. Based on our results, smokers can obtain equal amounts of nicotine from e-cigarettes regardless of if they choose menthol-flavour or tobacco-flavour.

ST 25

FDA-CTP and CDISC project to develop tobacco related standards to achieve efficiencies for all stakeholders

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The mission of FDA Center for Tobacco Product (CTP) is to protect Americans from tobacco-related disease and death by regulating the manufacture, distribution, and marketing of tobacco products and by educating the public, especially young people, about tobacco products and the dangers their use poses to themselves and others. To achieve this mission CTP performs science-based application review in addition to compliance outreach, enforcement, regulation and guidance formulation and other product regulation activities.

CDISC, for over 20 years, has developed standard models, concepts, terminologies, data exchange formats and implementation and user guides to improve the quality, efficiency and cost effectiveness of clinical research processes from protocol through analysis and reporting. CDISC standards also support interoperability and reusability in the form of meta-analysis to identify predictive, prognostic, and safety biomarkers and to apply artificial intelligence (AI) algorithms to facilitate analyses of large, pooled datasets. Machine-readable CDISC standards are available in Excel, JSON, RDF and XML. In addition, the Operational Data Model (ODM) XML standard for data sharing, provenance and archiving is also available.

CDISC standards are required for regulatory submission of clinical trial data in the US, Japan and are accepted in EU and China. These standards traditionally have been used solely for the submission of regulatory data.

This presentation will discuss CTP's commitment to the development and implementation of standards to speed regulatory review and decision making as well as the efficiencies that can be achieved for all stakeholders, introduce CDISC and the suite of CDISC standards, briefly describe the global regulatory environment and provide an overview of the collaborative standards development project recently commenced by CTP and CDISC to develop nonclinical and clinical data standards, new domain specifications, conformance rules and new terminology for tobacco studies.

ST 26

Application of innovative big data techniques for improving data processing and accelerating risk assessment in new tobacco products

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Data analyses are often performed on a per testing level by traditional data-processing applications involving data collection, data summary, validation, and generation of final outputs. Usually due to dispersed knowledge, testing results are interpreted in stand-alone reports, missing the ability to link with other informative data for improving reviews and decision-making processes (e.g., putting together data from consumption, chemical, toxicological, and clinical assessments).

Our objective was to evaluate available big data solutions for setting an automatic storage of datasets, for creating visualization tools and for running machine learning (ML) predictions in near real time.

Standard data management scripts have been written to import and structure original raw datasets received from various providers to make them ready for concatenation. The data lake: a unique repository with unlimited storage space is then used to centralise all the information and is linked to our artificial intelligence (AI) business applications where ML models are trained to produce predictions and feed dynamic dashboards. These are effective tools made for handling huge amounts of data to allow for continuous re-assessments while entering records into the lake.

Two examples will be shown regarding automation of pooled analyses when accumulating adverse events from clinical studies and on-live statistical comparisons from vapour chemistry emissions levels integrating toxicological risk assessments.

This big data platform was customised to fulfil specific needs regarding access to large volumes of data, to perform data enrichment and run instant analyses with the aim to optimise data driven reporting and accelerate safety assessments of novel tobacco products. Furthermore, this shift from traditional analytics platforms may enable changes to our ways of working, creating possibilities for new ways of thinki–g, new skills and new opportunities.

ST 27

Medium/long term variability of moist smokeless tobacco products from the United States marketplace

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The goal of this work is to characterize temporal variability of moist smokeless tobacco (MST) products from the United States marketplace with respect to harmful and potentially harmful constituents (HPHCs) and selected physical measurements for specific brands procured at seven timepoints from the marketplace over a 3-year period. A total of 20 products are included in the study, representing five different manufacturers and 70 % of the U.S. MST market. To separate product variability from potential method temporal variability, the products were frozen immediately after procurement and analyzed together after all the samples were collected. This approach closely matched the approach taken by the CORESTA CVAR Task Force examining cigarette variability. The MST products were analyzed for NNN, NNK, NAT, NAB, nicotine, acetaldehyde, formaldehyde, crotonaldehyde, arsenic, cadmium, pH, portion weight for portioned products, and moisture. Generally, per product moisture, pH, and portion weights were quite similar across the seven samples. Moisture, for example, varied over a range of about 3 % of the product average value ((max-min)/avg). Similar to the CVAR Task Force findings, most of the tobacco-related HPHCs varied considerably over the 3-year period. For example, on average, NNK showed a range that was more than 80 % of the product's average. These wide variations in the HPHC values should be considered when comparing one smokeless tobacco product to another.

ST 28

Harmful and potentially harmful constituents in two novel nicotine pouch products in comparison with regular smokeless tobacco products and pharmaceutical nicotine replacement therapy products

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Tobacco-free nicotine pouches, such as ZYN®, are relatively new products that may serve as a low-risk alternative to cigarettes or conventional, tobacco-based oral products among current tobacco users. In the absence of long-term epidemiological studies, chemical data may help to assess the potential for long-term health effects and to position ZYN® on the continuum of individual risk proposed for tobacco and nicotine-delivery products. The objective of this study was therefore to screen for and compare the presence of 43 compounds, including 36 harmful and potentially harmful constituents (HPHCs), in seven oral nicotine-delivery products. ZYN® (dry and moist), snus (pouched), two types of moist snuff (loose and pouched) and two pharmaceutical nicotine replacement therapy (NRT) products (lozenge and gum) were included in the study. The analyses were performed on triplicate samples and at external contract laboratories when possible.

The largest number (27) and generally the highest levels of HPHCs were quantified in the moist snuff products, which, for example, contained six out of the seven tested polycyclic aromatic hydrocarbons (PAHs), and seven out of the ten nitrosamines (including N'-nitrosonornicotine [NNN] and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK]).

A total of 19 compounds, none of which were PAHs, were quantified at low levels in the snus product. NNN and NNK levels were five to twelve-fold lower in snus compared to the moist snuff products.

Only low levels of acetaldehyde, ammonia, cadmium, chromium, lead, nickel, uranium-235, and uranium-238 were quantified in the NRT products.

The two ZYN® products contained no nitrosamines or PAHs but low levels of ammonia, chromium, formaldehyde, and nickel.

On a toxicant and individual risk continuum scale for nicotine delivery products, the current findings suggest that ZYN® should be classified close to NRT products.

ST 29

Determination of nicotine related impurities in nicotine pouches and tobacco containing products by liquid chromatography tandem mass spectrometry

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Nicotine-containing tobacco-free oral pouches have gained market shares in recent years. Instead of tobacco leaves they contain different non-tobacco fillers and nicotine. The long-term health effects of these products have not been established yet, but it is suggested that they are less harmful than cigarettes. The nicotine used in nicotine pouches usually comes from the extraction of tobacco, thus, related alkaloids may be found as impurities at low levels. Moreover, due to exposure to high temperatures, humidity, light etc. degradation of nicotine can occur, giving rise to the formation of nicotine degradation products.

There are several methods to determine the levels of nicotine and its degradants, metabolites, and alkaloids in various matrices, however, there are no published or recommended methods for the analysis of nicotine degradants in nicotine pouches. The scope of this study has been to develop a sensitive and selective liquid chromatography tandem mass spectrometry method for simultaneous determination of seven nicotine related impurities. All seven analytes and corresponding deuterated internal standards are separated within 3.5 min, including 1 min equilibration. The method is fully validated, showing good linearity with correlation coefficients > 0.996 for all analytes, good extraction yields ranging from 78 - 110 %, limits of detection between 0.08 - 0.56 µg/g and limits of quantification between 0.27 - 2.04 µg/g. Although the method is mainly developed to determine degradants of nicotine in nicotine pouches, it is validated and performs well on a broader range of tobacco containing products. All the analytes are detected in varying concentrations in the different matrices; however, the analytes concentrations are lower in the nicotine pouch products, compared to tobacco containing matrices. This method can be useful for quality control purposes, e.g., to check the purity of nicotine, as well as for stability studies of nicotine pouches by monitoring nicotine degradation.

ST 30

Modified QuEChERS method for the extraction of nicotine from oral traditional and innovative tobacco products using UPLC-MS/MS

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As the tobacco industry continues to emphasize tobacco harm reduction, an array of innovative potentially reduced-risk products have emerged as alternatives to smoking cigarettes. These innovative products include a wide range of smoke-free products with different matrix types. This has resulted in the need for developing and validating multiple matrix-dependent analytical methods for the determination of nicotine, which can only be applied to specific matrix types. In this study, we developed and validated a novel method for the extraction and accurate quantitation of nicotine from a variety of commercially available tobacco products with differing matrices (e.g., pouched and loose traditional smokeless tobacco, lozenge, gum, nicotine pouch, and e-liquids). The method employed a modified QuEChERs extraction technique consisting of a liquid-liquid extraction using sodium hydroxide and acetonitrile in conjunction with UPLC-MS/MS using isotopically labeled internal standard, nicotine-methyl-d_3. The method was validated according to the International Council on Harmonization (ICH) guidelines and guidance from the Center for Tobacco Products (CTP) at the Food and Drug Administration (FDA) on validation of analytical methods used for tobacco products. For all sample types evaluated in the validation study, the recoveries using isotopically labeled internal standard were found to be within 89 to 109 % for fortification levels that were based on the inherent nicotine concentration for each sample matrix. Intra-day and inter-day method precisions were both determined to be ≤ 7 % relative standard deviation (%RSD). This analytical method has the advantage to reduce extraction and analysis time, is easy to implement and maintain, and is applicable to a wide range of smoke-free tobacco products. We believe this method has potential for standardization.

ST 31

Overcoming the challenges inherent in effective quality assurance for modern oral pouches

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Modern oral pouches are increasingly being explored by the industry as a reduced risk nicotine delivery platform. When compared with conventional cigarettes, the science of manufacturing quality assurance (QA) for such products is in its infancy.

The practical measurement of key quality parameters for modern oral products, such as weight and tensile strength, are often confounded by the pouch design which results in short- and long-term variability.

The study objective was to develop methodologies that provide practical tools for line side QA of modern oral pouch manufacture to drive manufacturing consistency of the making process.

To overcome measurements variability, a robotic measurement tool was created that allowed measurement of key parameters. Time based phenomena concerned with moisture loss could be investigated as could an alternative to CORESTA Recommended Methods for determining pouch seal strength.

High humectant content contributes significantly to changes in test pouch weight from 0.1 % to 1 % weight loss per minute when exposed to factory conditions (brand dependent). A methodology is proposed that minimises errors due to moisture loss.

The key seam tensile strength parameter when using the CORESTA Recommended Method No. 90 (CRM90) is a time-consuming process best conducted in a laboratory. Measuring the tensile strength of a complete, full pouch was explored. A comparison is presented between the two methods. For the test piece, CRM90 yielded a seam strength slightly greater than the “full pouch” method (4.21N vs 4.15N) with slightly better variability (SD N = 18 0.388 vs N = 34 0.453).

The limitations in terms of time and batch size for such an automated QA tool are explored, concluding that for QA purposes, a sample size “sweet spot” of between 5 and 10 pouches is ideal for immediate sampling and analysis.

ST 32

Toxicology risk assessment: an approach for controlling the health risk of electronic cigarette

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Many harmful substances may be produced during the use of e-cigarettes, even though their levels may be lower than in cigarette smoke. These toxicants can potentially cause negative effects on human health, such as carcinogenicity, teratogenicity or reprotoxicity. The Food and Drug Administration (FDA) has established a list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke as required by the Federal Food, Drug, and Cosmetic Act (the FD&C Act). However, there is no specific guidance or regulation to define what levels in the aerosol can be acceptable for these substances in health risk assessments of tobacco products. Therefore, it is necessary to assess the human health risk and establish the toxicologically tolerable level for novel tobacco products such as e-cigarettes. The application of toxicological risk assessment (TRA) in evaluating the safety of e-cigarette products focuses on four parts. First, analysing the toxicological characteristics of the compound that consumers may be exposed to during the use of e-cigarettes and the toxicological characteristics of the different compounds after mixing. Second, combining with the potential exposure via reasonable and foreseeable lifetime usage of the product, assess potential health risks and establish tolerable exposure limits. Third, analysing the sources of substances of concern (e-liquid, thermal decomposition of e-liquid, extractables and leachables (E&L)), then control them to acceptable levels or replace them by other substances or disuse the source materials. Last, effectively combine physicochemical tests and biological tests to evaluate the health risks of e-cigarette aerosol systemically and scientifically. Through the application of this TRA framework, it ensures that the e-cigarette product minimises the human health risk to an acceptable level for use.

ST 33

Chemical analysis of selected harmful and potentially harmful constituents and *in vitro* toxicological evaluation of leading flavoured e-cigarette aerosols in Chinese market

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Adoption of electronic cigarettes (e-cigarettes) has increased significantly over the past decade due to consumer's perception that they may represent a safer alternative to combustible cigarettes (CS). E-liquids generally contain variable concentrations of vegetable glycerin (VG), propylene glycerol (PG), nicotine, organic acids, and a mix of flavouring additives. The harmful and potentially harmful constituents (HPHCs) are chemical compounds in e-cigarette aerosol or tobacco smoke that are required by regulators for control and monitoring. To quantify HPHCs in aerosols from commercial flavoured e-cigarettes in the Chinese market, a systematic study on the HPHCs levels, including carbonyls, other volatile organic compounds (VOCs), tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals from four leading market e-cigarettes with different flavours and a combustible cigarette mainstream smoke were analysed and compared under machine-puffed conditions. The results showed that the levels of the vast majority of the HPHCs in the e-cigarette aerosols were either below the level of detection or significantly lower than those in the commercial cigarettes and reported reference cigarette (3R4F) values. The neutral red uptake (NRU) and Ames reverse bacterial mutagenicity assays using aqueous extracts of the total particulate matter indicated that e-cigarette aerosols did not induce observable cytotoxicity and mutagenicity compared to those of CS. The levels of HPHC from these e-cigarette aerosols could lead to a significant reduction in exposure to these harmful substances for exclusive e-cigarette users, compared to CS. In tandem, the potential cytotoxicity and mutagenicity of the e-cigarettes might also be reduced although this will require further clinical validations.

ST 34

Extractables and leachables evaluation for the safety of materials in electronic cigarettes

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For e-cigarettes and medicinal products, the establishment of data-based safety thresholds for extractables and leachables (E&L) studies is helpful for making knowledge-based safety and risk assessments. As the inhalation route carries one of the highest safety concerns due to possible leachables from e-cigarette products, it could increase the risk of exposure-related respiratory and other diseases, systematically or locally. Hence, an E&L study is a requirement for ensuring the safety of materials in e-cigarette products. An E&L assessment framework for e-cigarettes products was established that has six parts. First, information collection on those materials directly in contact with the human body, aerosol generation during the device production and e-liquid processing. Second, design of appropriate or simulated extractables experiments covering types of solvents, extraction time and temperature based on the expected usage conditions of the product. Third, establishment of analytical evaluation thresholds (AET) through toxicological application safety thresholds. Fourth, selection of appropriate analytical techniques (e.g., instruments) for analysing extractables based on different chemical categories. Fifth, completion of qualitative and semi-quantitative analyses for those extractables above AET. Finally, the conduct of toxicological risk assessments based on regulatory guidance. Through this E&L approach, e-cigarette manufactures can identify the potential risks from leachables and select the suitable materials and processes to ensure the safety of e-cigarette products.

ST 35

Non-targeted analysis of ENDS – challenges, considerations and best practices

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There is a wide variety of e-cigarette devices and e-liquid formulations available on the consumer market. In addition to known toxicants, the identification of unknown compounds and estimation of their levels in electronic nicotine delivery system (ENDS) is critical for the assessment of potential user exposures. A set of non-targeted analysis (NTA) screening methods can help provide a comprehensive chemical characterization of the aerosol generated from ENDS products. However, care must be taken with sample preparation procedures, acquisition methods, and data processing to obtain reliable sample relevant results.

NTA data sets often contain experimental artifacts, many of which are introduced as part of the sample preparation process itself. An appropriate blank which has gone through the aerosol collection process is required to avoid overestimation of the number of chemical constituents in ENDS aerosol as a result of ambient air constituents. In addition, NTA data sets are information-rich and robust approaches are necessary to extract the relevant information from the vast amount of data generated. Sample-relevant compounds must be determined using multivariant techniques, as chemical contaminants that are captured by blanks may vary across data sets leading to misidentification of sample-relevant compounds.

This presentation will include comparison of results using appropriate and inappropriate NTA study design, including sample preparation and data processing for JUUL aerosol. The use of inadequate control and sample replicates resulted in up to 10× overestimation of the number of chemical constituents and insufficient cleaning measures resulted in sample cross-contamination. Additionally, different vendor consumables used for aerosol collection were determined to be additional sources for contamination, resulting in formation of sample irrelevant reaction products, such as long chain benzoates. It is necessary that NTA studies are conducted with attention to the appropriate controls and data handling for an accurate evaluation of ENDS aerosol.

ST 36

A 12-month stability study on JUUL2 Virginia tobacco and crisp menthol flavoured aerosols using targeted analytical methods

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The JUUL2® is a temperature-regulated, electronic nicotine delivery system (ENDS) designed to provide reduced-risk alternatives to adult smokers who would otherwise continue to smoke cigarettes. The JUUL2® System was launched within the United Kingdom in September 2021. The objective of this study was to characterize select chemicals and harmful and potentially harmful constituents (HPHCs) contained within the JUUL2® Virginia Tobacco and Crisp Menthol 18 mg/mL JUULpod aerosol over a 12-month period and to monitor resulting changes in chemistry and stability. Samples were manufactured, placed under long-term (25 °C / 60 % relative humidity [RH]) storage conditions, and removed from storage at 0, 3, 6, 9 and 12 months for analysis. Aerosols were collected using intense and non-intense puffing regimens and analysed for select chemicals and HPHCs based on the United States Food and Drug Administration Premarket Tobacco Application (USFDA; June 2019) and European Tobacco Products Directive (EUTPD; April 2014) guidance using validated methodologies. After 12-months of long-term storage, regardless of puffing regimen, the majority (36 out of 54) of the measured aerosol constituents were not present at levels above the limit of quantification. Nicotine, pH, water and other primary constituents remained consistent, exhibiting no more than a 20 % change from T0 levels. Thermal degradants increased slightly, and nicotine degradants remained below United States Pharmacopeia (USP) percent impurity limits. These results support at least a 12-month shelf-life for JUUL2® Virginia Tobacco and Crisp Menthol 18 mg/mL JUULpods.

ST 37

A 12-month stability study on JUUL menthol flavoured aerosols using two non-targeted analytical methods

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The United States Food and Drug Administration (FDA) Family Smoking Prevention and Tobacco Control Act, empowers the FDA to protect public health by regulating the manufacturing, distribution, and marketing of tobacco products. Beyond the analysis of target compounds, the FDA Premarket Tobacco Product Application guidance recommends that applicants evaluate chemical changes in their product over its shelf-life and provide complete product characterization. To address this, samples were aged using International Conference on Harmonization's climate zones II (25 °C ± 2 °C / 60 %RH ± 5 %RH) in their original packaging in environmental chambers. Aerosols were collected from three replicates, each from three production batches of JUUL Menthol 5.0 % nicotine by weight (Me5) using intense and non-intense puffing conditions and GC-MS and LC-MS non-targeted analysis methods were applied. The objectives of this study were to 1) thoroughly chemically characterize the aerosols over a 12-month stability study and monitor the changes in aerosol chemistry, and 2) compare the chemical complexity of aerosols to combustible cigarette smoke (CCS). Therefore, two non-targeted analytical methods were applied to initial aerosol (T=0); remaining samples were stored in their original packaging and aerosolized and analyzed after six months (T=6) and again after 12 months (T=12) of storage in their original packaging under the specified conditions. Trends from T=0 through T=12 show the formation of 16 additional chemical constituents compared to combined T=0 and T=6 results, which accounted for 0.009 % of the detected mass. After thoroughly evaluating Me5 for unique chemical constituents it was observed that the aerosols remained approximately 35-fold less chemically complex than CCS with only minimal chemical changes to the ENDS aerosols.

ST 38

Solvent-free squeezing extraction method to obtain higher concentrated test article formulation for e-cigarettes and heated tobacco products

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Many studies have been conducted on the chemical analysis and *in vitro* toxicological evaluation of aerosols from e-cigarettes and heated tobacco products (HTPs), indicating that they contain significantly fewer toxicants than cigarette smoke. Therefore, biological responses have not been confirmed in many assays, even at the highest concentration applied in *in vitro* testing, in accordance with the test guidelines. In contrast to traditional extraction by applying organic solvent to the particulate phase from cigarette smoke, a different approach was needed to formulate the more concentrated aerosol collected mass (ACM) from e-cigarettes and HTP aerosols. Accordingly, a solvent-free extraction method has been reported involving centrifugation of Cambridge filter pads (CFPs) on which ACM has been collected. In the present study, an alternative method to formulate ACM extract by squeezing several CFPs without extraction solvent was explored. After optimising the squeezing and centrifugation extraction methods without solvent, the main constituents and volatile or semi-volatile compounds in the ACM preparations from aerosols with different e-cigarette liquid matrix compositions were analysed. Furthermore, the extraction efficiency for each constituent obtained from CFPs using the two solvent-free methods and the traditional method were compared. Optimisation of the solvent-free extraction methods achieved a concentration almost ten times higher than that of the traditional method. No significant difference in the extracted efficiency of the main constituents of ACM was observed among the three extraction methods, regardless of the e-cigarette liquid matrix composition. However, chemical analysis showed that some volatile compounds exhibited significantly higher extraction efficiencies in the squeezing method than in the centrifugation method. Furthermore, application of the squeezing method to HTP ACM preparation found that the extraction efficiency was greatly affected by the polarity of the ACM constituents. These results demonstrated that our solvent-free squeezing method was applicable to formulating higher-concentration ACM from e-cigarettes, but not from HTPs.

ST 39

An analytical screening method to characterise changes of flavour ingredients in e-liquids

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E-cigarette users' sensory experience is greatly affected by the amount of flavour ingredients in e-liquids. Once an e-cigarette pod is opened, the volatilization of volatile flavour ingredients will result in the changes of perceived sensory stability and consumption experiences. Therefore it is important to investigate the flavour ingredients' reduction in pods. An analytical method was developed to determine the decrease of flavour ingredients. A grape flavour e-cigarette pod was selected as the sample for the study under two conditions, store mode and puffing mode. For store mode, pods were kept under normal storage situation for three days. For puffing mode, pods were vaped 200-250 puffs by a vaping machine in three consecutive days to simulate the average consumption. The e-liquids of the two modes were collected and diluted with methanol. Then a GC-MS method was applied for screening analysis and the main ingredients that might influence the consumption experiences were determined. The results showed that different classes of flavour chemicals had different variation trends under the two modes. Four esters (ethyl acetate, ethyl propionate, ethyl butyrate, ethyl 2-methylbutyrate) decreased significantly. The decreasing ratios were in the range of 41 % - 45 % under the store mode, and 64 % - 69 % under puffing mode, respectively. Based on those results, the formulation of e-liquid was modified through appropriate overdoses of these four esters. After formulation modification, the net promoter scores (NPS) rose from -10 % to 17 % (based on the feedbacks from 72 consumers' questionnaires for original formulation and 64 consumers' questionnaires for modified formulation). This method can be used to support the improvement of flavour stability and enhance the overall experiences of consumers.

ST 40

Non-target analysis of flavour ingredients in aerosols of electronic nicotine delivery systems by cryogenic trapping

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The flavour ingredients in aerosols of electronic nicotine delivery systems (ENDS) are mainly volatile or semi-volatile. In non-target analyses (NTA), the use of Cambridge filter pads for the trapping of volatile fraction in aerosols always causes low trapping rate and poor repeatability. In this study, a cryogenic trapping method for volatile aerosol collection was developed with glass collecting device treated in a cold (-100 °C) ethanol bath. The aerosols produced by a vaping machine entered the collecting device through a serpentine tube and then were cooled and collected on the inner tube wall. The collecting device was then rinsed with methanol to obtain the elutant. The flavour ingredients in the elutant were determined by GC-MS qualitatively and quantitatively. This method was validated using ethyl acetate as the recovery agent. The results showed that a satisfactory recovery rate (97 %) was obtained. For NTA, a tobacco flavour ENDS product was tested: aerosol samples from 100 puffs were generated by a vaping machine, the trapped aerosols were rinsed by 10 mL methanol. Then the methanol elutant was tested by GC-MS qualitatively and quantitatively. A total of 35 peaks (26 substances) were detected, and the concentrations of which ranged from 0.01-2926.32 µg/puff. This method is suitable for NTA and targeted analyses of ENDS aerosols. Compared with the method based on Cambridge filter pads, this method has the great advantage of detecting more components with low boiling points.

ST 41

An UPLC-MS/MS method for the determination of 11 amine compounds in electronic cigarette liquids and aerosols

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In order to monitor potential amines in e-liquids and aerosols, a quantitative method for the determination of 11 amines was established. This method can also be used for quality control of e-cigarette products. 1.0 g of e-liquid was weighed into a 10 mL volumetric flask, followed by dilution with 50 % methanol to obtain a test sample solution with a concentration of 100 mg/mL. A calibrated aerosol generating machine was used to produce aerosol at 3 s puff duration, 55 mL puff volume and 30 s puff interval. The e-cigarette device used was a RELX Pod Pro type, with 6.5W. After 100 puffs, the aerosol was captured by a glass fibre filter (GF), which was put into a flask containing 10 mL of extraction solvent (50 % methanol), then the flask was shaken at 200 rpm for 20 min.

The e-liquid sample solution and aerosol sample solution were filtered through a 0.22 µm filter membrane, and then UPLC-MS/MS was applied for determination. The results of e-liquids and aerosols showed that the linearities were good when the concentrations of 11 amine compounds were in the range of 0.1 – 5 ng /mL (R^2 = 0.993 - 0.999). RSDs in the repeatable test solution ranged from 1.3 % - 7.4 %. The recoveries of spiked solution were between 88.5 % - 102.6 %. The samples had good stability when they were under different storage conditions. This method is rapid, specific, and stable for detection of 11 amine compounds in e-liquids and aerosols, and it is suitable for quality control of e-cigarette products.

ST 42

An UPLC-MS/MS method for the determination of 11 carbonyl compounds in electronic cigarette liquids and aerosols

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A UPLC-MS/MS method was developed for the determination of 11 aldehydes and ketones in electronic cigarette liquid (e-liquid) and aerosol. 1.0 g e-liquid was weighed into a 10 mL volumetric flask, and 5.0 ml DNPH solution with concentration of 1.5 g/L was added, then diluted with 50 % acetonitrile to obtain 10 mL solution. A calibrated aerosol generating machine was used to produce aerosol at 3 s puff duration, 55 mL puff volume and 30 s puff interval. Two impingers were connected in series to collect 100 puffs of aerosol at room temperature. The e-cigarette device used was a RELX Pod Pro type with 6.5W. The e-liquids sample solution and aerosols sample solution were detected by UPLC-MS/MS and the analytical method was evaluated. The linearities of 11 compounds were satisfactory (R^2 = 0.990 - 0.999) when the concentrations were in the range of 0.002 - 0.1 µg/mL. And RSDs of 11 components in the precision test solution ranged from 1.6 % to 3.4 %. The recoveries of spiked solutions were 75.0 % ~ 119.0 %. The results showed that this method is rapid, specific, and stable, and can be applied for the detection of 11 carbonyl compounds in e-liquids and aerosols. Therefore it is also suitable for routine quality control of e-cigarette products.

ST 43

Sicrit-Q-ToF-MS for on-line monitoring of nicotine and cooling agents in ENDS aerosol

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Nicotine and other main components in the aerosols of electronic nicotine delivery systems (ENDS) are typically measured by GC-MS following machine-generated aerosol collection by Cambridge filter pads in fixed puff blocks, which represent the total amount of nicotine and other analytes for a given number of puffs and cannot characterise the variation of a component release in each puff of the aerosol. In order to study the release of various e-liquids substances of with a puff-resolved resolution, an online monitoring method for nicotine and typical cooling agents in the ENDS aerosol was developed. A RELX Pod Pro type e-cigarette device with 6.5W was used and the aerosol was produced by a calibrated aerosol generating machine at the puffing condition of 3 s puff duration, 55 mL puff volume and 30 s puff interval. A T-piece adaptor was connected to the outlet end of the aerosol generating machine (for air stream splitting, the splitting ratio was set at 25:1). The minor fraction of the puffing air stream was directly imported into a soft Ionization by chemical reaction in transfer (SICRIT) ion source for online ionization, and followed by a time-of-flight separation and mass spectrometry detection (TOF-MS). This online monitoring method could qualitatively and semi-quantitatively monitor a range of known aerosol components in the aerosol in real time without any other pre-treatment. The puff-resolved results of nicotine, flavour cooling agents such as WS-23 and WS-3 showed that their relative release behaviour were linked to the aerosolization mechanism. For these substances, a fluctuation range of ± 10 % could be obtained when compared with other quantitative methods. This online monitoring method is useful to study the dynamic aerosol formation during ENDS usage.

ST 44

Comparison of the toxicological potential of JUUL VT3 and ME3 ENDS products to reference cigarette 3R4F and filtered air in a 90-day OECD 413 nose-only inhalation toxicity study

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Electronic nicotine delivery systems (ENDS) that deliver nicotine via the inhalation route could serve as a viable alternative to combustible cigarettes. The JUUL System is a closed, cartridge-based ENDS, which comprises of two main components: a JUUL device and pre-filled JUUL pods (JUUL ENDS products).

The purpose of this work was to evaluate the potential toxicity of two JUUL ENDS products: one pre-filled with menthol flavor e-liquid at 3.0 % nicotine concentration (ME3) and the other with Virginia tobacco flavor e-liquid at 3.0 % nicotine concentration (VT3) compared to the reference cigarette (3R4F) and filtered air in a nose-only inhalation study using Sprague Dawley rats following OECD 413 guidelines.

Animals were exposed for 90 days to target mean aerosol concentrations of 0 µg/L (filtered air) for six hours, 250 µg/L 3R4F for six hours, or 1400 ug/L VT3 or ME3 for two, four and six hours, followed by a six-week recovery period.

Reductions in mean body weight gains during exposures were limited to males and more pronounced in 3R4F than in VT3- and ME3-exposed rats. Signs of respiratory inflammation observed in 3R4F group in both males and females at the end of exposure included elevated lactate dehydrogenase, pro-inflammatory cytokines, and neutrophils in bronchoalveolar lavage fluid with minimal to marked histopathological changes observed in the nose, turbinates, larynx and lungs; histopathological changes persisted at the end of recovery. In contrast, the findings in VT3- and ME3-exposed animals were generally limited to minimal to mild squamous metaplasia of the larynx which resolved in all except one mid-dose ME3-exposed female.

In conclusion, despite 5- to 6-fold higher mean aerosol concentrations, 90-day exposures to JUUL ME3 and VT3 product aerosols resulted in significantly less toxicity, including in the respiratory system, compared to 3R4F aerosols.

ST 45

Biological and proteomic changes in C57BL/6 mice after 10 weeks of inhalation of tobacco cigarette and electronic cigarette

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Electronic cigarettes (e-cigarettes) have been widely considered as an alternative to combustible cigarettes. However, the effects and mechanisms of e-cigarette aerosol on respiratory system function need to be fully elucidated, and *in vivo* studies can be used to evaluate e-cigarettes aerosol (ECA) vs. combustible cigarette smoke (CCS).

Thirty-two specific pathogen-free male C57BL/6 mice (eight weeks old) were randomly divided into four groups, including the air exposure (sham) group, an ECA low-dose (ECAL) group, an ECA high-dose (ECAH) group, and a CCS exposure group. Mice were orally and nasally exposed to the corresponding CCS and ECA, and compared with the differences induced in the mouse model. Changes in the trachea, lungs, tissue inflammation levels, and proteome of the lung were measured and analysed.

It was found that CCS exposure resulted in pathological changes in the airways and lungs, with decreased lung functions and increased levels of inflammation, whereas the ECAS exposure produced significantly less effects at equivalent nicotine levels. Proteomic analysis showed that CCS had more differentially expressed genes and complex regulatory mechanisms relative to ECAL and ECAH.

In this study different nicotine dose levels were selected and conducted a 10-week subchronic inhalation toxicity experiment was conducted. The results support the hypothesis that e-cigarettes aerosols have less harmful effects on the respiratory system than combustible cigarette smoke at the same nicotine dose under the animal model used, providing additional evidence for the relative safety of e-cigarettes.

ST 46

Comparison of *in vitro* human alveolar macrophage responses after exposure to cigarette smoke and e-liquids

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Alveolar macrophages are the first line of defence against inhaled substances/particulates in the respiratory airways and determine both short- and long-term lung responses. Both cigarette smoke (CS) and e-liquid products are established to deposit in the alveolar region, but there is limited understanding, particularly regarding e-liquids on their interaction with alveolar macrophages and their potential to generate adverse, toxicological or immunological responses. The objectives of this work were to compare the responses of alveolar macrophages exposed to CS and e-liquid products and evaluate if the phenotypic changes observed could assess their safety and/or identify a mechanism of toxicity. ImmuONE™ (human alveolar macrophages) and ImmuLUNG™ (human alveolar macrophage-epithelial co-culture) were provided by ImmuONE Ltd (Hatfield, UK). Both *in vitro* models were exposed to culture medium containing CS or e-liquid for up to 48 h (n = 12). Alveolar macrophages were assessed by high content image analysis and cell health and morphology parameters determined. Distinct phenotypic changes indicative of autophagy (reduced mitochondrial activity, increased cell size and vacuolation) were observed for cells exposed to CS in both mono- and co-culture models. In the presence of epithelial cells (ImmuLUNG™), macrophages were more sensitive to CS-induced changes. In contrast, the cell phenotypes observed after exposure to e-liquids were more in line with the untreated control indicating different mechanisms for how CS and e-liquids interact with alveolar macrophages. This technique demonstrates how a high content analysis approach can be applied *in vitro* to identify morphological changes in cells to provide a mechanistic-driven approach to inhaled safety and to further understand the differences in how human lungs respond differently to the inhalation of CS and e-liquids.

ST 47

A 3D *in vitro* electronic-cigarette flavours testing strategy using cigarette smoke as context

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The safety of vaping and its potential as a harm reduction alternative to smoking is currently being discussed, as is the role of flavours. Here we discuss an optimised approach for the assessment of e-cigarette aerosols using 3D reconstituted airway tissues (MucilAir), coupled with an LM4E Borgwaldt Aerosol generator. The optimised approach yields greater potential for differentiating between e-cigarette products with increased functional endpoints for more lung disease specific measures, higher throughput (due to decreased exposure times) and more human relevance (when comparing to average daily consumption). A 2D *in vitro* approach was used to screen e-liquids, whilst selected flavours were assessed in an optimised physiologically accurate and challenging 3D (MucilAir) whole aerosol exposure model. To contextualise vapour aerosol responses and to avoid misrepresenting these, we compared the vapour responses to those induced by cigarette smoke (1R6F research cigarette) and calculated the percentage reduction using a point of departure approach. We demonstrate that the toxicology of the most complex flavours remains significantly reduced when compared to cigarette smoke toxicity.

ST 48

A weight of evidence review on the potential acute and chronic risks of e-cigarette use

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In order to assess the potential acute and long-term effects associated with e-cigarette use and to guide future assessments, a comprehensive review of the literature was conducted, focusing on the potential harmful effects of e-cigarette use, more specifically, *in vitro*, *in vivo* and clinical studies were assessed.

The literature results show a compelling story. Over 14,000 e-cigarette manuscripts from 2016 onwards were identified upon an initial search. Once refined using targeting information, 300 relevant manuscripts were identified and grouped. Only 13 % of these manuscripts proposed that e-cigarette use is comparable, more hazardous or represents a new hazard compared to traditional cigarette smoking. The remainder (87 %) all present a consistent story; in that they substantiate e-cigarette use to be significantly less hazardous than cigarette smoking or that no risk was identified at all in their use. The latter is consistent with the e-cigarette position in the risk continuum of nicotine products, as being substantially less risky than cigarette smoking.

Inconsistencies in reporting, lack of dosimetry/chemical analysis of test articles, variable smoking regimens, lack of cigarette smoke context and specific details of e-liquid/aerosol formulations, ultimately limited to overall use and application of many of the reviewed datasets. Data suggests that addressing these study reporting inconsistencies would yield more powerful and applicable datasets on which appropriate conclusions can be drawn, thus limiting the overall misrepresentation of study outcomes.

ST 49

The use of ToxTracker for the toxicological assessment of tobacco and nicotine delivery products

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The reduced risk mandate in the field of nicotine delivery has driven innovation and expansion in new technologies which requires rapid toxicological assessment to keep pace. These new products (e.g., electronic cigarettes and tobacco heating products), vary greatly in vapour/smoke composition and bring about new toxicological assessment challenges.

To address the demand for increased throughput and modernised testing approaches, we have employed the genotoxicity screening platform ToxTracker®, to assess biomarkers of DNA damage, protein misfolding, oxidative and cellular stress, across the categories of cigarette (1R6F), tobacco heating product (THP1.4) and electronic cigarette (ePen3). We have also used two common extraction matrices for each product; gas vapour phase (GVP) and aqueous extracts (AqE).

Our data demonstrated a significant reduction in biomarkers of cellular (Btg2) and oxidative stress (Blvrb & Srnx1) and cytotoxicity for THP1.4 compared to cigarette (> 72 % reduction oxidative stress, > 59 % cellular stress and > 75 % cytotoxicity), with further reductions for ePen3 over 1R6F (> 97 % reduction oxidative and cellular stress, > 95 % cytotoxicity), when normalised for nicotine. We also demonstrated that while GVP and AqE responded equally across endpoints for the reference combustible cigarette, GVP had significantly less potency than AqE for THP (> 45 %) and ePen3.

Our data support a reduction in biological effect consistent with a potential harm reduction of alternative nicotine delivery technologies when compared with traditional cigarettes. As we aim to provide products with increasingly reduced toxicant profiles in the future, we should be mindful of the make-up of the smoke/vapour/aerosol from the different product categories, as the relative contributions of the vapour and particular phases can drastically vary between them. Test matrices should be used alone or in combination to facilitate assessment of both the particulate and vapour phases (e.g., GVP in combination with total particulate matter (TPM) preparations), with consideration on what is most appropriate for the product categories being evaluated and compared.

ST 50

In vitro toxicology assessment of ZYN®, oral nicotine pouches

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Nicotine pouches (NPs) are novel, tobacco-leaf free oral products. Swedish Match ZYN® products appear in different versions, having different nicotine strength, moisture, and ingredients. ZYN® extracts were evaluated to assess potential cytotoxic, mutagenic, and genotoxic response in neutral red uptake (NRU), Ames, and *in vitro* micronucleus (ivMN) assays respectively, in compliance with good laboratory practice (GLP). Additional mechanistic assessment of genotoxicity was done using ToxTracker®, a mammalian stem cell-based GFP-tagged reporter assay representing DNA damage, oxidative stress, protein damage and cellular stress. Study products for the GLP assays were two versions ZYN® (flavoured and unflavoured ZYN®), tobacco reference products were moist snuff (CRP2.1) and total particulate matter (TPM) from Kentucky Reference Cigarette 1R6F. The ZYN® products demonstrated non-mutagenic, non-genotoxic, and non-cytotoxic responses. The reference TPM extract displayed cytotoxic, mutagenic, and genotoxic responses, whilst reference moist snuff only revealed a cytotoxic response. Different varieties of ZYN® Peppermint, Nicorette Peppermint (NRT), a Swedish Snus reference product (CRP1.1) as well as nicotine and TPM extract were included in the ToxTracker® study. Five non-cytotoxic concentrations were tested and induction of the GFP reporters was determined after 24 h exposure. None of the oral products were induced DNA-damage (direct genotoxic). The ZYN® products and NRT were negative in all other assays, whilst CRP1.1 showed (weak) positive responses. Cigarette TPM was positive for all tested cellular responses. Overall, based on the results from the combination of *in vitro* assays it could be concluded that ZYN® has a similar toxicological profile as NRT product, and an improved toxicological profile compared to TPM and smokeless tobacco. These findings support that oral nicotine products display a reduced risk profile compared to traditional tobacco products.

ST 51

Comparative study of a 24-well miniaturised Ames test vs the standard Ames test for mutagenicity assessment of tobacco products

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Ames tests have been widely used to determine the mutagenicity of chemicals and tobacco smoke/vapor. Although the test can easily evaluate the mutagenicity, it takes some time to obtain data in accordance with authorised testing guidelines because of the variety of strains required. Recently, a retrospective analysis of the miniaturised Ames test (MAT), which uses a smaller format than the standard Ames test (SAT), was conducted by an OECD working group. However, there is still little knowledge regarding the sensitivity for cigarette smoke extracts and the accuracy of quantitative assessment with the MAT. The purpose of this study was to assess whether the relative mutagenicity of tobacco products can be quantitatively evaluated using the MAT. An MAT using 24-well plates was conducted using the standard bacterial strains listed in OECD TG 471. To confirm the responsiveness and discriminatory ability to cigarette smoke extracts, the test was conducted using the Kentucky reference cigarettes (3R4F/1R6F). In conditions with a clear dose–response effect, the slope values, which are indices to evaluate the strength of mutagenicity, were calculated from the linear portion of the dose–response curve and their intraday variations were compared with those of the SAT. In addition, test cigarettes consisting of different types of tobacco leaf (i.e., 100 % Burley or flue-cured) with 3R4F/1R6F were subjected to both tests. In the MAT, a clear positive response was observed with 3R4F/1R6F under similar conditions (i.e., strain and metabolic activation) as in the SAT. Although the MAT showed a wider range of variability than the SAT with TA98 and TA100 with or without S9, the MAT could discriminate the different cigarettes as well as the SAT. In conclusion, the MAT can be applied to a comparative evaluation of cigarettes for screening purposes, although with slightly less discriminatory power than the SAT.

ST 52

Comparing the cytotoxic potential between e-cigarette aerosols and cigarette smoke

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To investigate *in vitro* cytotoxicity of electronic cigarette aerosol (ECA) and cigarette mainstream smoke (CS), we conducted cytotoxicity evaluations using a cell counting kit-8 (CCK-8). The ECA was generated using two flavours including a tobacco (ECT) and mung bean (ECM) flavour in combination of a RELX pod device. The *in vitro* assays included human lung epithelial cells (BEAS-2b), human liver epithelial cells (LO2), human oesophageal epithelial cells (HET-1a), human lung cancer cells (A549, H1299), human liver cancer cells (HepG2, Huh7) and human oesophageal cancer cells (KYSE-150). A broad range of effects on cell growth, cell morphology and cell cloning ability was evaluated.

The results showed that the aerosols of the ECT and ECM had a weak effect on cell activity, with IC_{50} values greater than 100 µg/mL for all the cell types except H1299 cells. In contrast CS significantly inhibited the cell growth, with IC_{50} values between 7.26 and 80.66 µg/mL, and the cell activity was less than 25 % or absent of growth at a concentration of 100 µg/mL. At 100 µg/mL, the effects of ECT and ECM on cell morphology showed weak cell shrinkage and exfoliation, while the CS caused severe cell shrinkage, exfoliation, accompanied by extensive cell particles and cell death. At 50 and 100 µg/mL, the ECT and ECM exposures showed a slight inhibition of cell cloning, while the CS showed 75 % or 100 % inhibition, respectively. The results showed that the ECT and ECM aerosols showed a weak inhibitory effect on cells, with low cytotoxicity and little effect on cell morphology and cloning ability.

In contrast, the CS exhibited a strong inhibitory effect on cells and significant cytotoxicity, which reduced or stopped cell morphological growth and cloning ability. The results showed that the cytotoxicity of the electronic cigarette aerosols was less than that of cigarette smoke using the laboratory *in vitro* methods.

ST 54

Comparison of heated tobacco product aerosol to cigarette smoke in human bronchial epithelial tissues using high content screening

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Laboratory studies have shown heated tobacco product (HTP) aerosols have substantially reduced numbers and levels of harmful and potentially harmful constituents (HPHCs) relative to combustible cigarette smoke. The biological impact of these aerosols needs to be determined to assess their potential impact on consumer health. In the present study we utilised high content screening (HCS) on normal human bronchial epithelial (NHBE) cells to measure the biological impact of a range of HTPs, and a reference cigarette (1R6F).

HTP aerosols and cigarette smoke were bubbled through a series of three impingers each containing 10 ml of PBS to capture components of the smoke/aerosol. Both product categories were puffed in accordance with ISO 20778 (55 ml, 30 sec interval, 2 sec puff duration), yielding aqueous extract concentrations of 1.8 puffs/ml for 1R6F and 4.8 puffs/ml for HTPs. Chemical characterisation quantified nicotine levels as a dosimetry marker. Cells were treated with increasing concentrations of PBS. NHBE cells were treated for four hours (two hours for glutathione (GSH) content) and 24 hours to investigate the impact on six endpoints (five for 24 hours), cell count, GSH content (representing oxidative stress), H2AX phosphorylation (double DNA strand breaks), c-jun phosphorylation (cell stress), cytochrome C release (Apoptosis) and NF-κB translocation (inflammatory and other pathways).

The 1R6F bubbled PBS, caused a significant dose dependent decrease in cell count and significantly altered γ-H2AX, NF-κB, p-c-Jun and GSH (at concentrations > 0.2 µg/ml nicotine). A partial overlap with endpoints induced by the HTP solutions was observed but at concentrations considerably higher (> 1.61 µg/ml nicotine higher than 1R6F). The fold change difference in responses as measured by minimum effective concentrations was between 1.89 - 10.38 fold higher for the HTP when compared to the 1R6F. These results add to the growing body of evidence that HTPs are likely to be considerably less harmful than combustible cigarettes and offer significant harm reduction potential for those adult smokers who choose to transition to these products as an alternative to continued smoking.

ST 55

Bridging fundamental cigarette combustion science to drive innovative heated tobacco products

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On a continuum of potentially reduced risk tobacco products, heated tobacco products (HTPs) represent an important novel tobacco category that is closer to smokers' ritual and sensory expectations. Commercial HTPs have set high intellectual property barriers on new and existing players wanting truly innovative HTP products. Digging deeper into fundamental tobacco thermophysics and thermochemistry can be a source of inspiration to drive better HTP performance. In this work, tobacco thermophysical principles in combustible cigarettes are explained and used to demonstrate the design behind a closed-ended HTP prototype. By bridging the combustion science established from a burning cigarette, this innovative HTP solution is able to enhance overall product performance on reducing toxicants while improving the deliveries of key sensory chemicals as compared to its open-ended equivalent. Specifically, the physics behind the air flow to release entrained aerosol constituents without undesirable cooling of the heated tobacco bed is demonstrated, and the effects on aerosol chemistry and particle size distribution are presented. In summary, innovative HTP designs can be achieved by tapping into decades of fundamental tobacco research that has been built on the basis of cigarette combustion science.

ST 56

A strategy to bridge between THP product variants using exemplar data

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Alternative tobacco and nicotine products, including tobacco heating products (THPs), vaping products and nicotine pouches, are undergoing extensive scientific evaluations to demonstrate their reduced risk compared to combustible cigarettes. At the same time, they also show a high pace of continuous innovation that is required to meet the consumer demands, to support their efforts in switching to these new alternatives.

Bridging constitutes a pragmatic approach to ensure that changes to the specifications of a product do not impact its reduced risk potential. According to this concept, after an extensive foundational dataset has been collected on a reference version of a product, future iterations of the same product can be benchmarked against this foundational dataset to ensure changes do not adversely affect its risk potential.

Here we explore a bridging concept using THPs as an example product category and how the risk potential of continuous product evolution and refinements can be effectively evaluated without re-establishing a foundational dataset. Using three exemplar datasets taken from the published literature, covering chemical emissions, whole aerosol *in vitro* assessment and 21st Century *in vitro* toxicology approaches, we demonstrate that bridging can be a consumer-centric method to assess product risk in an effective manner.

ST 57

Simulation study on the effect of perforation on mainstream smoke temperature in an heated tobacco product stick

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As with combustion cigarettes, the effect of perforation for a heated tobacco product (HTP) stick is considered important. It is noted that air ventilation rate is one of the key parameters in terms of tobacco design for both a combustion cigarette and an HTP stick. In case perforations played a role in reducing tar in a combustion cigarette, air ventilation in an HTP stick seems to play an important role in providing the users with an appropriate sense of warmth by lowering the temperature of the mainstream smoke by diluting the high temperature of the vaporised and aerosolized moisturizer components with outside air. In the present study, the effect of perforation in an HTP stick design was investigated in various ways using numerical simulation analysis. The effect on mainstream smoke was investigated by changing various physical properties such as air dilution rate, shape, size, number, and arrangement of perforations. The effect of the temperature profile was also examined numerically. It is hoped that this result will help understand the effect of perforation on the mainstream smoke temperature of an HTP stick.

ST 58

Numerical simulation of heat transfer processes and releases of key components in electrically heated tobacco products

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To establish a numerical model to describe the dynamic heat transfer processes and the releases of key components in the tobacco section of electrically heated tobacco products, the porous medium theory and tobacco reaction kinetics were combined to probe the heat transfer, aerosol generation and transfer, the behaviours of key aerosol constituents (moisture, glycerol and nicotine) from the heated tobacco rod. The model was established and solved numerically by employing computational fluid dynamic software (Fluent). The temperature variations inside the tobacco section and the residual contents of the key components after a given puff number were determined by the scaled model and experimentally measured by the electrically heated tobacco products. The experimental data showed satisfactory agreement with the simulated values. The average errors of the model simulations for the contents of moisture, glycerol and nicotine residuals in the tobacco section under a puff-by-puff basis were 5.02 %, 12.41 % and 19.97 %, respectively. The temperature for 76.51 % volume fraction of the tobacco materials was below 150 °C during the entire working session of the product, causing lower release rates of glycerol and nicotine. This numerical model can be used to assist the design optimisation for temperature programme regulation and tobacco ingredient formulations, and therefore helping the development and quality improvement of electrically heated tobacco products.

ST 59

Research on preparation technology of heated tobacco core material based on powder forming process

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Various types of tobacco materials including reconstituted tobacco have been used in heated tobacco products (HTPs), in order to match the system requirements and to satisfy consumer expectations. Given their unique properties, paper-making reconstituted tobacco (RT) and band-cast RT have been the most common selections as core materials in tobacco rods for the mainstream designs of HTP on the market. Following the fast growth of the HTP market, and to meet the increasingly diversified needs in product designing and manufacturing processes, a new type of RT is being manufactured by converting tobacco raw materials into ultrafine powder (UFP) and utilising it in the paper RT manufacturing process. In this study, samples of the UFP RT were analysed for their hot water soluble content, nicotine content, tobacco sheet density, thermal conductivity, thermal weight loss, tensile strength, and other properties. The analytical results were compared with those of band-cast RT and paper-making RT manufactured with the conventional process. For many of the analysed properties, the results of UFP RT lie between the other two. It indicates that the UFP RT appears to be a significantly different material. Prototype HTPs were produced and evaluated with different heating systems. The outcomes further suggest the new type of RT product can be a versatile and powerful tool for the design and development of HTPs, either individually or mixed with other tobacco materials including conventional paper-making RT and band-cast RT.

ST 60

Application of “cooling and low retention” filter rods made of cellulose acetate microspheres for heated tobacco products

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Decreasing cigarette smoke temperature while maintaining an adequate amount of smoke is important for the development of heated tobacco products and also one of the greatest challenges to filter rods design. A new type of porous filter rod with effects of cooling and low retention of components in the cigarette smoke was made with cellulose acetate microspheres by CelFX™ filter rod forming technology. The quality and processability of the new microsphere filter rods were investigated, and the effects of pressure drop and length of the filter rods on the temperature and components of cigarette smoke were studied. The results showed that: 1) The microsphere filter rods had a 3D porous structure, and their porosities were in the rage of 64 % - 68 % and pressure drops were lower than 3.0 Pa/mm with CV value of 5 %. The filter rods were resistant to thermal collapse during the puffing process. 2) Compared with a control sample (IQOS™), the smoke temperature of heated tobacco products with microsphere filter rods decreased by 3 °C and the amounts of nicotine released were similar. 3) Heated tobacco products with balanced smoke temperature and amount of smoke could be achieved by the optimisation of the filter rod structure through adjusting the unit pressure drop and length of microsphere filter rods. This study proposes a new type of porous filter rod and its application scheme, which provides references for the filter rod design and product development of heated tobacco products.

ST 61

A comparative study on delivery of nicotine, humectants and endogenous aroma constituents from reconstituted tobacco materials in granule and sheet form under heat-not-burn condition

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There are a great variety of heat-not-burn products (HnBs) available on the market. The heating devices are various and the tobacco materials in heating rods also come in different forms.

To determine the delivery of the nicotine and endogenous volatile aroma constituents from different material forms, two different forms of reconstituted tobacco materials (A: granule and B: sheet) were prepared using the same tobacco leaf blend, and the same flavour formula was designed to ensure a similar content of humectants and tobaccos. Nicotine, humectants and aroma constituents in prepared material were quantitatively determined. Tobacco rods filled with either reconstituted tobacco materials form A or B were heated by the same device with a central heating blade, and emissions were generated by linear smoking machine. Nicotine, humectants and aroma constituents in emissions were collected by Cambridge filter pad and quantitatively determined. Puff-by-puff analysis was carried out to study delivery of the compounds of interest on puff basis.

The total delivery of nicotine from A into emission was higher (58.9 %) than that of B (49.8 %). The delivery of propyl glycol and glycerin was also found to be higher from material A (A:39.2 %, 5.1 % vs B: 17.9 %, 3.8 %). More endogenous volatile aroma compound types were detected in emissions from material A too, which were 38 compared with 33 different types from material B. The material form A also showed 10 % higher delivery of the amount of volatile aroma compounds compared with material form B. Puff-by-puff analysis showed that nicotine delivery from material B was much more stable compared with material A.

It was observed that under the central heating system, the form of reconstituted tobacco materials is a critical factor that affects delivery of nicotine, humectants, and endogenous aroma compounds. Findings of the present study provided useful information for development and optimisation tobacco materials for HnB use.

ST 62

Studies on analytical method and transfer rate for minor alkaloids in tobacco filler and aerosol of heated tobacco products by GC-MS

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There are various types of alkaloids in tobacco such as nicotine, nornicotine, myosmine, anabasine, anatabine, ß-nornicotyrine, cotinine and ß-nicotyrine. Among tobacco alkaloids, nicotine accounts for approximately 95 % of total alkaloid content. Other alkaloid compounds have only 5 % of total alkaloids. Despite the small amount, these minor alkaloids can affect smoke quality of tobacco and can be precursors of tobacco specific nitrosoamines (TSNAs). Accordingly, it is necessary to determine and control contents of minor tobacco alkaloids. This study focused on heated tobacco products (HTPs) that are showing a rapid market growth since they were introduced in 2017. For a few years, research related to HTPs has been performed in many institutes, however, less attention was paid to minor tobacco alkaloids of HTPs than to other constituents. Therefore, the aim of this research was to develop and validate the analytical method for minor tobacco alkaloids in the tobacco filler and aerosol of heated tobacco product based on the CORESTA Recommended Method No. 87 (CRM87) using GC-MS. All calibration curves were linear ($R^2 > 0.99$) within the range, and good recovery (85 % ~ 120 %) and repeatability (S.D. < 20 %) were obtained for all alkaloid compounds. Subsequently, using this validated method, the concentration of minor alkaloids in tobacco filler and aerosol of HTPs was determined, and the transfer rate (%) from tobacco filler to aerosol of HTPs was investigated. These data could be used for minor alkaloid related quality control of heated tobacco products.

ST 63

Development of an analytical method for carbonyls in aerosol of aHTP

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Unlike an electrically heated tobacco product (eHTP), an aerosol heated tobacco product (aHTP) includes a liquid cartridge. ISO 21160, which is a standard method well known for analysing carbonyls in aerosol of combusted cigarettes by 2,4-dinitrophenyl-hydrazine (2,4-DNPH) reagent after trapping the aerosol in an impinger. However, ISO 21160 is not optimised for analysing carbonyls in the aerosol of aHTPs but only in combusted cigarettes. Therefore, in this study, the ISO 21160 method (only impinger) was compared to the trapping method of the Cambridge filter pad (particulate matter) with impinger (gas phase) to establish an optimised method for carbonyls in the aerosol of aHTPs. In addition, 2,4-DNPH and 2,4-DNPH HCl reagents were used in order to compare the degree of the carbonyl derivatisation effect in aerosol. For aHTP, formaldehyde was collected more in Cambridge filter pad than in the impinger, whereas other carbonyls were mostly collected in the impinger. Also, no significant overall differences between 2,4-DNPH and 2,4-DNPH HCl in NGP carbonyls were found.

In conclusion, the combined trapping method of particulate matter with gas phase is suitable for analysing carbonyls in the aerosol of aHTP and both derivatisation reagents could be used in aHTP carbonyls analysis.

ST 64

Development and validation of a GC-MS method for the analysis of PAHs in heated tobacco product aerosols

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Heated tobacco products (HTPs) are designed to deliver a tobacco/nicotine-containing aerosol with significantly reduced toxicant exposure for users relative to combustible cigarettes. polycyclic aromatic hydrocarbons (PAHs) are a diverse range of organic compounds containing two or more fused aromatic rings. PAHs are known inducers of drug-metabolising enzymes, which are present in cigarette smoke due to incomplete combustion of tobacco matter. Sixteen PAHs are included on the U.S. Food & Drug Administration (FDA) list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke. FDA PMTA/MRTP application regulatory pathways for new tobacco products require manufacturers to submit robust product characterisation data to demonstrate a relative reduction in PAH toxicant exposure for smokers switching to “reduced-risk” HTP.

The development and validation of a sensitive and selective gas chromatography with mass spectrometry (GC-MS) method for the quantitative determination of 17 PAHs in HTP aerosol is reported. The work was partially based on a Hall Analytical in-house PAH method that has been successfully adapted for HTP aerosol analysis.

Generated eHTP aerosols were trapped in 8 - 12 puff collections, 2 second durations, 55 mL puff volumes, 30 s puff intervals, bell curve profiles. The tobacco aerosol generation was performed on a Cerulean SM450e puffing machine set up for HTP analysis with Cambridge filter pad aerosol collection. The chromatographic method achieves limits of quantitation (LOQ) of 1 ng/mL (in solution) for benzo[a]pyrene, with a working range of 1 - 250 ng/mL for target PAHs.

The method has demonstrated a suitable degree of accuracy, precision, and robust quantitation. Initial experimental results obtained from commercially available eHTP demonstrate a significant reduction in PAH levels compared with the literature data for a 3R4F reference cigarette.

ST 65

Application of TD-GC×GC-TOFMS for the comparison of the emissions profiles of non-tobacco based substrates in tobacco heating systems

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One of the greatest advances in the field of gas chromatography has been the development of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS). In contrast to conventional one-dimensional chromatography, GC×GC allows for a greater peak capacity and greater separation of coeluting compounds. GC×GC-TOFMS is a powerful tool and has been applied to the untargeted screening of different types of matrices including tobacco smoke, e-cigarette and heated tobacco products (HTPs) emissions. Recently, non-tobacco based substrates for use in tobacco heating systems (THS) have come onto the market. The application of untargeted screening by GC×GC-TOFMS to this type of substrates is not documented in the literature. To address this gap, the aim of this study was to compare the 2D chromatographic emissions profiles of six different commercial non-tobacco based heat-not-burn (HnB) consumables with a eHTP as a control. The aerosol of the six non-tobacco based samples and the control was generated on a Borgwaldt RMD20D smoking machine using the ISO intense regime (55/2/30), bell shape, 1 puff, no vent blocking. The emissions were collected in triplicate on thermal desorption (TD) tubes (Markes®, UK) and analysed by GC×GC-TOFMS. Mass spectrometry data deconvolution, integration and sample comparison was done using ChromSpace® and the advanced chemometrics ChromCompare+® platform (SepSolve®, UK). A thousand peaks/compounds were found with a library mass spectral match = 750 and of those, 21 compounds were detected in all sample replicates in addition to glycerol, propylene glycol and nicotine. Menthol and other flavouring compounds were detected only in some samples. These results are important to understand the chemistry of non-tobacco based consumables and provided information that advanced analytical techniques such as TD-GC×GC-TOFMS combined with innovative data handling platforms are a valuable screening tool for the chemical characterisation of HTPs and can be used for non-tobacco based HnB emissions.

ST 66

Rapid determination of nicotine content of reconstituted tobacco based on near-infrared spectroscopy technology

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Heat-not-burn tobacco with an external heating source is a cleaner alternative to conventional cigarettes due to its lower emission of nicotine, CO and tar in the smoke. Reconstituted tobacco is one of the most important components of heat-not-burn tobacco. The nicotine content of the reconstituted tobacco determines the flavour and the safety of the heat-not-burn cigarette product. In order to improve the detecting efficiency of the nicotine content, a novel near-infrared spectroscopy (NIR) combined with extreme learning machine (ELM) algorithm was applied to build the prediction models. Compared to the traditional partial least squares (PLS) model and principal component regression (PCR) model, the experimental results showed that the ELM model could obtain the best results for prediction sets. It indicated that the models could be applied for the rapid and accurate determination of the value of the nicotine content of the reconstituted tobacco of heat-not-burn cigarette product. The method proposed lays a foundation for the further implementation of online analysis of the nicotine content and rapid determination of other quality parameters of reconstituted tobacco of heat-not-burn cigarette products.

ST 67

Ion-exclusion chromatography coupled to tandem mass spectrometry for quantification of multiple organic acids in tobacco and reduced-risk products

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Organic acids are used as preservatives, as well as aroma- and taste-additives, in traditional tobacco products. Recently these acids have also been used as additives in reduced risk products like e-cigarette liquids. The study of these compounds has been cumbersome, however, due to the poor retention and peak-shape observed in reversed phase or hydrophilic interaction chromatography and the limited usability of GC-MS. While ion chromatography is a viable option, specialised equipment might not be readily available in research labs, in addition conventional detectors lack the selectivity of mass spectrometric methods.

In this study the analysis of organic acids using standard HPLC-MS/MS instrumentation by coupling ion-exclusion liquid chromatography (IELC) to tandem mass spectrometry (MS/MS) is reported.

Two factors remained key during method development activities: (1) To obtain good retention the eluent pH must be below the respective organic acid's pKa value. (2) For optimal sensitivity the pH should be above the pKa value of the respective organic acid. By variation of different eluent additives, multiple IELC-MS/MS methods were developed to quantify twelve organic acids, namely acetic acid, butyric acid, citric acid, formic acid, glycolic acid, lactic acid, levulinic acid, malic acid, oxalic acid, pyruvic acid, succinic acid and tartaric acid in tobacco products and e-liquids. Detected acid concentrations ranged from < 1 µg/g to > 1000 µg/g for various product types, while essential method performance parameters, like Instrument LOQ (0.003 µg/ml to 16.5 µg/ml) and calibration ability (linear or quadratic) met our quality criteria. It was shown that IELC-MS/MS enables the sensitive detection of multiple organic acids in tobacco products and e-liquids without need for derivatisation or tedious sample preparation.

ST 68

Development of an analytical method for nitrogen compounds in mainstream cigarette smoke using GC-MS

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Pyridine-based nitrogen compounds are present in tobacco leaf and smoke. This study developed and validated a simultaneous analytical method for nitrogen compounds such as pyridine, 2-Picoline, 3-Picoline, 4-Picoline, 2,4-Lutidine, 2,5-Lutidine, 2,6-Lutidine, pyrrole, 3-Vinylpyridine and indole in mainstream smoke using gas chromatography-mass spectrometry (GC-MS). In validation of the analysis, nitrogen compounds were detected within 43 minutes using GC-MS and the correlation coefficient (R^2) of the calibration curve was 0.999 or higher, showing high linearity. All 10 nitrogen compounds obtained good recovery (83 % ~ 110 %) and repeatability (1.1 % ~ 10.5 %). In addition, the compounds with higher molecular weight (MW) were collected more in the Cambridge filter pad than in the impinger and among the similar MW were affected by vapour pressure. This study shows that this method would be applicable for selected nitrogen compounds in mainstream cigarette smoke. Furthermore, it is expected that the method could be extended to the analysis of other nitrogen compounds.

ST 69

An improved method for the determination of carbonyls in cigarette smoke and butyraldehyde co-elution

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Carbonyls, including formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde crotonaldehyde, methyl ethyl ketone, are included in the FDA established list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke. CORESTA published the Recommended Method No. 74 (CRM74), "Determination of selected carbonyls in mainstream cigarette smoke by HPLC" for the determination of carbonyls in cigarette smoke. CRM74 includes the seven aforementioned carbonyls that are HPHCs and also includes butyraldehyde, an inhalation irritant. CRM74 was later developed into ISO 21160:2018 and ISO 23922:2020 for non-intense and intense smoking, respectively. CRM74 and the ISO standards have a total run time of 45 minutes. We developed a high throughput method based on CRM74 with a run time of 10 minutes. This method uses ultra-high pressure liquid chromatography (UHPLC) with a sub-2 µm InfinityLab Poroshell 120 Bonus-RP analytical column. Data generated with our improved method were consistent with CRM74. All requirements for method validation were met. The repeatability for each analyte was ≤ 10.7 %, intermediate precision was ≤ 8.6 % over three days, linearity was demonstrated with a coefficient of determination of ≥ 0.999 for all analytes, and recoveries were 80 % to 109 % for 1R6F non-intense and CM8 intense samples. This optimized method significantly reduced instrumental run time. Most importantly, our study demonstrated that butyraldehyde, as reported with the standardized methods and our method, is actually a mixture of isomers that cannot be effectively separated. Based on our validation, we suggest reporting butyraldehyde as a mixture of butyraldehyde and iso-butyraldehyde when using CRM74 and the ISO standards.

ST 70

Evaluation on accuracy improvement of GC-MS determination of seven minor alkaloids in mainstream cigarette smoke by novel analyte protectants

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Tobacco minor alkaloids are important precursor substances of TSNAs. Therefore, their accurate quantifications are of great significance. In order to realise determinations of seven minor alkaloids in mainstream cigarette smoke with high accuracy and robustness, the compensation effects of novel suitable analyte protectants (APs) for matrix effects were investigated. Through comparing the heights and shapes of chromatographic peaks before and after the additions of APs in standard solutions prepared in CH_2Cl_2 and cigarette smoke solution, the compensation effects of 12 APs and their combinations on the matrix effects of seven minor alkaloids were evaluated, and the best combination of 2-(pyridine-3-yl) ethan-1-amine (2 mg/mL) + 1,2-decanediol (1 mg/mL) was identified. This AP combination could effectively improve the shapes and increase the heights (by 7 % - 337 %) of chromatographic peaks for standard solutions prepared in CH_2Cl_2 and cigarette smoke solution. Before the addition of this AP combination, slope ratios of calibration curves for the two types of standard solutions of seven target chemicals were 71.4 % - 159.8 %, while after the addition, the slope ratios were 87.4 % - 105.6 %, indicating this AP combination reduced the matrix difference between pure solvent and cigarette smoke solution. After the addition of the AP combination, the standard curves of solutions prepared in CH_2Cl_2 showed good linearity ($r^2 \geq 0.999$), the spiked recoveries were between 80.9 % - 119.6 %, and the inter- and intra-day precisions were between 1.5 % - 9.5 % and 3.1 % - 8.5 % respectively. Three commercial cigarette samples and one mixed standard solution were also tested under five different instrument working conditions to verify the intermediate precision of the method, and the results showed that the RSD values were higher (3.5 % - 25.4 %) without the AP combination and smaller (< 6.7 %) with the AP combination. Due to its high accuracy, precision, and robustness, this method has good application prospects.

ST 71

Analysis of six aromatic amines in the mainstream smoke of tobacco products

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Tobacco smoke is a complex mixture that contains thousands of compounds, including more than 60 carcinogens that were identified by the year 2000. Aromatic amines are a class of carcinogenic compounds in tobacco smoke that are listed on the FDA list of harmful and potentially harmful constituents (HPHCs). The objective of this study was to develop a method using solid-phase microextraction-coupled to gas chromatography-triple quadrupole mass spectrometry (SPME headspace GC-MS/MS) for the quantitative determination of six aromatic amines, including 1-aminonaphthalene (1-AN), 2-aminonaphthalene (2-AN), 3-aminobiphenyl (3-ABP), 4-aminobiphenyl (4-ABP), o-toluidine (o-TOL), and o-anisidine (o-ANI), in the mainstream smoke of cigarettes, cigars, and heated tobacco products. The method developed here combines high sensitivity with simple sample preparation and has demonstrated satisfactory linearity for all six aromatic amines with correlation coefficients greater than 0.9994. The limits of detection range and the limits of quantitation range were 12 - 96 pg/mL and 41 - 320 pg/mL, respectively. Their recoveries and coefficients of variation (CV %) were 90 - 112 % and 2.1 - 6.6 %, respectively. The new SPME headspace GC/MS/MS method has been successfully applied to measure the contents of the six aromatic amines in the mainstream smoke of cigarettes, cigars, and heated tobacco products.

ST 72

Analytical approach to replacing a number of sensory panel based tests

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BAT has started to investigate a move to chemical or physical analyses, migrating away from human sensory panels, in order to standardise measurements and have more consistent data.

With the Covid pandemic, BAT faced some challenges with carrying out face-to-face sensory assessments, as well as the on-going search for faster responses and easier decision-making processes. This led to the need to investigate correlations using advanced analytical techniques between human sensory panels and analytical approaches. This study aimed to search for chemical or physical analyses to replace some existing sensory panels. Therefore, different studies were performed aiming to replace two different panels: the capsule crushing panel used to evaluate the impact of aging of capsules on crush strength and the sniffing panel used to ensure the quality of flavours that are applied in cigarettes at the BAT Flavour Center.

For the capsule crushing panel, the physical analyses of deformation and strength to crush were performed using a compression testing machine. Using different cigarettes with capsules, the human sensory panel was conducted at different time points during the aging process, while physical analyses were performed on these same products. Using statistical tools, different combinations of the results were carried out to simulate the best fit for the correlation between the analytical approach and the results of the human sensory panel.

For the sniffing panel, the analytical approach chosen was Infrared (MIR). The chemical fingerprint of different flavour mixtures was analysed and, using chemometric and machine learning techniques, some specifications were created to set the limits for quality control for concentration, composition and cross-contamination deviations. If the samples are within the limits, it is not necessary to perform analyses in the sniffing panel.

As a result, it was possible to replace the sensory panels (one in a partial way) with the analytical approaches tested.

ST 73

Quantitative determination of 23 flavour compounds related to potential additives in tobacco products and regulatory reporting requirements

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In 2018, the National Agency of Health Oversight in Brazil (ANVISA) set forth provisions for the reporting of 165 compounds in tobacco products, to address potential attractiveness or enhanced addictiveness of these products. The objective of this study was to develop an approach for the determination of multiple compounds, suitable for 'routine' analysis with 'adequate' method performance characteristics (i.e., 'fit for purpose'). This study describes the quantitative determination of 23 of these compounds using gas chromatography - mass spectrometry (GC-MS).

The methodology used two grams of ground tobacco product extracted with methyl tert-butyl ether (MTBE) and a saturated potassium chloride (KCl) aqueous solution. An internal standard solution containing seven deuterated compounds, was added prior to the extraction. After extraction and phase separation, an aliquot of the organic phase (MTBE) is used for GC-MS analysis. The analysis was performed using a 30 m DB-17MS column and single ion monitoring (SIM) data acquisition mode. Compound identification was done by comparing the retention times, quantifier and qualifier ratios, to those of standard reference materials. For positive identification, at least one of the qualifier ions must be within 50 - 150 % of the target ratio, and no qualifier ions below 50 % of the target ration.

Calibrations were prepared using calibration standards ranging from 0.05 µg/mL to 10 µg/mL, with a nominal internal standard concentration of 1 µg/mL. Recoveries of all target compounds from a laboratory fortified matrix (LFM) were within 79 - 119 %, with one exception for (E)-Hexen-2-al (69 %). The LFM samples were also used to evaluate method precision with the coefficients of variation (CV) for all analytes ≤ 15.

A suitable method for the quantification of 23 compounds with adequate sensitivity has been developed. However, the method does not differentiate between compounds naturally present in tobacco, those chemically bound to the matrix, and those that might have been added.

ST 74

Quantitative determination of 28 flavour compounds (lactones and pyrazines) related to potential additives in tobacco products

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Many regulatory bodies require or will require the quantification of various flavour components in tobacco products addressing potential attractiveness or enhanced addictiveness issues related to these products. The objective of this study was to develop 'fit for purpose' methodology for the determination of a series of lactone and pyrazine flavour components. This study describes the quantitative determination of 28 compounds using gas chromatography - mass spectrometry (GC-MS).

The methodology used two grams of ground tobacco extracted with methyl tert-butyl ether (MTBE), a saturated potassium chloride (KCl) aqueous solution and internal standard solution containing four deuterated compounds (plus 4,4'-Dibromobiphenyl). After extraction and phase separation, an aliquot of the organic phase (MTBE) was used for GC-MS analysis using a 30 m DB-17MS column and single ion monitoring (SIM) data acquisition mode.

The methodology had many challenges related to commercially available reference materials limited to a mixture of isomers (e.g., 2-ethyl-3,5(or 6)-dimethyl pyrazine and 6,10-Dimethyl-5,9-undecadien-2-one). A change in the instrument response was also observed for some compounds (4-Vinyl guaiacol, trans-β-ionone, isoamyl phenylacetate and sclareolide), requiring a 'bracketing standards' approach for quantitation. Calibrations were prepared using standards ranging from 0.05 µg/mL to 10 µg/mL. However, a low S/N ratio for (-)-β-Citronellol, and poor level recoveries caused by matrix effects for γ-Valerolactone, required the lowest standard be 0.10 µg/mL. Recoveries of all target compounds from laboratory fortified matrix (LFM) at three different levels of fortification were within 79.5 - 123 %, except γ-Valerolactone (55 - 62 %) related to the matrix effects. The LFM samples were also used to evaluate method precision with the coefficients of variation (CV) for all analytes ≤ 15.

A suitable method for the quantification of 28 compounds with adequate sensitivity has been developed. However, the method does not differentiate between compounds naturally present in tobacco, those chemically bound to the matrix, and those that might have been added.

ST 75

Waterpipe bowls and heaters: does the ISO standard reflect what is available to consumers of waterpipe tobaccos?

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The waterpipe bowl and electric heater specified in ISO 22486:2019 “Waterpipe tobacco smoking machine — Definitions and standard conditions” are not typical of those available to users of waterpipe (shisha) tobaccos. There are no electric heaters available to the consumer that resemble the electric heater specified in ISO 22486, which does not use perforated foil, and the Egyptian-style bowl (ESB) specified in ISO 22486 does not have dimensions similar to those we have been able to purchase in the US (volume, number and spacing of holes in the bottom of the bowls). ESBs are not suitable for commercial dark air-cured (DAC) shishas. These products have much smaller particle size than their flue-cured (FC) counterparts and particles of tobacco and surrounding liquid are drawn through the holes and down into the base of the bowl during heating and puffing. Thus sample integrity is compromised during testing. Indeed, manufacturers and vendors of DAC shishas recommend use of the phunnel-style bowls. With the phunnel bowl, hot air from the glowing charcoal or from an electric heater passes over the bed of tobacco before exiting down the elevated center hole in the bowl. Using an electric heater taken from a large-size Hady E-Shisha Smokepan and a regulated DC power supply set at 12.5 VDC (~32 W), we were able to compare the ESBs with three sizes of phunnel bowls during runs of 180 three second puffs every 20 s, with puff volume similar to that specified in ISO 22486 and sample weight of 10 g. Shisha temperature was much less with the phunnel bowls, but loss of mass during use was lower with the larger bowls. Optimum bowl was a Tangiers small phunnel bowl. Test substance was a commercial mint-flavored DAC shisha and similar effects have been observed for flavored FC shishas.

ST 76

Do residues after use provide more information on in-use product chemistry than do emissions? A study with waterpipe tobaccos

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Over the past 30 years, there has been much focus on emissions from heated or combustible tobacco products, but little focus on what is left after use. Contemporary waterpipe (shisha) tobaccos contain only 10 - 30 % tobacco with the remainder being glycerol (or glycerol and propylene glycol), sugar syrups, flavor carriers (such as ethanol, triacetin, and propylene glycol), flavors, and coloring. Processing aids and preservatives include water and citric acid. Tobaccos used commercially include flue-cured (FC), dark air-cured (DAC), and Burley (BUR), with the majority of commercial products being FC, with DAC used in most other products. Particle size distribution and the amount of stem found in products can vary by manufacturer. Thus, surrogate samples were made from KY RT2 Ground FC tobacco or KY RTDAC Ground DAC tobacco. Other ingredients, glycerol, high fructose corn syrup (HFCS-42), 42 DE corn syrup, and water, are commercially available. Thus, anyone can duplicate these surrogate products and/or vary the ratio of glycerol to the amount and kind of sugar syrup. The formula for the FC surrogate was 50 % glycerol, 32 % HFCS-42, and 18 % KY RT2. The formula for the DAC surrogate was 41 % glycerol, 18 % 42 DE corn syrup, 29 % KY RTDAC, and 12 % water. Since ground tobacco was used, the surrogate shishas must be heated/puffed using a phunnel bowl such as Tangiers small phunnel bowl with a heater taken from a large Hady E-Shisha Smokepan and powered by a regulated power supply set at 12.5 VDC. Results were comparable to commercial FC or DAC products, respectively. Parameters measured included aerosol pH, shisha temperature during heating/puffing, and LC analyses of extracts of residues using a Luna sugar column (85/15 ACN/H_2O). Residue of DAC surrogate showed Levoglucosan, while the residue of the FC surrogate showed fructose, glucoses, and other decomposition products but little levoglucosan.

ST 77

Effect of filter ventilation hole characteristic on cigarette ventilation rate

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Laser perforation is an important method for online perforation of cigarette filters. The quality of the laser perforation is a key factor to ensure the stability of cigarette quality. Nowadays, the hole quality with different laser perforation parameters is evaluated using only high magnification microscopes to observe the circumference of the holes, without further research on the shape, size and depth of the hole. Therefore, to investigate the effect of laser perforation stability on filter ventilation, this study characterises the shape, size and depth of filter ventilation holes under different pulse durations (15 µs ~ 120 µs) by using a 3D focus variation optical microscope, measuring the filter ventilation of the cigarette samples and determining the relationship between them. The results show that the laser pulse duration has a strong effect on the hole characteristics: 1) With the increase of pulse duration, the diameter of the ventilation holes increases linearly, R^2 = 0.986, and the variation coefficient was within 7 %. 2) Similarly, the filter ventilation increases, and the correlation between filter ventilation and pulse duration was in line with the logistic curve, R^2 = 0.997. And the relative deviation between the simulated value and the test value was within 6 %. 3) The relationship between hole diameter and filter ventilation was also in line with the logistic curve, R^2 = 0.998. 4) The reasons for the above two observations are that when the pulse duration is shorter, the depth of the ventilation holes is small, and there were ventilation holes that did not penetrate the paper. When the pulse duration is longer, the ovality of the holes gradually decreases, and the equivalent area of the elliptical holes is smaller than the area of the circular holes under the same laser perforation conditions.

ST 78

Microscopic analyses of filtering media

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Considering that 80 % of marine litter is made of plastic, the EU adopted in 2019 the Single Use Plastic Directive (SUP). It targets ten single use plastic items representing 70 % of the marine litter.

Cigarette butts made of cellulose acetate is among the top ten. Therefore, there is an interest in replacing cellulose acetate filters by a non-plastic alternative, such as, for instance, paper.

As the use of non-plastic cigarette filters are growing, it is important to have characterisation methods in order to understand their physical structure and how it compares to a cellulose acetate one.

Optical microscopy, scanning electron microscopy (SEM) and X-Ray tomography were used in this study. Optical microscopy allows the visualizing of the filter rod and cigarette butt appearance post consumption. The paper crimping process used for the filter rod formation, especially pitch size and paper expansion, can also be characterised by this technique. SEM allows deep analysis of fibres and paper cross-section appearance. Today, several types of cellulosic filter media can be used. Optical and SEM microscopy allow the differentiation of these different types.

The cigarette filter 3D structure can be determined with X-Ray tomography that shows the distribution of the filtering media inside the filter. Crimped paper is not homogeneously distributed in a filter rod with the presence of channels. 3D tomography can look inside the filter rod and it highlights that the channels are discontinuous all along the filter, which contributes to the higher paper filter smoke/aerosol retention. By changing the resolution of 3D tomography, we can obtain more in depth information about the distribution of cellulosic fibres inside the filter.

The above three imaging techniques are complementary and, when selected properly, can address various targets: visual appearance, crimping characterisation, fibre composition or scientific understanding.

ST 79

Development of pack tactile consumer usage test method

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Cigarette packs are handled by each consumer in different ways. However, there exists a common sequence of handling of the cigarette pack. While holding, the thumb will be placed on the front panel of the pack roughly at the centre, along with the rest of the fingers on various pack panels. During the above action, the cigarette pack experiences compression forces applied by the consumer at different areas of the pack. Similarly, the consumer also experiences tactile forces at his palm and fingers. The objective of this study was to develop a pack tactile and consumer usage (PTCU) test as a comprehensive measurement of the cigarette pack strength, simulating the consumer usage in the hand. PTCU of a cigarette pack is measured using equipment, which can test the tensile strength and compressive strength of materials, with specialised parts designed to simulate the above mentioned compressive forces. Compressive forces are measured for a predetermined deflection. This is done in order to obtain a measurement of the pack strength in the consumer's hand during usage. PTCU tests were carried out for different cigarette pack styles like square edge, bevelled edge, round corner edge etc., and pack sizes used in various cigarette sizes. The overall pack compression and width compression forces for square edge packs (200 N) are three times higher than all other pack styles (ranging from 30 N to 50 N). However, the width and lid compression forces range from 5 N to 7 N between the pack styles. PTCU tests were used to evaluate and differentiate the performance of the pack and the manufacturing packing technologies for new launches, material changes, design changes and development, competitor bench marking and cost reduction.

ST 80

Analysis of absorption hysteresis of different cigarette blends based on adsorption thermodynamics method

QIU Changgui(1,2); LIU Ze(3); WEI Qing(1); HE Banghua(3); YANG Qianxu(3); LIU Jing(1,2); LI Siyuan(4); WANG Zeyu(4); ZUO Wen(4); YANG Lan(4); GUO Miaomiao(3)

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(2) Yunnan Comtestor Co., Ltd., Kunming 650106, China
(3) China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, China
(4) Hongyun Honghe Tobacco (Group) Co., Ltd., Kunming 650231, China

In order to study the isothermal adsorption characteristics for cut tobacco, cut stem and cut reconstituted tobacco, the adsorption hysteresis was quantified using the definite integral expression of GAB model, and the isosteric adsorption heat of the adsorption and desorption process to characterise the binding capacity of water and cut tobacco samples was analysed by Clausius-Clapeyron equation. The relationship between the difference of isosteric adsorption heat, adsorption hysteresis and equilibrium moisture content was analysed. The results showed that: 1) Under the same adsorption temperature, the adsorption hysteresis of cut tobacco was the largest, followed by cut stem, and the adsorption hysteresis of cut reconstituted tobacco was not significant. 2) With the increase of adsorption temperature, the adsorption hysteresis of cut tobacco and cut stem weakened and adsorption hysteresis content decreased. 3) The trend of isothermal adsorption heat of different tobacco blend formulations was significantly different. With the increase of adsorption quantity, the adsorption heat of cut tobacco decreased rapidly at first and then tended to the heat of water vapor liquefaction, while that of cut stem and cut reconstituted tobacco increased slowly at first and then tended to the heat of water vapor liquefaction. 4) The difference of initial adsorption heat between the desorption process and the adsorption process is significant, and the larger the difference of adsorption heat, the more significant the hygroscopic hysteresis. The thermodynamic causes of adsorption hysteresis of cut tobacco and cut stem are also analysed by this method.

ST 81

Influence of leaflet shape and size on processing properties of reconstituted tobacco

LIU Jing; LI Zhongren; ZHU Ting; LU Jin; MAO Junjie; QIN Qi; CHEN Hua; ZHANG Lizhong; LI Junchao

China Tobacco Schweitzer (Yunnan) Reconstituted Tobacco Co., Ltd., Yunnan Yuxi 653100, China

Along the cigarette manufacturing process, tobacco leaves go through various treatments such as moisturising, drying, heating, and cooling. For reconstituted tobacco (RT) used in the process, these conditions may cause linting and dusting issues. Historically, the final yield of RT in cigarettes is between 71.6 % - 90.8 %. Thus, the range and level of using RT in cigarettes is limited, and potentially resulting in a waste of resources. To tackle the issues, RT leaflets with different shapes and sizes were tested. In this study, two different RT products, one in a hexagonal shape and one in an irregular shape, were compared. The comparative analysis was carried out for cut tobacco both from the individual cutting process and mixed cutting process, for their cut uniformity, cut edge microscope imaging, whole filament rate and shredded filament rate. The results show that the shredding rate of irregular-shaped leaflets is 2.4 times higher than hexagonal-shaped leaflets. The total amount of dust produced by the hexagonal-shaped leaflets, either from the individual cutting process or from the mixed cutting process, is lower than that of the irregular-shaped leaflets. Hexagonal-shaped leaflets provide a potential route for improving the processing performance of RT, especially reducing the occurrence of linting and dusting problems, and, therefore, enhancing the stability of cigarette product quality.

ST 82

Establishment of an algorithm model for internal sensory quality and key process parameters of paper-making reconstituted tobacco

LI Zhongren; FENG Tao; WANG Zhipeng; LI Hailong; ZHANG Wenjun; ZHOU Jun; YUAN Jue; WU Jianlin; CHEN Yuchao

China Tobacco Schweitzer (Yunnan) Reconstituted Tobacco Co., Ltd., Yunnan Yuxi 653100, China

Intelligent manufacturing is one of the core topics for the future development of the tobacco industry, and big data is the main driving force for the realisation of intelligent manufacturing. In view of the quality fluctuation caused by the fluctuation of process parameters in the production process of paper-making reconstituted tobacco, the entry of substandard products into cigarette formulations is to be avoided. Through real-time online data collection (six monitoring results per second) of pre-coating moisture, coating liquid concentration, coating liquid temperature, coating rate, tunnel drying temperature, and finished product moisture, from a database, linear regression analysis was performed on the above process parameters and internal sensory product quality using minitab software. The correlation impact score was obtained to eliminate the process parameters with Pearson impact score ≤ 0.4 (weak correlation and no correlation). The LightGBM algorithm was used to analyse the correlation between the above process parameters and the internal sensory quality of the product, extract features and complete the establishment of the data model. The results showed that the established algorithm model has an accuracy rate of 87.21 % for the individual score and 92.78 % for the total score of the internal sensory quality evaluation derived from production data. The algorithm model can simulate the internal sensory quality of each box of products produced online. Through the setting of the early warning system, the production process parameters can be monitored and trended in real time, and then the adjustment of the production process parameters can be guided.

ST 83

Preparation of flavour metal complexes with adjustable pyrolysis temperature by metal ion selection

ZOU Peng; HE Zengyang; ZHANG Jin; SHU Junsheng; WANG Wenbin; NING Yong; SHAO Ning

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Tobacco flavours, as important tobacco additives, have attracted extensive attention to their stability. The demand for the stability of tobacco flavours is growing especially with the popularity of novel tobacco products. Volatile flavour molecules can be coordinated with metal ions to prepare flavour metal complexes and to improve the thermal stability of flavours. In this study, four ethyl maltol-containing metal complexes were synthesised by the coordination between ethyl maltol and ions of Fe^{3+}, Al^{3+}, Zn^{2+} and Ca^{2+}. The structures of those ethyl maltol-metal complexes were characterized by nuclear magnetic resonance (NMR) spectroscopy, single-crystal and powder X-ray diffractions and ultraviolet-visible (UV-Vis) spectrophotometry. The thermal behaviour and pyrolysis products were then investigated by thermogravimetry, single photoionisation mass spectrometry (SPIMS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The NMR and other spectral results confirmed that four metal complexes were synthesised with proper and specific structures. The results of thermal analysis showed that the four metal complexes could release ethyl maltol at temperatures higher than the phase transition temperature of ethyl maltol, indicative of the good thermal stability of synthesised complexes. The differences among metal ions caused the temperature of maximum weight loss rate of each complex to change in the range of 270 °C - 350 °C. The peak temperatures of ethyl maltol released from Fe^{3+}, Al^{3+}, Zn^{2+} and Ca^{2+} metal complexes were 279 °C, 346 °C, 294 °C and 337 °C, respectively. The above results indicate that the exploitation of metal complexes as flavour precursors is a feasible strategy not only to enhance the thermal stability but also to regulate the release temperature of flavours.

ST 84

Population balance – Monte Carlo simulation for aerosol evolution of glycerol

SHI Dantong; ZENG Fan; LIU Chuang; JIANG Xingtao

Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China

Electronic cigarettes (e-cigarettes) are used to deliver nicotine for inhalation through aerosolization of e-liquids composed mainly of propylene glycol and glycerol. The process of e-liquid aerosolization involves a detailed interaction between nucleation, condensation/ evaporation and coagulation. In this study, a numerical simulation of e-cigarette aerosol evolution is described by nucleation-condensation growth and subsequently condensation-coagulation growth based on different specific times for glycerol aerosol systems. A three-body collision model from the molecular kinetic theory was applied to calculate the glycerol nucleation rate, since the saturation pressure of glycerol is low. The Hertz-Knudsen droplet growth model was then adopted to account for the condensation/evaporation process. With the assumption of a homogeneous control volume, a time-driven Monte Carlo method with equally weighted simulation particle for population balance was used to simulate the coagulation behaviour of glycerol droplets. The results showed that particle number and mass distribution of the droplets followed a lognormal distribution similar to the experimental data, with count mean diameter ranging from 0.8 nm - 1.2 nm and the particle number concentration in the order of $1.0 \cdot 10^{14}$ per m^3. An increase of the evolution time was shown to increase the particle count mean diameter and decrease the particle number density. The effect of puff volume was investigated, indicating that the aerosol particle size decreased as the puff volume was increased.

ST 85

Capillary evaporation model of binary mixed solution of propylene glycol and glycerin in e-cigarette atomizer

CHEN Jingbo(1,2); WANG Zhiguo(1); PAN Zhenhai(3); CAO Jun(1); SUN Zhiwei(1,2); LIU Wei(1); KONG Bo(1); DU Wen(1)

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(3) College of Mechanical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

For the current e-cigarette products on the market, the main method to generate e-cigarette aerosol is by heating the atomiser where the vaporization-condensation process occurs. E-liquid is mainly composed of a mixed solution of propylene glycol (PG), glycerin (VG) and additives (nicotine, flavours, etc.). By establishing an energy conservation equation, one can predict the vaporisation rate of the e-liquid, the temperature distribution and the evolution of the aerosol composition. PG and VG in the e-liquid were initially believed to follow the phase transition law of non-azeotropic mixtures, which means that the evaporation of PG is prior to that of VG. However, studies indicate that the ratio of PG to VG in aerosol is close to the ratio in raw e-liquid due to the working temperature of 200 °C in an e-cigarette atomiser. To understand the above phenomenon, a capillary evaporation model for the binary mixed solution of PG and VG at the microscopic scale was established by using Ansys Fluent software. According to the simulation, a fluctuation-stabilisation process of the aerosol composition was observed. During this process, PG preferentially evaporated near the capillary gas-liquid interface (meniscus). However, owing to the limitation of the mass transfer effect of capillaries, the PG liquid could not be replenished immediately to the meniscus, and consequently a local area with VG enrichment was formed. The above complex process promotes the evaporation of VG, and increases the proportion of VG in the aerosol, followed by a steady state. In addition, this model also predicts the effects of heating temperature on the wall, the ratio of PG to VG in the solution and the capillary size on the fluctuation duration, respectively. The results show that by increasing heating temperature on the wall and the proportion of VG as well as decreasing the capillary diameter, the steady state form can be reached more efficiently.

ST 86

Numerical simulation of aerosol evolution for e-cigarette

SHI Dantong; ZENG Fan; LIU Chuang; JIANG Xingtao

Shenzhen RELX Tech. Co., Ltd., Shenzhen, Guangdong 518000, China

Electronic cigarette (e-cigarette) aerosols result from instantaneous cooling of vaporised e-liquid, which is typically composed of solvents such as propylene glycol and glycerol. The dynamics of formed e-cigarette aerosols can be described by nucleation, condensation/evaporation and coagulation. Aerosol growth is a combination of nucleation-condensation and condensation-coagulation, due to a larger specific time for dynamic coagulation events rather than for nucleation dynamic events. In this work, an extended classical nucleation approach was used to account for nucleation from multispecies in a vapor mixture. The Hertz-Knudsen droplet growth model and the Monte Carlo method for population balance were adopted to calculate aerosol droplet growth and their number separately. Simulation results for the gaseous mixture of air and propylene glycol showed that the particle number and mass distribution of droplets followed a lognormal distribution and count mean diameter reached 0.66 nm, 0.89 nm and 1.03 nm for evolution times of 1 s, 2 s and 3 s, respectively. The average particle size was larger for a glycerol-air mixture than that of the propylene glycol-air mixture at a given ratio of air flow to the vapour flow. Numerical calculations were also performed on a gaseous mixture of propylene glycol, glycerol and air, showing a similar tendency for the particle size and number distribution. As the proportion of propylene glycol vapour among the mixture decreased, the count mean diameter increased and the partition coefficient for propylene glycol and glycerol decreased.

ST 87

Brand verification and counterfeit identification of e-liquid based on electronic nose technology

SONG Zheng; LIU Peixian; JIANG Jingjing; JIANG Xingtao

Shenzhen Matrix Technical Service Co., Ltd., Shenzhen, Guangdong 518000, China

Electronic cigarettes (e-cigarettes) are a novel type of nicotine delivery system (ENDS) which has developed rapidly in recent years. There are many brands and flavours of e-liquids on the market. The cost and safety of different brands of e-liquids are different. The better-known brands of ENDS on the market are prone to counterfeiting, which disrupts the market order and carries unknown health risks for consumers. This study established an electronic nose-based model that could be used to identify different brands of e-liquids with the same or similar flavouring profiles. Multivariate analytical methods including principal component analysis (PCA) and discriminant function analysis (DFA) were used to record and analyse the characteristic flavour components from 39 flavour A samples of different batches and 20 flavour B samples of different batches. An identification model was then built. This model could distinguish different brands of e-liquids effectively. Ten e-liquid samples of different brands but with the same flavour profiles were randomly selected as blind samples to verify the accuracy of the identification model. The results showed that the accuracy of the model using two flavour types, flavour A and flavour B, reached close to 100 %. The model is an effective and convenient method for brand authenticity verification.

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PROGRAMME SUMMARY

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 1

Monday 10 Oct

Session 1

Chair: JACKSON
Mod.: SCOTT

PRODUCTION

CET	
13:30-13:45	**AP01** DEERGASI
13:45-14:00	**AP02** REED
14:00-14:15	**AP03** VANN
14:15-14:30	**AP04** SHORT
14:30-14:45	**AP05** CHINAMO
14:45-15:00	**AP06** ZENG Weiai
15:00-15:15	**AP07** DU Haina

DAY 2

Tuesday 11 Oct

Session 1

Chair: SCOTT
Mod.: JACKSON

SUCKER CONTROL

CET	
13:30-13:45	**AP09** BAILEY
13:45-14:00	**AP10** TAGA
14:00-14:15	**AP11** VANN
14:15-14:30	**AP12** ROUSSOS
14:30-14:45	**AP13** RICHMOND

Session 2

Chair: DIMBI
Mod.: ZHANG

TECHNOLOGY

CET	
15:15-15:30	**AP14** ZVOBGO
15:30-15:45	**AP15** ZHANG Jianqiang
15:45-16:00	**AP16** YUAN Cheng

DAY 3

Wednesday 12 Oct

Session 1

Chair: XU
Mod.: FISHER

GENETICS / BREEDING

CET	
13:30-13:45	**AP17** MALPICA
13:45-14:00	**AP18** LEWIS
14:00-14:15	**AP19** PRZYBYS
14:15-14:30	**AP20** HU Zhengrong
14:30-14:45	**AP21** FREDERICK
14:45-15:00	**AP22** IWAI
15:00-15:15	**AP23** WANG Zhong
15:15-15:30	**AP24** TAKEUCHI
15:30-15:45	**AP25** CHEN Hongyu

DAY 4

Thursday 13 Oct

Session 1

Chair: FISHER
Mod.: DIMBI

PEST & DISEASE

CET	
13:30-13:45	**AP26** HOSSEINI
13:45-14:00	**AP27** KEENEY
14:00-14:15	**AP28** KEENEY
14:15-14:30	**AP29** MILLER
14:30-14:45	**AP30** MALPICA
14:45-15:00	**AP31** B SAILAJA
15:00-15:15	**AP32** B SAILAJA
15:15-15:30	**AP33** ZOU Congming

DAY 5

Friday 14 Oct

Session 1

Chair: JACKSON
Mod.: SCOTT

CPAs

CET	
13:30-13:45	**AP34** RICHMOND
13:45-14:00	**AP35** VANN
14:00-14:15	**AP36** VANN
14:15-14:30	**AP37** BECKER
14:30-14:45	**AP38** STRAUMANN
14:45-15:00	**AP39** PERKINS

Session 2

Chair: ZHANG
Mod.: JACKSON

MICROB. IMPACT ON CURED LEAF

CET	
15:30-15:45	**AP40** WANG Zhen
15:45-16:00	**AP41** CAI Wen
16:00-16:15	**AP42** HUANG Kuo

Full Session Titles

Day 1: **Session 1 -** Production: impact of nutrition, soil and management
Day 2: **Session 1 -** Sucker control: management and genetic control
Session 2 - Technology application in genetics and physiology
Day 3: **Session 1 -** Genetics: impact of breeding and biotechnology
Day 4: **Session 1 -** Pest and disease management
Day 5: **Session 1 -** Crop protection agents (CPAs) in tobacco production and residue analysis
Session 2 - Microbial impact on cured leaf

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

DAY 6

	Monday 17 Oct
	Session 1
	Chair: JACKSON Mod.: LALANDE
CET	**SUSTAINABILITY**
13:30-13:45	**AP43** IRVING / GEOVANNELLO
13:45-14:00	**AP44** WANG Yi
14:00-14:15	**AP45** FRANTZ
14:15-14:30	**AP46** LIU Yanxia
14:30-14:45	**AP47** JAZI
14:45-15:00	**AP48** SHAZDEHAHMADI
15:00-15:15	**AP49** SAJJADI
15:15-15:30	**AP50** MUNA

DAY 7

	Tuesday 18 Oct
	Session 1
	Chair: FISHER Mod.: XU
CET	**LEAF CONSTITUENTS**
13:30-13:45	**AP51** KUDITHIPUDI
13:45-14:00	**AP52** ZHAO Yuanyuan
14:00-14:15	**AP53** LI Yingxue
14:15-14:30	**AP54** WANG Lan
	Session 2
	Chair: FISHER Mod.: XU
CET	**LOW NICOTINE TOBACCO**
15:00-15:15	**AP55** FISHER A.
15:15-15:30	**AP56** DEWEY
15:30-15:45	**AP57** FRANCESCHETTI

Full Session Titles

Day 6: Session 1 - Sustainability through integrated programmes and production practices

Day 7: Session 1 - Leaf constituents: TSNA and other chemistry

Session 2 - Low nicotine tobacco

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 8

CET	Wednesday 19 Oct
	Session 1
	Chair: CAHOURS Mod.: YOSHINO
	PERCEPTION AND BEHAVIOUR
13:30-13:45	**ST01** WADKIN
13:45-14:00	**ST02** GRAY
14:00-14:15	**ST03** KUN Duan
14:15-14:30	**ST04** SHETTY
14:30-14:45	**ST05** AL MOOSAWI
14:45-15:00	**ST06** SHETTY

CET	Session 2
	Chair: CAHOURS Mod.: HARP
	BIOMARKERS
15:30-15:45	**ST07** WANG Yangzhong
15:45-16:00	**ST08** MARTIN
16:00-16:15	**ST09** KANOBE
16:15-16:30	**ST10** TROFIMOV

DAY 9

CET	Thursday 20 Oct
	Session 1
	Chair: STEVENS Mod.: PANI
	E-VAPOUR: PROD. DESIGN & CHEMISTRY
13:30-13:45	**ST11** LI Xinduo
13:45-14:00	**ST12** LIU Shengyi
14:00-14:15	**ST13** YOU Rui
14:15-14:30	**ST14** SHI Dantong
14:30-14:45	**ST15** JABLONSKI
14:45-15:00	**ST16** COCCIARDI

CET	Session 2
	Chair: STEVENS Mod.: WAGNER
	NICOTINE SCIENCE
15:30-15:45	**ST17** DULL
15:45-16:00	**ST18** CHEETHAM
16:00-16:15	**ST19** YANG Ji
16:15-16:30	**ST20** BUSSEY

DAY 10

CET	Rev. XC - ok Friday 21 Oct
	Session 1
	Chair: HARP Mod.: CAHOURS
	CLINICAL STUDIES
13:30 - 13:45	**ST21** McEWAN
13:45 - 14:00	**ST22** GUO Yi
14:00 - 14:15	**ST23** HONG Kyung-Soo
14:15 - 14:30	**ST24** JACOBSON
14:30 - 14:45	**ST25** CONNOLLY / MALLA
14:45 - 15:00	**ST26** LARROQUE

CET	Session 2
	Chair: WAGNER Mod.: CAHOURS
	ORAL TOB. PROD.: PROD. ANALYSIS & METHODS
15:30 - 15:45	**ST27** MORTON
15:45 - 16:00	**ST28** REDEBY
16:00 - 16:15	**ST29** AVAGYAN
16:15 - 16:30	**ST30** LOPEZ
16:30 - 16:45	**ST31** TINDALL

Full Session Titles

Day 8: **Session 1 -** Perception and behaviour: understanding how nicotine products are perceived and used
Session 2 - Biomarkers: method improvement and effects of product switching

Day 9: **Session 1 -** E-vapour: product design and chemistry
Session 2 - Nicotine science

Day 10: **Session 1 -** Clinical studies: nicotine effects and data analysis
Session 2 - Oral tobacco products: product analysis and methods

SMOKE SCIENCE and PRODUCT TECHNOLOGY

DAY 11 — Monday 24 Oct

Session 1
Chair: WAGNER
Mod.: STEVENS

E-VAPOUR: PROD. ASSESS.

CET	
13:30-13:45	**ST32** HUANG Yilan
13:45-14:00	**ST33** LU Rui
14:00-14:15	**ST34** LI Xinduo
14:15-14:30	**ST35** CROSSWHITE
14:30-14:45	**ST36** COOK
14:45-15:00	**ST37** CROSSWHITE

Session 2
Chair: STEVENS
Mod.: WAGNER

E-VAPOUR: ANALYT. METH.

CET	
15:30-15:45	**ST38** ITO
15:45-16:00	**ST39** HE Yi
16:00-16:15	**ST40** HE Yi
16:15-16:30	**ST41** LIANG Jingjing
16:30-16:45	**ST42** LIANG Jingjing
16:45-17:00	**ST43** LIANG Jingjing

DAY 12 — Tuesday 25 Oct

Session 1
Chair: YOSHINO
Mod.: HARP

E-VAPOUR: IN VITRO TOX

CET	
13:30-13:45	**ST44** WADHWA DESAI
13:45-14:00	**ST45** KUN Duan
14:00-14:15	**ST46** HUTTER
14:15-14:30	**ST47** BISHOP
14:30-14:45	**ST48** THORNE
14:45-15:00	**ST49** SMART

Session 2
Chair: HARP
Mod.: YOSHINO

NOVEL TOB. PROD.: *IN VITRO* TOX

CET	
15:30-15:45	**ST50** MOSES
15:45-16:00	**ST51** TAKAHASHI
16:00-16:15	**ST52** LIU Qianyuni
16:15-16:30	**ST54** TRELLES STICKEN

DAY 13 — Wednesday 26 Oct

Session 1
Chair: EITZINGER
Mod.: HU

HTP: PROD. DESIGN, MOD. & TESTING

CET	
13:30-13:45	**ST55** LIU Chuan
13:45-14:00	**ST56** MIAZZI
14:00-14:15	**ST57** JUNG Yongmi
14:15-14:30	**ST58** GAO Yihan
14:30-14:45	**ST59** ZHANG Wenjun
14:45-15:00	**ST60** SONG Xiaomei
15:00-15:15	**ST61** LIU Shengyi

Session 2
Chair: EITZINGER
Mod.: PANI

HTP: ANALYTICAL METHODS

CET	
15:45-16:00	**ST62** KIM Hye-Won
16:00-16:15	**ST63** LEE Seon-Bong
16:15-16:30	**ST64** GIBBONS
16:30-16:45	**ST65** PINTO

DAY 14 — Thursday 27 Oct

Session 1
Chair: PANI
Mod.: HU

TOB. & CIG. SMOKE: ANALYT. METH.

CET	
13:30 - 13:45	**ST66** ZHANG Jianqiang
13:45 - 14:00	**ST67** WINDISCH
14:00 - 14:15	**ST68** JU Soyoung
14:15 - 14:30	**ST69** JIN Xiaohong Cathy
14:30 - 14:45	**ST70** WANG Xiaoyu
14:45 - 15:00	**ST71** JI Huihua

Session 2
Chair: WAGNER
Mod.: PANI

FLAV. & WATER PIPES: ANALYT. METH.

CET	
15:30 - 15:45	**ST72** SCHAEFER
15:45 - 16:00	**ST73** RODRIGUEZ-LAFUENTE
16:00 - 16:15	**ST74** RODRIGUEZ-LAFUENTE
16:15 - 16:30	**ST75** LAUTERBACH
16:30 - 16:45	**ST76** LAUTERBACH

DAY 15 — Friday 28 Oct

Session 1
Chair: YOSHINO
Mod.: EITZINGER

CIG. COMP. TESTING & DESIGN

CET	
13:30 - 13:45	**ST77** ZHANG Qi
13:45 - 14:00	**ST78** CHERKAS
14:00 - 14:15	**ST79** REJIMON
14:15 - 14:30	**ST80** QIU Changgui
14:30 - 14:45	**ST81** LIU Jing
14:45 - 15:00	**ST82** FENG Tao
15:00 - 15:15	**ST83** ZOU Peng

Session 2
Chair: HU
Mod.: EITZINGER

E-VAP. & E-LIQUID MODEL

CET	
15:45 - 16:00	**ST84** SHI Dantong
16:00 - 16:15	**ST85** DU Wen
16:15 - 16:30	**ST86** SHI Dantong
16:30 - 16:45	**ST87** SONG Zheng

Full Session Titles

Day 11: **Session 1 -** E-vapour products: product assessment
Session 2 - E-vapour products: analytical methods
Day 12: **Session 1 -** E-vapour products: *in vitro* toxicological assessment
Session 2 - Novel tobacco products: *in vitro* toxicological assessment
Day 13: **Session 1 -** HTP: product design, modeling and testing
Session 2 - HTP: analytical methods
Day 14: **Session 1 -** Tobacco and cigarette smoke: analytical methods
Session 2 - Flavours and water pipes: analytical methods
Day 15: **Session 1 -** Cigarette component testing and design
Session 2 - E-vapour and e-liquid modeling