1. INDUSTRY ENGAGEMENT WITH THE SCIENTIFIC COMMUNITY. Willie J. MCKINNEY
Amy E. Brannan and Donna C. Smith; Altria Client Services, Richmond, VA, USA

Collaborations between industry scientists and the broader scientific community are common. Tobacco industry collaboration with public health scientists presents unique opportunities and challenges. The convergence of i) an FDA regulatory environment where FDA can make science and evidence based decisions about tobacco products and communications; ii) innovations by tobacco companies and concurrent evolving adult tobacco consumer expectations; and iii) divergent perspectives in the public health community relative to tobacco harm reduction all serve to create a nexus for tobacco industry, FDA and academic scientists to effectively collaborate. We all have a role advancing harm reduction. For example, industry and academic scientists may gather the scientific evidence to demonstrate the risk reduction potential of new innovative tobacco products. Their knowledge of cigarette smoke attributable-risk and adult tobacco consumer preferences is important for this analysis. Public health, including FDA, play a pivotal role in advancing harm reduction by educating adult smokers about the identified risk differential between tobacco products, so that adult tobacco consumers can make informed decisions. Although productive, these collaborations face several challenges. Most notably, the history of the tobacco industry may sometimes negatively impact effective scientific engagement efforts. However, regardless of potential barriers, several new forums are facilitating productive communications between academic harm reduction enthusiasts, pessimists, public health and the tobacco industry. These forums serve to diminish barriers such as a lack of trust. This paper will present some of the conditions necessary for successful and productive tobacco industry engagement with the scientific community.

2. THE ROLE OF INDUSTRY IN ENGAGING WITH INDUSTRY TO DEVELOP REGULATORY-APPROVED METHODS FOR TOBACCO AND NICOTINE PRODUCT ASSESSMENT. Christopher J. Proctor, Ian M. FEARON, Derek Mariner, Christopher Wright and James Murphy; British American Tobacco (Investments) Limited, Southampton, UK

Industry consensus methods for product assessment are commonplace across many industrial sectors and often form the basis of regulatory approaches. In the pharmaceutical industry for example, the foundations for the assessment of the safety, quality and efficacy of new medicines were laid almost 30 years ago in cross-industry collaboration with regulators, leading to the inception of the ICH guidelines which are now applicable globally.

In the tobacco industry, when an EU expert scientific panel advised that more scientific data was needed on specific additives used in cigarettes it suggested that tobacco manufacturers could carry out joint studies to collect the required data. This is a similar approach to that taken with chemical manufacturers to achieve compliance with European chemical regulations.

There are some long-standing cross industry fora that work on consensus methods, such as the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), and more recently a broad church of interested parties was involved in creating the British Standards Institute (BSI) product standards for e-cigarettes. Yet in other areas, such as the development of population models, individual companies have created their own models independently of one another.

This paper looks at the importance of cross-industry collaboration in non-competitive contexts, the value of consensus standards for both industry and regulators, and some of the conditions necessary to create standards trusted by all interested parties.
3. FACTORS AFFECTING MEDIA COVERAGE OF TOBACCO RESEARCH NEWS. Cheryl K. Olson; Cheryl K. Olson ScD, LLC, San Carlos, CA, USA

Scientific publications with perceived relevance to public health or safety are often picked up by the popular press. The accuracy of such coverage by electronic and print media varies substantially. Reviewing examples of coverage of recent scientific publications on health effects of tobacco, nicotine or e-cigarettes reveals patterns in the quality and nature of press coverage, as well as its apparent motivation. This presentation focuses on the process by which journalists decide whether to cover a health-related news story; common points of confusion that affect quality of coverage (e.g., correlation vs. causation, or statistical vs. real-world significance); and ways that researchers might act to improve the likelihood and quality of media coverage of research.

4. VAPERS AND THE VAPING INDUSTRY ARE THE PRIMARY AGENTS OF TOBACCO HARM REDUCTION IN THE UNITED STATES. Christopher Russell; Centre for Substance Use Research, Glasgow, UK

The most commonly used method of quitting smoking in the United States, substituting e-cigarettes for cigarettes, was not conceived by, is not recommended by, and was, until very recently, not controlled by the U.S. Food and Drug Administration (FDA). The increasing popularity of a method of quitting smoking that is not recognised by FDA as an effective smoking cessation method highlights a divergence between how tobacco harm reduction has been conceptualised within the medical science and health policy communities and how tobacco harm reduction is being exercised in the real world by more and more smokers.

In this presentation, I argue that, for the past decade, tobacco harm reduction in the real world has been driven by manufacturers, vendors, consumers and advocates of vapour products. Testimony reveals that smokers rarely initiate vaping without first researching the products or talking to a vaper or a vape shop sales assistant about why they vape, how the vaping experience compares to smoking, or how their life and health has changed since becoming a former-smoking vaper. The knowledge, practical advice and encouragement that have been transferred to smokers through millions of such interactions have likely rationalised and motivated millions of attempts to substitute e-cigarettes for conventional cigarettes that may not have occurred in the absence of these peer interactions.

The increasing preference of U.S. smokers to use nicotine-containing products as a method of quitting smoking suggests progress towards a smoke-free society may be further accelerated by a nicotine regulatory system that encourages an expansion and diversification of the market in new tobacco and nicotine products that appeal to and reduce harm to smokers, and maximises the user community’s opportunities to interact with smokers.

5. USE OF FORMALIN IN E-VAPOR PRODUCTS TO MONITOR FORMALDEHYDE DELIVERY. Kathleen Spanangler, James Wilkinson, Matt Melvin, John H. Miller IV and Georgios D. Karles; Altria Client Services, Richmond, VA, USA

Guidance provided by the FDA (May of 2016) for Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems proposes full chemical characterization of the e-cigarette aerosol. One class of compounds the FDA recommends to be reported in aerosol are the carbonyl containing compounds, formaldehyde, acetaldehyde, acrolein and crotonaldehyde. There currently is no CORESTA recommended method for measuring carbonyls in e-vapor products; however, the CORESTA E-vapor Subgroup is currently working on evaluating multiple methods for the analysis of these carbonyl compounds in e-vapor products (e-liquids and aerosols). In order to evaluate if a new method is suitable for e-vapor products, experiments such as trapping efficiency, recovery, repeatability and reproducibility need to be assessed. Since the levels of carbonyls in e-vapor aerosols are typically very low with variable consistency, evaluation of the fore mentioned method performance parameters present unique challenges. In our lab, we have assessed the addition of formalin to a control e-liquid at known levels to serve as a monitor for formaldehyde analysis in the aerosol of e-vapor products. We have demonstrated the stability of formalin measured in e-vapor aerosol for at least six weeks when refrigerated at 4 °C. Additional work will be presented to reinforce that the addition of formalin to e-vapor monitor cartridges provides a direct way for
laboratories to evaluate method robustness for carbonyl analysis and to monitor laboratory performance over time.

6. CHARACTERIZATION OF AEROSOLS GENERATED FROM A PROTOTYPE E-VAPOR PRODUCT AND A CAPILLARY AEROSOL GENERATOR. Jingjie ZHANG, Christopher Tucker, K. Monica Lee and Michael Oldham; Altria Client Services, Richmond VA, USA

E-Vapor products, which are gaining steady popularity among tobacco consumers, are composed of various types of e-liquid formulations and aerosol generating devices. There are situations that formulations need to be tested separately from devices. The purpose of this study was to characterize the chemical composition and particle size distribution of the aerosols generated from prototype one-channel CAG and a prototype e-vapor product using an identical e-liquid formulation. Prototype e-vapor products were puffed (55 ml volume, 5 second duration, every 30 seconds) using a 20-port linear smoking machine. The CAG temperature was set to 250 °C to match the measured coil temperature during puffing of the prototype e-vapor product. Aerosols from each device were collected on Cambridge filter pads and analyzed (GC/FID; GC/TCD) for major formulation ingredients (propylene glycol [PG], glycerin, water, and nicotine). Analysis (UPLC/MS) of selected carbonyls (formaldehyde, acetaldehyde, acrolein, and crotonaldehyde) was performed by collecting aerosols using a Cambridge filter pad and an impinger in series. Particle size distribution was measured using a cascade impactor. Similar levels of PG, glycerin, and nicotine were detected in the aerosol from both devices. When detected above the limit of quantification, the levels of selected carbonyls were lower in the prototype e-vapor product aerosols. Both devices generated similar particle size distributions. The CAG and the prototype e-vapor product are comparable in terms of aerosol output (chemical composition and particle size distribution). Therefore, the CAG can be used as a surrogate device to generate and test e-vapor formulations in in-vitro and in-vivo studies.

7. NEUTRAL RED UPTAKE CYTOTOXICITY ASSAY IN A549 CELLS UNDER DIFFERENT CULTURE CONDITIONS. Bonnie G. COFFA, Utkarsh Doshi, Jingjie Zhang, Willie J. McKinney, K. Monica Lee; Altria Client Services, Richmond, VA, USA and Pooja Desai; Enthalpy Analytical, Richmond, VA, USA

The OECD guideline (TG129) recommends mouse fibroblasts (3T3) & normal human epidermal keratinocytes (NHK) for performing in vitro cytotoxicity assays but also mentions limitations of these cells, such as having little to no metabolic capacity or possibility of inappropriate interpretation of data if these cells are unrelated to the target organ of interest. Considering the primary target organ for inhalable products, we conducted this study to evaluate the suitability of a human derived alveolar cell line A549 for the neutral red uptake (NRU) assay under various testing conditions. First, positive control sodium laurel sulfate (SLS) and DMSO extracted cigarette smoke total particulate matter (TPM) were tested for cytotoxicity in A549 and 3T3 under submerged condition. Secondly, A549 were grown at the air-liquid interface (ALI) and assessed for cytotoxicity following 24 and 48 hr. of treatment with SLS in media supplied from basal side, containing 0% or 5% serum. Dose-dependent cytotoxicity was observed in 3T3 and A549 in response to both SLS and TPM and the corresponding IC50 were estimated. Under submerged conditions containing serum, A549 displayed lower IC50 (SLS) and higher IC50 (TPM) in comparison to the values of mouse 3T3 cells, suggesting species-dependent differences in sensitivities. No significant difference in cytotoxicity was observed in A549 cells between submerged and ALI cultures in response to SLS. Enhanced cytotoxicity was evident in A549 cells exposed to SLS under serum free environmental conditions. In summary, 1) A549 cells could be effectively used in the TG129 NRU assay in submerged as well as ALI conditions and 2) media composition such as presence of serum, should be carefully controlled especially when using human-derived cells such as A549.
8. ARE AVAILABLE TEST METHODS FOR THE DETERMINATION OF CARBONYLS IN MAINSTREAM CIGARETTE SMOKE FIT FOR THE ANALYSIS OF CIGARS? Regina M. Ballentine, Karen C. Avery, Matt S. Melvin, Jennifer H. Smith and Karl A. Wagner; Altria Client Services, Richmond, VA, USA

In May 2016, the U.S. Food and Drug Administration (FDA) issued a final rule to deem cigars to be subject to the Federal Food, Drug, and Cosmetic Act, as amended by the Family Smoking Prevention and Tobacco Control Act. As part of this regulation, the FDA will require manufacturers to report the quantities of Harmful and Potentially Harmful Constituents (HPHCs) in cigar filler and smoke. Standardized methods do exist for the analysis of carbonyls in cigarette smoke; however, these methods have not been shown to be fit for purpose for the analysis of cigars. CORESTA Recommended Method No. 74, “Determination of Selected Carbonyls in Mainstream Cigarette Smoke by High Performance Liquid Chromatography” was based on Health Canada method T-104 and is the basis of ISO/CD 21160:2017, “Determination of selected carbonyls in the mainstream smoke of cigarettes -- Method using High Performance Liquid Chromatography”. Due to the fact that it may take an hour or more to collect cigar smoke, we hypothesized that the carbonyl–DNPH derivative would degrade over time, resulting in decreased carbonyl yields. Cigar smoke was collected and time studies were conducted to determine carbonyl stability in the acidic DNPH trapping solution. The result of this work indicates that there is degradation of the DNPH derivative during smoke collection. Results will be presented for different cigar blend types. Furthermore, these results indicate that specialized methods need to be developed for the robust analysis of carbonyls in cigar.

9. THE CHALLENGES OF MACHINE SMOKING THE DIVERSE CIGAR PRODUCT CATEGORY. Tammy L. Blake¹, Regina M. Ballentine², Karen C. Avery³, Anthony P. BROWN⁴, Karl A. Wagner¹, Michelle J. Carpenter⁵ and Kerry L. Stutt¹; ¹Altria Client Services, Richmond, VA, USA; ²Eurofins Lancaster Laboratories, Richmond, VA, USA

In May 2016, the U.S. Food and Drug Administration (FDA) issued a final rule to deem cigars to be subject to the Federal Food, Drug, and Cosmetic Act (the FD&C Act), as amended by the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act). As part of this regulation, the FDA will require manufacturers to report the quantities of Harmful and Potentially Harmful Constituents (HPHCs) in cigar filler and smoke. The ability to machine smoke cigars is critical for generating meaningful HPHC data. The CORESTA Cigar Smoking Methods Subgroup has been the main driver for the development of cigar smoking methods. This Subgroup has published several CORESTA Recommended Methods (CRMs) which enable the determination of nicotine-free dry particulate matter (NFDPM), nicotine, water, and carbon monoxide. The diversity in shape and size of the cigar product category creates challenges for smoke collection and often requires product specific solutions to achieve acceptable analytical results. Custom cigar holders that meet the requirements of CRM No. 64 will be described and compared to the commercially available cigar holders. The custom cigar holders enable the collection of smoke from untipped and tipped cigars and provide for improved ease of use and reduced variability over the commercially available holders. A comparison between the two styles of cigar holders for the determination of smoke yields for 9 commercial cigar products will be presented.

10. DIFFERENCES IN PLASMA NICOTINE PHARMACOKINETIC PROFILES FOR VARIOUS E-VAPOR PRODUCTS USED BY ADULT SMOKERS UNDER AD-LIBITUM VS. CONTROLLED USE CONDITIONS. Jianmin LIU, Qiwei Liang, Maria Gogova, Yuxi Zhao and Mohamadi Sarkar; Altria Client Services, Richmond VA, USA

Plasma nicotine pharmacokinetic (PK) profiles are often used to characterize nicotine exposure from e-vapor products (EVP). The purpose of this analysis was to determine whether PK profiles are different when adult smokers (AS) use EVPs under ad libitum or controlled use conditions. We conducted a 6-way randomized crossover study in twenty-four AS smoking ≥10 cigarettes/day and had not used EVPs in the past month. AS used six different types of EVPs (tank- or cartridge-based) with different flavors and levels of nicotine under two use conditions (10 hours apart) – controlled use of 10 inhalations of 4-second duration with 60-second intervals (over ~10 minutes) and ad libitum use for 10 minutes. Nicotine plasma levels were measured periodically for 120 minutes. The maximum concentration (Cmax0-2hrs) and area
under the curve (AUC0-2hrs) were higher under ad libitum vs. controlled use. On average, AS took ~twice as many puffs under ad libitum compared to controlled use and average puff duration ranged from 3.0 to 3.6 s for the six EVPs. The inter-individual variability (CV%) for Cmax0-2hrs was larger under ad libitum (53%) than under controlled use (28%). The identical use conditions under controlled use may provide a better method to compare nicotine PK from different types of EVPs. The high inter-individual variability under ad libitum use conditions may reflect behavioral aspects e.g. flavor preference and satisfaction. The pros and cons of each test condition for assessing nicotine PK will be discussed. These variability estimates may be used to design future studies with EVPs.

11. MARKTEN® E-VAPOR STABILITY STUDY: DESIGN AND INITIAL DATA. Susan PLUNKETT, Michael Morton, William Gardner, Karl Wagner and Diana McKinney; Altria Client Services, Richmond, VA, USA

The Food and Drug Administration’s (FDA’s) Draft Guidance for Industry Premarket Tobacco Product Application for Electronic Nicotine Delivery Systems states that an application should include data informing “the established shelf life of the product and changes in pH and constituents (including HPHCs and other toxic chemicals) over the lifespan of the product.” The FDA has not published industry guidance on stability testing protocols for e-vapor products, and no scientific literature has been published to date on the design and execution of e-vapor product stability studies. Although e-vapor products are not pharmaceutical products, in the absence of specific guidance, we have adopted the stability study design from the FDA Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products.

We describe the design and share initial data from an e-vapor stability study on MarkTen® e-vapor products analyzing for 50 constituents in both liquid and aerosol. We determined these constituents in 3 batches of product with 10 replicates each in both liquid and aerosol over multiple time points. Two vaping regimens and 2 storage conditions were tested at select time points.

We will discuss constituent levels, trends, and possible constituent sources. Due to the lack of standardized methods, we suggest best practices for data collection and interpretation including blanks to distinguish background levels from the very low levels of e-vapor constituents. Finally we discuss the statistical utility of 10 replicates.

12. NEW SMOKING MACHINE APPROACH FOR CIGARETTE SMOKING UNDER INTENSE AND ISO 3308 CONDITIONS. (Presented by Whitley Lee) Nils Rose; Borgwaldt KC, Hamburg, Germany

ISO 3308 specifies the definitions and conditions for routine analytical smoking machines, defining the puffing regime as a 35ml bell shape puff with a 2s duration taken every 60s. It also specifies two machine types “A” and “B” dependent to the adjustment of the termination device but misleadingly distinguished as “rotary machine” and “linear machine”. These were harmonized regarding their tar and CO deliveries in 1995 and 2002.

In 2007, by demand of the WHO, ISO TC 126 started the preparation of a more intensive smoking regime based on a 55ml bell shape puff with a 2s duration taken every 30s and sealed ventilation holes.

In 2010 ISO TC 126 WG10 carried out a study to measure the tar, nicotine and carbon monoxide yields of cigarettes using both smoking regimes. The outcome of this study has shown significant differences in water and corresponding deliveries (TPM, ) with a trend to higher deliveries on linear machines. One possible explanation for this phenomenon was assumed to be the distance between the cigarette and the filter pad causing loss due to pre-condensation. The study also shows lower variation in the data gathered for rotary machines.

In consideration of this, a 4-channel hybrid machine was developed. It is tailored to the requirements of the new smoking method and combines the advantages of both machine types in an ideal and most efficient manner.
This poster briefly describes the design approach of the machine and discusses first validation data for both smoking regimes compared to the yields delivered from standard linear and rotary smoking machines.

13. “TRACK AND TRACE” IN THE SMOKING LABORATORY. (Presented by Whitley Lee) Nils Rose; Borgwaldt KC, Hamburg, Germany

“Track and Trace” is one of the major requirements regarding “Good Laboratory Practice”. During the whole production process, from incoming goods up to the packed product, cigarettes are well tracked. But in the smoking laboratory this becomes more problematic. The products have to be linked carefully to complex smoking plans including multiple machines and different parameter sets. And finally the products are converted physically into loaded smoke traps continuing their way to the chemical analysis.

Along the way there are plenty of possibilities to mix up samples or data or to lose the link between the traps and the primary product.

This poster describes a technical solution of consistent tracking during the whole procedure to avoid errors and to gain information about the current status of the product during the whole process, which is an important topic for a simplified laboratory accreditation. It also simplifies the laboratory process and offers not only the possibility for higher efficiency but also for greater process automation.


Electronic cigarettes (ecigs) have become a popular mode of nicotine ingestion. They are often promoted as being less harmful than traditional cigarettes, but research supporting that claim is incomplete. The main chemical components of ecigs are propylene glycol and glycerol and they are usually found in a 70:30 or 50:50 ratio with 0.6 to 3.6% nicotine. Recently, Celerion Inc. was granted a patent for using isotopic propylene glycol to monitor how much PG is ingested from ecig use (vaping). The use of isotopic propylene glycol made clear delineation of ingestion of propylene glycol from foods versus ecigs. A similar method was explored for the quantitation of glycerol. Glycerol has the added challenge that lipids from the plasma – specifically triglycerides - break down into glycerol, greatly increasing the basal glycerol concentrations compared to propylene glycol concentrations. Literature values for free glycerol concentrations range from 6500-12000 ng/mL(2-4) and after breakdown of the triglycerides, total glycerides are more than 10X that. Assuming uptake similar to the propylene glycol, the Cmax for glycerol vaping would be 600-2000 ng/mL. The method detailed here deftly avoids isotopic contribution from endogenous glycerol; the result is an accurate, reproducible method for isotopic glycerol in human plasma.

16. WEB-BASED SYSTEM FOR ORDERING TOBACCO REFERENCE PRODUCTS AND MANAGING PROFICIENCY STUDIES. Bront DAVIS and James Hunsucker; University of Kentucky, Lexington, Kentucky, USA

The Center for Tobacco Reference Products in the College of Agriculture, Food and Environment at the University of Kentucky has developed a website (https://ctrp.uky.edu) to streamline the sale and distribution of tobacco reference products and proficiency test kits. Users must request access to the secure website, and once granted, they are able to purchase products and manage purchases via the website. Significant design considerations and user feedback have been incorporated in the proficiency testing functionality to improve the reporting process for participants, including: (1) creation of the Excel data templates for proficiency studies; (2) purchasing proficiency studies; (3) accessing the Excel data template; (4) uploading Excel data with an opportunity to review before final submission; (5) accessing interim report and commenting on the process; and (6) accessing final reports. This online system has significantly improved the data collection, analysis and reporting process associated with conducting proficiency testing programs.
17. STABILITY OF THE CERTIFIED 1R6F REFERENCE CIGARETTE. Huihua Ji, Ying Wu and Franklin Fannin; University of Kentucky, Lexington, Kentucky, USA

University of Kentucky, Center of Tobacco Reference Products (CTRP), has provided reference cigarettes for almost 50 years. These reference cigarettes are widely used as control samples in tobacco research including analytical method development and modified risk tobacco product development. In 2014, CTRP obtained a service agreement with the US Food and Drug Administration (FDA) to produce a certified reference cigarette. The first certified reference cigarette, 1R6F, was manufactured in March, 2015. However, there are no data showing the stability of 1R6F during long-term storage. The objective of this project is to study the stability of the cigarette tobacco filler and resulting mainstream cigarette smoke of 1R6F. Cigarettes were stored at -20°C, 4°C and room temperature (~22°C) for 1, 2, 3, 6, 9 and 12 months. Before they were analyzed, cigarettes stored in -20°C were transferred to 4°C for 24 hr then room temperature for at least 2 hr. The reference cigarettes were conditioned for 48 hr at 22°C and 60% relative humidity, prior to analysis in tobacco filler and mainstream cigarette smoke. Filler analysis included oven volatiles, alkaloids and TSNAs. Smoke analysis included the measurement of alkaloids, TSNAs, CO and TPM under ISO smoking regime. There were no significant changes for the oven volatiles, nicotine and NNN in the filler at 4°C and -20°C conditions. However, oven volatiles decreased significantly when cigarettes were stored at room temperature. There were no significant changes for puff/cigarette, CO, TPM, alkaloids and TSNAs in mainstream smoke of cigarettes under -20°C, 4°C and room temperature conditions. Our experimental data demonstrated 1R6F cigarettes were stable after one-year storage at 4°C and -20°C for selected constituents. This study will continue to monitor the stability of the 1R6F cigarette supply for the duration of this research project.

19. VALIDATION OF A LIMIT TEST METHOD FOR ANALYSIS OF PRIMARY AROMATIC AMINES (PAA) IN ELECTRONIC CIGARETTE LIQUID AND AEROSOL. Elizabeth R. Anderson, Jason D. Beane, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

Primary aromatic amines (PAAs) are compounds of interest that are routinely found in mainstream tobacco smoke. With the increasing interest in e-cigarette aerosol and liquid products, there is requirement for sensitive methods of measurement for PAAs when evaluating these matrices. The method described here is a modification of a method used for combustible cigarettes for PAA analysis in e-liquid and aerosol.

This method is a limit test, and utilizes a low level standard and a matrix spike to demonstrate realistic low level responses for comparison against samples. The low level standard is used as a clean matrix reference while the matrix spike represents a true representation of the responses of each analyte in various formulations of Electronic Cigarette e-liquid and aerosol. The target PAAs analyzed are 1-aminonaphthalene, 2-aminonaphthalene and 4-aminobiphenyl. Electronic cigarette aerosol is collected using a 44-mm Cambridge filter pad (CFP) which is then extracted with 5% HCL through both MCX and HLB cartridges. The final fraction is collected in toluene and derivatized with heptafluorobutyric anhydride. The sample is analyzed by GCMS using negative chemical ionization (NICI). The method has been validated as a limit test in concordance with the ICH guidelines.

20. SIMPLE METHOD FOR PARTICLE SIZE DISTRIBUTION DETERMINATION OF SMOKELESS TOBACCO PRODUCTS. Salem Chouchane, Paul A. Lineberry and Luke D. VanHorn; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

The particle size of smokeless products such as moist snuff can be used to characterize the products. The goal of this study was to determine the particle size distribution of different smokeless products. The particle distribution was determined using a simple sieve method and was validated. Validation parameters include precision, and intermediate precision. Challenges encountered in the method validation were in determining the appropriate sieve size and the degree of moisture in the samples.
22. VALIDATION OF A LIMIT TEST METHOD FOR BENZO(α)PYRENE IN ELECTRONIC CIGARETTE LIQUID AND AEROSOL BY LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION. Darius GRISSOM, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

Benzo(α)pyrene is on the list of HPHC’s in the recent FDA draft guidance for Premarket Tobacco Applications (PMTA) for Electronic Nicotine Delivery Systems (ENDS). Because this analyte is not typically detected in electronic cigarettes, a limit test offers an more efficient and cost effective analysis. A validated quantitative benzo(α)pyrene method for traditional combustible cigarettes was modified to include electronic cigarette liquid and aerosol matrices incorporating a limit test. The limit test approach reduces solution preparation, chemical cost, and analysts experimental and review time.

The method was developed using UPLC chromatographic separation through an Agilent Zorbax Eclipse XBD-C18 column, 4.6 x 50 mm, 1.8 μm particle size. The method has been validated and the results of the method validation will be reported.

23. VALIDATION OF A QUANTITATIVE METHOD FOR THE TRACE LEVEL ANALYSIS OF ETHYLENE GLYCOL AND DIETHYLENE GLYCOL IN ELECTRONIC CIGARETTE LIQUID AND AEROSOL SAMPLES. Darius GRISSOM, Joe Kennaday, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston Salem, NC, USA

USP employs a GC-FID method to test for the Limit of Diethylene Glycol and Ethylene Glycol in raw materials Prolylene Glycol and Glycerin (NMT 0.1%, each analyte). Eurofins Lancaster Laboratories has developed and validated a GCMS method that can report values down to trace levels in e-liquid (0.002% ethylene glycol, 0.003% diethylene glycol) and e-liquid aerosol (0.006% ethylene glycol, 0.003% diethylene glycol) products.

The method was developed using a GC/MS Chromatographic separation through a Restek Stabilwax column, 30m x 0.25mm x 0.25μm. This poster highlights the validation parameters used to successfully validate this method, the calibration range of the method, and critical factors discovered during the validation. One specific critical factor was the successful completion of recovery studies with high product variability.

24. VALIDATED METHOD FOR THE QUANTITATIVE DETERMINATION OF CARBONYLS IN ELECTRONIC CIGARETTE AEROSOL AND LIQUID. Nancy QIAN, Jonathan Wilkins, Angela Seamans, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

Carbonyls are typically found in tobacco and cigarette smoke. The Food and Drug Administration have included carbonyls in the list of HPHC’s in the recent draft guidance for premarket tobacco application for electronic nicotine delivery systems for both e-liquid and aerosol. The level of carbonyls in electronic cigarettes is typically found at trace levels and current methods used for analysis of carbonyls in tobacco and cigarette smoke are not sensitive enough to detect trace levels of carbonyls. As a result, Eurofins Lancaster Laboratories developed and validated a method to detect and quantitate trace levels of carbonyls in electronic cigarette e-liquid and aerosol. The carbonyls that are measured are formaldehyde, acetaldehyde, acrolein, and crotonaldehyde.

The Carbonyls are analyzed using a Waters Acquity UPLC with Waters Xevo MS/MS detector. The separation uses an Acquity UPLC BEH Shield RP18 column with 1.7 μm particle size, 100 mm length x 2.1 mm diameter. The Xevo TQ-S Triple Quadrupole with electrospray in negative ion mode and MRM is used for the detection. This method is validated to quantitate at an LOQ of 5ng/mL for Formaldehyde, Acetaldehyde, Acrolein, and Crotonaldehyde. The method validation parameters include specificity, linearity, precision, accuracy, range, robustness, solution stability and determination of LOD and LOQ.
25. TRACE LEVEL AMMONIA ANALYSIS IN E-LIQUID AND AEROSOL PRODUCTS. Nolan D. SPANN, Jonathan Wilkins, Angela Seamans, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

A method was established to detect trace levels of ammonia in E-liquid and E-cigarette aerosol matrices. The new method includes both a limit test and a quantitative analysis step if ammonia levels are present above the limit test level (50 ng/mL). The method was developed using Ion chromatography. Chromatographic separation was achieved using a Dionex™ IonPac™ CS16 column, 250 x 3 mm, 5 µm particle size. The method validation results will be presented.

26. VALIDATION OF A QUANTITATIVE METHOD FOR THE ANALYSIS OF ANABASINE IN ELECTRONIC CIGARETTE LIQUID AND AEROSOL. Norman E. FRALEY, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

Anabasine has been identified on both the FDA HPHC list for Tobacco Products and Tobacco Smoke as well as on the list of compounds for a Premarket Tobacco Application (PMTA) for Electronic Nicotine Delivery Systems (ENDS). With the increasing interest in electronic cigarette aerosol and liquid products, there is a need for quantitating low levels of anabasine. A method was developed and validated for the determination of the presence of anabasine in electronic cigarette liquid and aerosol using liquid-liquid extraction and GC-MS-MS. Samples are dissolved in alkaline aqueous salt solution, extracted with dichloromethane and quantified based on the anabasine MRM transitions. The method validation results will be reported.

27. VALIDATION OF A LIMIT TEST METHOD FOR SELECTED VOLATILE ORGANIC COMPOUNDS IN VAPOR BY GC-MS. Norman E. FRALEY, Carl J. Adams and Salem Chouchane; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

There is interest to analyze trace levels of 1,3-butadiene, isoprene, acrylonitrile, benzene and toluene, in electronic cigarette liquid and aerosol. These analytes are not typically present in these systems, so limit tests offer a more efficient and cost effective approach for analysis. A limit test method was developed and validated for 1,3-butadiene, isoprene, acrylonitrile, benzene and toluene in electronic cigarette liquid and aerosol using gas bubbler collection followed by analysis by GC-MS. Samples are dissolved in methanol and quantified based on the compound specific SIM transitions. The method validation results will be presented.

28. A COMPARISON OF A HIGH AND LOW RESOLUTION ICP-MS FOR THE ANALYSIS OF TOXIC METALS IN PLANT MATRICES. Derek D. BUSSAN1, Timothy L. Danielson2 and Karl A. Wagner2: 1Eurofins Lancaster Laboratories, Richmond, VA USA; 2Altria Client Services, Richmond, VA USA

Inductively coupled plasma mass spectrometry (ICP-MS) is the analytical tool of choice for routine analysis of trace metals. For this work, we determined nine elements (As, Be, Cd, Co, Cr, Hg, Ni, Pb, and Se) in plant matrices using a high-resolution magnetic sector instrument (HR-ICP-MS) and a quadrupole instrument that was equipped with a collision and dynamic reaction cell. These elements were targeted because they are listed on the FDA’s established list of Harmful and Potentially Harmful Constituents (HPHCs) for cigarettes and smokeless tobacco. As, Co, Cr, Ni, and Se are known to suffer from polyatomic and/or molecular spectral interferences which are commonly addressed using collision and/or reaction cell technology in quadrupole based systems. However, high resolution (HR)-ICP-MS instruments have the ability to resolve these polyatomic and/or molecular spectral interferences with a resolving power of 10,000 atomic mass units. The objective of this study was to compare the analytical figures of merit, i.e. limits of detection (LOD), precision, and accuracy using both types of mass spectrometers. Certified plant reference materials were used to evaluate the figures of merit. It was found that the quadrupole based instrument had superior detection limits for As and Se when using collision and reaction cell technology as compared to HR-ICP-MS due to the trade off in sensitivity when the HR-ICP-MS is operated in high resolution mode. However, the quadrupole ICP-MS had lower precision than the HR-ICP-MS for elements that do not suffer from polyatomic atomic interferences.
41. DETERMINATION OF SELECT VOLATILE ORGANIC HYDROCARBONS IN CIGAR SMOKE. Charles BROOKS; ITG Brands, Greensboro, NC, USA

Testing for select volatile organic hydrocarbons has been in common practice for cigarette smoke for many years. Recently, testing for cigar smoke has become of potential interest. While methods for smoking and analysis for TNCO for cigars are in place, no standardized methods exist for VO determination in cigar smoke. An analytical method was developed and applied to a range of cigar styles including machine-made little cigars (large filter cigars) and cigarillos, and larger hand-rolled products. Cigars were smoked according to existing cigar smoking regimes recommended by CORESTA for TNCO analysis (CRM No. 46, CRM No. 64, CRM No. 65). During smoking, the volatile smoke components were collected in methanol contained in -70°C cold-trapped impingers, to which the pad and 200 µL of Benzene-d6 ISTD was added prior to mixing and transferring aliquot to amber GC vials for analysis. A DB-5ms column (60m x 0.25µm x 1.0µm) connected to a DB-Wax column (1.1m x 0.25µm x 0.25µm) was used for separation with a constant flow rate of 1.2 mL/min. Extracts were analyzed for 1,3-butadiene, isoprene, acrylonitrile, toluene, benzene, and styrene via gas chromatography with single quad Mass Selective Detection in EI/SIM mode. In addition to determining the levels of analytes present in smoke for a range of cigars, the impact of lighting technique and conditioning time were explored.

42. COMPARISON OF PROBABILITY OF RISK ASSOCIATED WITH CIGAR EXPOSURE. Karshak KOSARAJU, Felix Ayala-Fierro and Rob Stevens; ITG Brands, Greensboro, NC, USA

Cigars are unique tobacco products of variable physical and chemical properties. Limited information is available about exposure to smoke and probability of risk, if any, to smoke constituents. Cigar smokers can be exclusive or dual smokers of cigars and conventional cigarette products. Literature data indicates that exclusive cigar smokers do not inhale smoke into their lungs in contrast to dual smokers. Approximately one-third of total cigar nicotine is taken into the smoker’s mouth as mainstream smoke and available for absorption mainly from the buccal mucosa. For dual smokers the overall exposure to a cigar is represented by both inhalation and buccal whereas for exclusive cigar smokers the overall exposure includes buccal and transdermal absorption through direct contact with lips. The objective of this study was to conduct a comparative probability risk assessment (PRA) of cigar products using cigar-specific “input” variables.

Quantitative risk assessment (QRA) of two cigar products resulted in differences of 10-30% (depending on the analyte) between products; however, probabilistic risk assessment (PRA) revealed the change was too small and may not be considered significant. In fact, PRA showed > 90% overlap between probability distributions of the two products. Sensitivity analysis suggested that smoking behavior and other exposure parameters contribute to > 90% of the observed variability associated with the analyte of concern between the products. Results overall indicates that PRA is a useful tool when comparing the probability of risk of cigar products.

43. ANALYSIS OF AROMATIC AMINES IN MAINSTREAM CIGARETTE AND CIGAR SMOKE BY GC-MS. Jeff ZHU, Charles Brooks and Lee Pittaway; ITG Brands, Greensboro, NC, USA

Polyaromatic amines have been routinely tested for cigarette smoke for several years. Typical methods employed require complex sample extraction and clean up procedures, long run times, and extensive instrument maintenance. We sought to develop an improved method for analysis of 1 & 2-aminonaphthalene and 3 & 4-aminobiphenyl with application to cigarette and cigar smoke testing. Simplified sample preparation steps employed in the 2016 CORESTA Aromatic Amines collaborative study for seven aromatic amines using a method from BAT – Souza Cruz were adopted for use with this method. Based on the referenced technique; steps for liquid-liquid extraction, neutralization, and drying and concentrating steps were eliminated. For rapid analysis of the target compounds, gc column choice and parameters were optimized to allow for a short run time while maintaining or improving selectivity and sensitivity over our previous internal method. The optimized method was demonstrated to be applicable to cigarette and cigar smoke. Results for a range of products along with validation results will be discussed. A comparison between existing methods and the new method will be provided, as well.
44. ASSESSING POPULATION HEALTH EFFECTS OF TOBACCO PRODUCT USE FOR REGULATORY COMPLIANCE: A COMPARISON OF AVAILABLE METHODS. Florian TEISCHINGER; JT International, Geneva, Switzerland, and Bill Poland; Certara USA, Menlo Park, CA, USA

The US FDA encourages use of statistical models to project effects of a new tobacco product on population health, for Modified Risk Tobacco Product Applications (MRTPAs) and Pre-Market Tobacco Applications (PMTAs). We review different methods chosen for recently developed models. “Cohort models” follow a single cohort through the death of each member, while full (“cross-sectional”) population models follow a population of mixed ages and tobacco exposures over a specified period, including births as well as deaths. Both types either calculate expected counts of members of each product use category, or use summary statistics from Monte Carlo simulation of tobacco use histories of many individuals. Output metrics include the difference in cumulative deaths between a reference scenario and a scenario where a new product is added, or may use measures such as smoking-attributable deaths or life years, perhaps quality-adjusted. Each choice has advantages; we give examples of each model type and review pros and cons.

Full cross-sectional population models have been criticized as overly complex, subject to bias, and limited to inadequately short time periods, unlike cohort models. However, we demonstrate that a full population model does not need not to have any of these limitations, and can even reduce to a cohort model if birth rates are set to zero (and initial ages are set to a single age if desired). Thus, additional complexity becomes the user’s choice. In return for the birth rate requirement, full population models project annual prevalence of use of each product and mortality. This allows validation to include running over historical years to check evolution of the total population, smokers, and former smokers, all with age and sex breakdowns.

45. HIGHLY SENSITIVE NICOTINE PLASMA AND METABOLITE ASSAYS FOR CLINICAL TRIALS BY LC-MS/MS. Gene RAY, Yansheng Liu, Marsha Luna, X. Steven Yan, Julie Showalter and Kimberly Jackson; KCAS Bioanalytical and Biomarker Services, Shawnee, KS, USA

Human plasma assays for nicotine and its key metabolites (cotinine and trans-3’-hydroxycotinine) have been validated in compliance with FDA guidelines in support of clinical trials. Success in establishing these assays is careful screening of all reagents and mitigating nicotine contamination throughout the laboratory. Two separate assays were validated for nicotine. A low curve, high sensitivity assay with quantitation limit of 0.2 ng/mL and high curve assay with a quantitation limit of 0.5 ng/mL.

The low curve nicotine assay had precisions (% CV) of ≤ 7.3% CV and accuracies (% bias) of ≤ 3.9% above the LLOQ. The precision and accuracy for nicotine at the LLOQ were 12.3% and 3.9%, respectively. The high curve nicotine assay had precisions of ≤ 4.4% and accuracies of ≤ 4.9% above the LLOQ; the precision and accuracy at the LLOQ were 8.6% and 4.6%, respectively. The precision and accuracy for cotinine and trans-3’-hydroxycotinine methods were comparable to that of nicotine.

Stability was documented for each analyte in plasma for 18.5 hr at ambient temperature, following 4 freeze/thaw cycles and for up to 48 days at -20 oC. Stability was also documented in whole blood for 1.5 hrs at room temperature.

Assay specificity was confirmed in the presence of menthol (as glucuronide metabolite) and in presence of lipemic and hemolytic plasma. These methods meets or exceeds FDA regulatory requirements and acceptable for clinical trial support.

47. THE IMPACT OF 2, 4-DINITROPHENYLHYDRAZINE (DNPH) BACKGROUND IN THE DETERMINATION OF CARBONYLS COMPOUNDS IN E-CIGARETTE VAPOR. Carmen DONISA, Peter Joza, Angel Rodriguez-Lafluente and William Rickert; Labstat International, Kitchener, ON, Canada

Carbonyl compounds, are known to be present in some e-liquid formulations and can be formed during aerosol generation. Analysis of these compounds, as their hydrazones (2,4-dinitrophenylhydrazine; DNPH) are routinely performed using high performance liquid chromatography (HPLC) with either ultraviolet
(UV) or tandem mass spectrometry (MS/MS) detection. However, the variability associated with the background carbonyl levels found in the DNPH solutions, can impact the interpretation of analytical data, particularly in long term stability and simulated use studies. Differences in background carbonyl levels have been found amongst DNPH suppliers, as well as variability from lot-to-lot and container-to-container. In recent years, increased background levels of carbonyls have been found for formaldehyde, acetaldehyde, acetone and methyl ethyl ketone (MEK). Background carbonyl levels (on a µg/collection basis) have ranged from 1.4 to 12.2 for formaldehyde, and non-detected to 10.7 for acetaldehyde. When analyzing low delivery products, it is increasingly difficult to distinguish between the background levels from the DNPH solution, background associated with the vaping environment, and that generated by the product during the vaping process. Consequently, DNPH is routinely re-crystallized in the laboratory. Although this can reduce levels of some compounds like formaldehyde, other compounds such as acetone and MEK may be present in trace amounts in the solvents used during the recrystallization process, increasing the background levels. Purchasing of a custom DNPH recrystallized 5 times by the supplier, was found to have comparatively low amounts of formaldehyde and acetaldehyde. DNPH hydrochloride has been evaluated as another approach to background reduction with some success.

48. A COMPARISON OF TWO DIFFERENT EXTRACTION METHODS FOR AROMATIC AMINES IN MAINSTREAM SMOKE USING GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (GC/QQQ). Andy STINSON1, Sherry Gilliland1, Andrew Huckins1, Sherri S. Brown2 and I. Gene Gillman2; 1Liggett Group, Mebane, NC, USA; 2Enthalpy Analytical, Durham, NC, USA

The established GC-MS methods for the analysis of aromatic amines (o Anisidine, o Toluidine, 2, 6 Dimethylaniline, 1 Aminonaphthalene, 2 Aminonaphthalene, and 4 Aminobiphenyl) involve complex sample preparation and analyzing the samples on a gas chromatograph with a single-quadrupole mass spectrometer. In this study, we have elected to use a triple-quadrupole mass spectrometer that greatly improves the selectivity of detection of the compounds of interest.

The objective of this study was twofold: (1) compare the establish two –step SPE extraction method verse a one-step extraction method and (2) compare the results using a triple-quadrupole mass spectrometer under electron impact ionization, a triple-quadrupole mass spectrometer using chemical ionization and the established single-quadrupole method (GC-MS).

We will present our finding that the triple-quadrupole mass spectrometer enables simplification of the extraction method offers superior limits of detection compared to the quadrupole mass spectrometer based standard method. We will present instrument/ method precision, accuracy and LOD & LOQ of both methods.

49. DETERMINATION OF NICOTINE, NNN, AND NNK IN A NEW CIGARETTE TOBACCO FILLER STANDARD REFERENCE MATERIAL (SRM 3222) AND ITS SMOKE. Walter B. WILSON, Jeanita S. Pritchett and Lane C. Sander; National Institute of Standards and Technology, Gaithersburg, MD, USA

The United States has an annual production of approximately 800 million pounds of tobacco with estimated domestic sales of around 260 billion cigarettes/year. The National Institute of Standards and Technology (NIST) has recently collaborated with the Center for Tobacco Products at the Food and Drug administration (FDA) to develop a new low nicotine tobacco filler Standard Reference Material (SRM 3222). SRM 3222 was prepared from air-cured, low nicotine tobacco and is intended primarily for use in evaluating the accuracy of current and new analytical procedures for measuring nicotine, N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl-1-butane (NNK), and moisture in cigarettes sold daily. The certified values for nicotine, NNN, and NNK are based on the results of measurements made by NIST, U.S. Centers for Disease Control and Prevention (CDC), and commercial laboratories using isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS) with different sample preparation techniques. Briefly, 0.250 g portions of finely ground SRM 3222 were vortex in a 15 mL polypropylene tubes with either 10 mL of 100 mM aqueous ammonium acetate or 5 mL of methanol extraction solution for a total of 1 h and 18 h, respectively. In the case of the smoke analysis, preliminary studies have been
conducted using gas chromatography/MS and LC-MS/MS to identify nicotine, NNN, and NNK in the tobacco smoke collected from burning approximately 10 g of SRM 3222.

50. MENTHOL CONTENT IN CANADIAN CIGARETTES. Ivana KOSARAC, M. M. Patel, A. L. Doane, R. Scholtz, N. Mladjenovic and T. K. Mischki; Health Canada, Ottawa, Canada

In 2009, the Government of Canada prohibited the addition of flavours to cigarettes as a measure to reduce the use of cigarettes by youth. At the time this prohibition did not include menthol, a flavour known to impart cooling and analgesic properties to cigarette smoke. Recently, the sale of flavoured tobacco has been prohibited in provinces such as Alberta, Nova Scotia, and New Brunswick and the Government of Canada has published a regulatory amendment to remove menthol from its list of permitted ingredients as of October 2, 2017. Currently, menthol cigarettes represent 4.7% of total cigarette sales in 2014. The objective of this study was to examine the menthol content and emissions from menthol cigarettes and compare this to new “green” branded cigarettes, introduced in the Alberta market after prohibition of flavourings in tobacco. Menthol content averaged 2.8 +/- 1.2 mg/g tobacco while menthol emissions averaged 0.38 +/- 0.16 and 1.1 +/- 0.30 mg/cigarette for ISO and Health Canada emissions protocols respectively. “Green” branded cigarettes had below LOD and LOQ levels of menthol in tobacco and emissions measurements. Nicotine and tar composition in tobacco and emissions varied across all brands but were comparable to previously analyzed non-menthol cigarettes.

52. BACILLUS SPP ISOLATED FROM SMOKELESS TOBACCO PRODUCTS. Casandra WEST1 and Amy Devlin2; 1RAI Services Company, Winston-Salem, NC, USA; 2R. J. Reynolds Tobacco, Winston-Salem, NC, USA

Globally, there are an estimated 300 million users of smokeless tobacco. Since smokeless tobacco products are placed in direct and prolonged contact with the oral mucosa, researchers are beginning to evaluate the associated microbiomes of such products. Presently, there have been no reports describing the intra-species diversity of cultivable (viable) bacilli isolated from smokeless tobacco and very few studies describing intra-species Bacillus diversity in general. Yet, over 90% of cultivable microorganisms from tobacco products belong to the genus Bacillus. This group of organisms is ubiquitous and incredibly diverse. Bacillus species have been isolated from soil, air, sediment, aquatic and marine environments as well as from humans, cattle, fish and birds. There are over 170 species recognized to date and countless subspecies; many of which have important industrial, agricultural and medicinal uses. Several strains have been employed as probiotics for humans, livestock and aquaculture operations. Recently, marker gene amplification meta-genomic studies have been applied to evaluate these products and can support paths to more specific genetic investigations. Such studies provide valuable, all-encompassing views of the DNA of bacterial families that may be present in smokeless tobacco products; however, the presence of DNA does not necessarily indicate the presence of a living organism. Herein, this investigation focuses on the characterization of representative Bacillus strains isolated from smokeless tobacco products through evaluations of inter- and intra-species relatedness, genetic similarity and relatedness to previously studied and published strains.

53. ASSESSMENT OF FLAVOR TRANSFER TO AEROSOLS OF ELECTRONIC NICOTINE DELIVERY SYSTEMS. Courtney G. CULBERT1, David T. Szabo1 and Amanda G. Hudson2; 1RAI Services Company, Winston-Salem, NC, USA; 2R.J. Reynolds Tobacco, Winston-Salem, NC, USA

In the evaluation of electronic nicotine delivery systems (ENDS), reliable analytical methods are needed to acquire flavor data for consumer exposure risk assessment calculations. Without accurate transfer data, the conservative default assumption that 100% of the flavor added to the e-liquid is being transferred to the aerosol may be an overestimation. Flavor transfer values can be calculated by comparing the amount of a flavor in an e-liquid to the amount observed in a generated aerosol. These flavor amounts can be determined using both quantitative and semiquantitative gas chromatography mass spectrometry (GC-MS) methods. In the quantitative method, absolute flavor amounts are calculated using known standards, while a semiquantitative approach estimates the amount of flavor by comparing to an internal standard. Because the inherent error associated with semiquantitation of flavors in both e-liquids and aerosols is expected to be similar, the semiquantitiated flavor transfer values would also be expected to be similar to the quantitated
flavor transfer values. Testing this assumption on a variety of flavors, the flavor with the lowest transfer between e-liquid and aerosol was measured at 18% (quantitated) versus 17% (semiquantitated), while the flavor with the highest transfer was 78% (quantitated) versus 80% (semiquantitated). This trend held for all flavors investigated, suggesting that using flavor transfer data from the semiquantitative analysis would be predictive of quantitative analysis. Since semiquantitation is less labor and cost intensive than quantitation, semiquantitative flavor transfer studies are a valuable tool for the evaluation of ENDS flavors and consumer exposure.

54. CHARACTERIZATION OF INHALATION EXPOSURE TO CIGARETTE SMOKE. Charlene LIU and Kristin Marano, RAI Services Company; RAI Services Company, Winston-Salem, NC, USA

Quantitative risk assessment (QRA) may inform regulatory decisions regarding tobacco products. Evaluation of human health risks from cigarette smoking requires an adequate assessment of the exposure, which is a challenging task in part because the concentration of chemicals in the respiratory tract is not constant. No regulatory guidance currently exists for exposure assessment of tobacco products, although examples exist in the peer-reviewed literature. The U.S. Environmental Protection Agency (EPA) provides guidance that addresses methods for quantitative evaluation of exposure and risk, which is useful and can be applied to tobacco products. Two different methods were developed to quantify inhalation exposure with machine-generated smoke yields from a market survey of U.S. cigarettes. The first method treats exposure to a chemical in smoke as a continuous process and estimates an exposure concentration by averaging the yields of the chemical from cigarettes consumed over the average daily volume of air inhaled by a user. The second method treats exposure to the chemical as discrete smoking sessions and estimates a respiratory concentration of the chemical via summation of discrete smoking sessions over the course of a day. Both methods incorporate standard exposure parameters to derive an upper-bound lifetime average exposure to the chemical. For simplicity and conservatism, both methods assume 100% retention of the chemical in the smoker’s body. The two methods provide risk estimates with relative percent differences within 30%; the first method was more conservative (i.e., risk-maximizing). Exposure assessment of tobacco products should be consistent with available evidence, guidance, and state of the science for risk assessment. These findings indicate that incremental modifications to exposure input assumptions do not materially affect the QRA results.

55. QUANTITATIVE RISK ASSESSMENT: THE PROPOSED NNN STANDARD. Kristin MARANO1, P. Robinan Gentry2, and Charlene Liu1; 1RAI Services Company, Winston-Salem, NC, USA; 2Ramboll Environ, Monroe, LA, USA

The US Food and Drug Administration (FDA) published a proposed standard for N-nitrosonornicotine (NNN) content in finished smokeless tobacco (ST) products. The proposed NNN limit of 1 part per million dry weight (ppm dw) was derived based on a target excess lifetime cancer risk (ELCR), although the rule states the increased risk of oral cancer was the impetus for the rule. A review of the quantitative risk assessment (QRA) conducted by FDA was undertaken, with findings from this review indicating deficiencies in FDA’s QRA. First, the methods used to estimate the NNN cancer slope factor are inconsistent with derivation methods recommended by the US Environmental Protection Agency (EPA) and methods supported by FDA. Second, key input assumptions (i.e., body weight and lifespan) are inconsistent with current EPA recommendations in risk assessment practice. Third, the absorption factor estimated by FDA was incomplete. Fourth, the estimated ELCR in the proposed rule conveys an unrealistic level of precision, inconsistent with EPA recommendations. Finally, it is not clear that an ELCR calculation is relevant for the establishment of an NNN limit: ELCR is inadequate as a measure of excess cancer deaths in the population, because it does not account for competing mortality. However, if the ELCR is calculated in accordance with current EPA risk assessment guidance, an NNN level ≥5 ppm dw would result. This range is consistent with, or lower than, historical NNN levels in the ST products used by participants in Swedish epidemiological studies demonstrating no meaningful increase in oral cancer risk. There is no evidence that suggests setting the product standard to a ≥5-fold lower concentration of NNN will further reduce cancer risks in ST users.

Note that the 71st TSRC had to be rescheduled due to a hurricane. As a result, several papers were withdrawn, and several had to be presented by the author’s colleague.
56. EPIDEMIOLOGICAL EVIDENCE AND FDA’S PROPOSED NNN STANDARD. Tiffany PARMS\textsuperscript{1}, Sandra Sulksy\textsuperscript{2}, Greg Mariano\textsuperscript{3} and Kristin Marano\textsuperscript{1}; \textsuperscript{1}RAI Services Company, Winston-Salem, NC, USA; \textsuperscript{2}Ramboll Environ, Amherst, MA, USA; \textsuperscript{3}Ramboll Environ, Arlington, VA, USA

The US Food and Drug Administration (FDA) has advanced a proposed product standard of 1µg/g N-nitrosonornicotine (NNN) content in finished smokeless tobacco (ST) products, citing selected findings from epidemiology to support the proposed limit. An independent review of FDA’s application of epidemiological literature identified a number of inaccuracies. First, FDA combines oral cancer relative risk (RR) estimates for men and women, which is inappropriate given the RR between genders are widely different. Furthermore, men are the predominant users of ST. Second, FDA relied upon Swedish epidemiology to indicate current (low) levels of NNN in modern Swedish ST products are not associated with increased risk of oral cancer; however, NNN levels in Swedish ST in use during the time of the epidemiology studies were higher than levels in current products. Third, FDA relied on studies of international ST products (e.g., Asia and Africa), yet the composition and use behaviors associated with ST products unique to Asia and Africa differ markedly from those of US products, and are not relevant to US ST products and users. Fourth, NNN concentrations in products used by study participants in the available epidemiology studies cannot be estimated precisely, and there is substantial heterogeneity in the concentration of NNN and other toxicants across and within ST product types. Finally, FDA’s conclusion that NNN is the predominant driver of excess oral cancer risk among ST users is inconsistent with existing scientific data, as urinary levels of NNN are generally higher among ST users compared with smokers, yet smokers incur a substantially higher risk for oral cancer than ST users. Thus, considered objectively, the available epidemiology data do not support the proposed NNN standard.

57. AKR1C1 AS A BIOMARKER FOR DIFFERENTIATING THE BIOLOGICAL EFFECTS OF COMBUSTIBLE FROM NON-COMBUSTIBLE TOBACCO PRODUCTS. Quynh T. TRAN\textsuperscript{1}, Sangsoo Woo\textsuperscript{2}, David Henderson\textsuperscript{3}, Hong Gao\textsuperscript{3}, Wolfgang Zacharias\textsuperscript{3}, Gang Liu\textsuperscript{1} and G.L.Prasad\textsuperscript{1}; \textsuperscript{1}RAI Services Company, Winston-Salem, NC, USA; \textsuperscript{2}Axio Research, Seattle, WA, USA; \textsuperscript{3}University of Louisville School of Medicine, Louisville, KY, USA

Smoking has been established as a major risk factor for developing oral squamous cell carcinoma (OSCC), but less attention has been paid to the effects of smokeless tobacco products. Our objective is to identify potential biomarkers to distinguish the biological effects of combustible tobacco products from those of non-combustible using oral cell lines. Normal human gingival epithelial cells (HGEC), non-metastatic (101A) and metastatic (101B) OSCC cell lines were exposed to different tobacco product preparations (TPPs) including cigarette smoke total particulate matter (TPM), whole-smoke conditioned media (WS-CM), smokeless tobacco extract in complete artificial saliva (STE), or nicotine (NIC) alone. We performed microarray-based gene expression profiling and found 3456 probe sets from 101A, 1432 probe sets from 101B, and 2717 probe sets from HGEC to be differentially expressed. Gene Set Enrichment Analysis (GSEA) revealed xenobiotic metabolism and steroid biosynthesis were the top two pathways that were upregulated by combustible but not by non-combustible TPPs. Notably, aldo-keto reductase genes, AKR1C1 and AKR1C2, were the core genes in the top enriched pathways and were statistically upregulated more than 8-fold by combustible TPPs. Quantitative RT-PCR results statistically support AKR1C1 as a potential biomarker for differentiating the biological effects of combustible from non-combustible tobacco products.

58. THE TOXICOLOGICAL EVIDENCE FOR THE PROPOSED NNN STANDARD. Robinan GENTRY\textsuperscript{4}, Tracy Green\textsuperscript{1} and Kristin M. Marano\textsuperscript{3}; \textsuperscript{1}Ramboll Environ, Monroe, LA, USA; \textsuperscript{2}R. J. Reynolds Tobacco, Winston-Salem, NC, USA

The US Food and Drug Administration (FDA) has published a proposed standard for N-nitrosonornicotine (NNN) in smokeless tobacco (ST) products. An independent review of the toxicological evidence was conducted. Results of this review suggested that FDA failed to consider relevant scientific evidence. First, NNN concentrations associated with increased incidence of cancer in animals are much larger than expected human exposure from use of ST products. Second, differences in metabolism of NNN have been shown across species, which is important in understanding the relevance of animal results to humans. Third, there is no evidence of concordance between target tissue tumor formation across species to support
conclusions regarding the overall toxicological evidence for oral and esophageal tumors. Fourth, esophageal tumors reported in rats, and characterized as malignant in the proposed rule, are largely benign, based on reported histopathological evaluations, and may not be representative of oral cancer in humans; when only malignant tumors are considered, the incidence of oral tumors is statistically increased in only one of five studies. Fifth, there were no repeated dose oral animal toxicity studies identified that included multiple dose treatments; therefore, there is a lack of evidence to describe the dose-response relationship between NNN oral exposure and oral cancer. Finally, in the only published study conducted according to methods comparable to Organization for Economic Cooperation and Development (OECD) guidelines for chronic toxicological assays, rats exposed to NNN in diets containing actual ST or ST extract showed no tumors of the oral cavity, esophagus, or pharynx. Based on an assessment of the available toxicological data, evidence does not support that the proposed NNN limit in ST products would be protective of public health.

59. MEASURING PARTICLE SIZE DISTRIBUTIONS OF ELECTRONIC CIGARETTE AEROSOLS UNDER VARIOUS PUFFING FLOW RATES AND DILUTION RATIOS. Chen SONG; Reynolds American, Winston-Salem, NC, USA

Particle size distributions (PSD) of electronic cigarette (e-cigarette) aerosols have lately been measured using a variety of instruments, such as electric mobility instruments, light scattering and extinction instruments and cascade impactors. These studies usually diluted the e-cigarette aerosol samples significantly to avoid exceeding the detection thresholds of the instruments. The measured e-cigarette aerosol particle size was therefore smaller due to the volatile nature of the chemical composition of e-cigarette aerosol. In addition, various puffing flow rates and e-cigarette liquid chemical compositions were used. The purpose of the current study is to systematically investigate the changes of e-cigarette aerosol PSD under different dilution ratios and puffing flow rates. The e-cigarette aerosols were generated using both ciga-like and tank type e-cigarettes. Dilution ratios ranged from 1:1 up to 1:10, while three different puffing flow rates were tested. The test matrix also included e-cigarette liquid chemical compositions prepared with different propylene glycol and glycerin ratios. A multi-angle light scattering instrument and a mini-MOUDI cascade impactor will be employed to measure the e-cigarette aerosol PSD. Results from preliminary experiments showed that e-cigarette aerosol particle size decreased with increasing puffing flow rates. Furthermore, a slight decrease in the e-cigarette aerosol particle size was observed under low dilution ratios. The findings of this study have implications for accurately measuring e-cigarette aerosol particle size and the dynamic changes of e-cigarette aerosol under various conditions such as the human respiratory tract.

60. HANDMADE PREMIUM CIGARS SMOKE EMISSIONS - LIMITATIONS RELATED TO TNCO DETERMINATION VARIABILITY. Beatrice TEILLET1, Christian Schulz2 and Stephane Colard1; 1SEITA, Fleury-les-Aubrais, France; 2Reemtsma Cigarettenfabriken Hamburg, Germany

To date, few studies have been published for handmade premium cigars. The assembly process of a premium cigar involves combining natural leaves which are then rolled by hand. This results in a variable product. In this study, a range of handmade premium cigars were analysed for tar, nicotine and carbon monoxide (TNCO) according to CORESTA recommended methods. These methods were initially developed for testing machine made cigars. Smoke testing of cigars by these methods was challenging and led to variable results. Challenges were faced due to variation in the diameters of the cigars, measured at 33 mm from the mouth end after cutting. Discrepancies between calculated puff volumes of up to 3 mL per puff were observed for a given ring gauge. In addition, puff counts were highly variable, as high as double for a given product. The yield variability observed ranged from 52 to 160% for tar, 60 to 160 % for nicotine and from 32 to 120% for carbon monoxide.

As a consequence of the substantial variability when smoked, it was not possible to distinguish between different handmade premium cigars, with the exception of the TNCO value for the smallest cigar format when compared to a large cigar. The limitations of TNCO results for premium cigars and the need for method refinement and further investigations are discussed.
The U.S. Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for collecting Federal excise taxes on tobacco products. Tobacco products in the U.S. may fall into several taxable categories including cigars, cigarettes, sniff, chewing tobacco, pipe tobacco and roll-your-own. The existence of these taxable categories means that the TTB is also responsible for the determination of proper tax classification. Not only does proper classification determine the amount of tax owed, comprehensive classification procedures must also determine if a consumer product is subject to the tobacco excise tax. Since a product must contain tobacco to be subject to the excise tax, laboratory methods that test for the presence of tobacco can provide useful information to ascertain taxable status of a product.

To test for the presence of tobacco, an analytical method was developed that permits the simultaneous determination of nicotine and related alkaloids, tobacco specific nitrosamines (TSNA), and solanesol in methanolic extracts of tobacco. The method utilizes ultra performance liquid chromatography with electrospray ionization – tandem mass spectrometric detection (UPLC-ESI-MS/MS) and was optimized for the analysis of nicotine, cotinine, nornicotine, anatabine, myosmine, anabasine, isonicotene, nornicotyrine, nicotyline, N-nitrosanatabine (NAT), N-nitrosanabasine (NAB), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK), N’-nitrosonornicotine (NNN) and solanesol. The analytical method was designed to attenuate the instrument response of nicotine, which is overwhelming, to permit simultaneous analysis of all analytes.

**67. DEVELOPMENT OF AN ANALYTICAL METHOD TO DETECT METHYL SALICYLATE IN HUMAN PLASMA.** Sandra MILLER\(^1\), Kirk Newland\(^2\), Jianmin Liu\(^1\) and Mohamadi Sarkar\(^1\); \(^1\)Altria Client Services, Richmond, VA, USA; \(^2\)Celerion, Lincoln, NE, USA

Wintergreen is used as a common flavor in many moist smokeless tobacco (MST) products and is also widely used as a food flavorant as well as an over-the-counter analgesic to relieve muscle aches. MST products, including wintergreen flavored products, have been in the commercial market for decades. Unlike food, MST is not ingested and it is important to understand how much of the flavor is absorbed during use. Therefore, a method of detecting methyl salicylate (the main chemical component of wintergreen flavor) in human plasma is needed to estimate the absorption of this compound during use of wintergreen flavored MST. We report a novel high sensitivity LC-MS/MS method for the determination of methyl salicylate in human plasma. An aliquot (0.0750 mL) of human plasma containing the analyte and internal standard, d4-Methyl Salicylate is extracted using a protein precipitation with derivatization procedure. The extracted samples are analyzed by an HPLC equipped with an AB SCIEX API 4000™ triple quadrupole mass spectrometer using an ESI source. Positive ions are monitored in the multiple reaction monitoring (MRM) mode. Quantitation is determined using a weighted linear regression analysis (1/concentration2) of peak area ratios of the analyte and internal standard. The range of quantification is 1.75 - 50.0 ng/mL. In a clinical study, peak plasma levels during a single 40-minute use of 2 g wintergreen flavored MST ranged from < LOQ - 4.62 ng/mL. Development of this method is an important step in being able to assess the absorption of methyl salicylate during wintergreen MST use. This method also has broader application and may be used to characterize methyl salicylate uptake from other consumer products as well.

**68. THE SCIENTIFIC EVIDENCE FOR THE PROPOSED NNN STANDARD.** Kristin MARANO\(^1\), Charlene Liu\(^1\), Geoffrey M. Curtin\(^1\), Tiffany Parmis\(^1\), Annette Bachand\(^2\), Sandra I. Sulsky\(^2\), Greg Mariano\(^2\), Tracy Greene\(^3\) and P. Robinan Gentry\(^3\); \(^1\)RAI Services Company, Winston-Salem, NC; USA, \(^2\)Ramboll Environ, Amherst, MA, USA; \(^3\)Ramboll Environ, Monroe, LA, USA

The US Food and Drug Administration (FDA) published a proposed standard of 1 µg/g (dry weight) N-nitrosonornicotine (NNN) in finished smokeless tobacco (ST) products. FDA’s rationale for the standard is that NNN is a potent carcinogen, NNN is a major contributor to the elevated cancer risks associated with ST use, and establishing such a limit is appropriate for the protection of public health. However, a public health benefit associated with the proposed NNN limit is not supported by the available scientific evidence,
which includes toxicological and epidemiological data, results of quantitative risk assessment (QRA), and modeling projections of population mortality based on potential changes in use behaviors. Specifically, animal experimental designs, including tested exposure concentrations and observed outcomes, are not relevant to humans. Historical levels of NNN in Swedish products used to estimate health risks, and which demonstrate no meaningful risk of oral cancer, were well above the proposed standard. FDA’s QRA approaches do not conform with US Environmental Protection Agency recommendations in risk assessment practice, and results are implied to be representative of oral cancer risk in humans when they are not. Finally, changes in use behaviors likely to be associated with the proposed standard - and the resulting impact on overall population health - are not appropriately considered by FDA. Realistic scenarios for use behaviors likely to result from the proposed standard (e.g., increased use of cigarettes) suggest an increase in mortality that offsets the benefits proposed. Based on an independent evaluation of the epidemiological and toxicological evidence, QRA, and likely effect on population health, the proposed limit of NNN in ST products would not benefit, or otherwise be protective of, public health.

69. CRITICAL ASSESSMENT OF THE ANALYTICAL METHODS MEASURING NNN IN SMOKELESS TOBACCO PRODUCTS. Candice K. CUNNINGHAM; RAI Services Company, Winston-Salem, NC, USA

In January 2017, the U.S. Food and Drug Administration (FDA) issued the proposed rule, “Tobacco Product Standard for N-nitrosornicotine (NNN) in Finished Smokeless Tobacco Products.” Within the proposed rule, FDA recommended an analytical method for measuring NNN in smokeless tobacco products (with provisions for “alternative methods”). The FDA’s laboratory information bulletin (LIB), “Determination of N-nitrosornicotinie in Smokeless Tobacco and Tobacco Filler by HPLC-MS/MS” referenced CORESTA Recommended Method (CRM) 72, an established, consensus method for analyzing smokeless tobacco products to quantify NNN.

The release of the proposed standard, including the LIB, highlights the need for readily available, robust, high-throughput analytical methods for determination of NNN in order to comply with regulatory requirements. This presentation will discuss the figures of merit and the challenges of analytical methods for determining NNN in the smokeless tobacco matrix as it relates to the proposed product standard and analyses currently performed in the tobacco industry. Discussion will include sample handling protocols, analytical instrumentation selection, moisture versus water content determination, and dry weight basis NNN calculations. FDA’s LIB will be compared to well-established methods such as CRM 72, CRM 76, and those available through the Centers for Disease Control. Additionally, best practices regarding robust method development will be assessed (ASTM, FDA draft guidance, etc.) as these relate to our shared understanding of future proposed standards in a regulated industry.

70. GENETIC APPROACHES AND RESOURCES FOR ENABLING COMPLIANCE TO PROPOSED NNN STANDARDS. Ralph E. DEWEY1, Ramsey S. Lewis1, Jianli Lu1, Lucien Bovet2, Simon Goepfert2 and Prisca Campanoni2; 1North Carolina State University, Raleigh, NC, USA; 2Philip Morris International, Neuchatel, Switzerland

Tobacco-specific nitrosamines (TSNAs) are formed through the nitrosation of tobacco alkaloids. Over the past decade, we have demonstrated how altering the genetics of the tobacco plant can result in dramatic reductions in TSNA formation. This has been accomplished by reducing either the levels of the alkaloid precursor of a given TSNA, or the nitrosating agent involved. By introducing, then combining, mutations in the three nicotine demethylase genes responsible for production of the NNN precursor nornicotine, we have observed reductions in the levels of nornicotine and NNN in air-cured tobacco leaves by as much as 80% in comparison to varieties lacking these mutations. As an alternative strategy, we have demonstrated that the expression of a constitutively active nitrate reductase enzyme can mediate substantial reductions in all classes of TSNAs within the leaves of air-cured plants. In addition to reviewing these previously published means of minimizing NNN accumulation, we will also present the results of a new strategy of TSNA reduction. By inhibiting the expression of a specific nitrate transporter gene, we observed an approximate 66% reduction in NNN within air-cured leaves. An additional advantage of the latter two strategies for TSNA reduction is that along with NNN, substantial reductions are observed in the levels of NNK, another TSNA that could be the subject of mandated reductions by the FDA in the future. The
utilization of tobacco varieties containing gene mutations that greatly decrease the propensity for NNN production likely represents the most effective means for ensuring compliance to NNN standards while retaining important leaf quality characteristics.

71. NNN LEVELS IN STABLE REDUCED CONVERTER (SRC) AND LC LINES CURED UNDER CONDITIONS THAT FAVOR NNN FORMATION. Marcos LUSSO, Kenworth Lion, Andrew Adams, Whit Morris, Ujwala Warek and James Strickland; Altria Client Services, Richmond, VA, USA

N-nitrosonornicotine (NNN), a tobacco specific nitrosamine (TSNA) found in cured leaf, filler and smoke, is formed by nornicotine nitrosation. We previously demonstrated a ~75% reduction of cured leaf NNN content in burley and dark tobaccos having stable reduced convertor status (SRC) compared to Low Converter plants (LC). Here we present NNN data for burley and dark tobacco SRC and LC lines cured under normal conditions (control) and under conditions favourable for TSNA formation (experimental). SRC and LC burley were housed in a control barn and air cured according to recommended practices. Additionally, an experimental burley barn was packed with about ~1/3 more SRC and LC burley tobacco plants than the control barn and managed to retain high relative humidity during curing. For dark tobacco, SRC and LC lines were housed in a control barn and fire cured according to recommended practices. An experimental Dark barn was packed with SRC and LC Dark tobacco and cured at temperatures up to 173˚F having open flames during curing. At the end of curing, leaf samples were collected and analyzed for TSNA. SRC plants had ~63% and 64% less NNN than LC plants in control barns for burley and dark tobacco, respectively. NNN levels increased in SRC and LC tobaccos when cured in experimental barns compared to control barns. However, SRC NNN levels in experimental barns were ~76 and 61% lower relative to LC plants cured under identical conditions for burley and dark tobacco, respectively.

72. N-NITROSONORNICOTINE REDUCTION IN DARK TOBACCO VARIETIES AND SMOKELESS PRODUCT PROTOTYPES. Marcos LUSSO, Andrew Adams, Benjamin Lewis, Timothy Poyner, Kenworth Lion, Lionel DeLoach, Timothy Danielson, James Franke, Ujwala Warek and James Strickland; Altria Client Services, Richmond, VA, USA

During the past decades there has been a substantial effort to reduce the level of the tobacco specific nitrosamine N-nitrosonornicotine (NNN) and its precursor nornicotine in tobacco and tobacco products. Research on the mechanism of nornicotine formation led to the identification of three tobacco genes (CYP82E4, CYP82E5 and CYP82E10) encoding for cytochrome P450 nicotine demethylases that convert nicotine to nornicotine. Through conventional breeding, we developed dark tobacco varieties (Stable Reduced Converter/SRC varieties) containing the three non-functional nicotine demethylase genes. Tobacco varieties containing this new technology, named ZYVERTT™ technology, were grown on-of-farm experiments and, leaf produced from these varieties, were used to make smokeless product prototypes. Control product prototypes were produced using leaf from commercial low converter (LC) varieties. Our results indicate that the presence of non-functional alleles of the three genes reduces the level of nornicotine and NNN in dark tobacco varieties by about 70% compared to the level observed in commercial LC dark tobacco varieties. The reduced levels of NNN in the cured leaf of SRC varieties was also observed in smokeless product prototypes.

73. COMPARISON OF INTRA-CROP YEAR VARIABILITY IN NNN IN TOBACCO AND NNN LEVELS IN SMOKELESS TOBACCO PRODUCTS. Michael J. OLDHAM, David J. Phillips, Jason L. Jordan, Michael J. Morton, Kenworth E. Lion III, Marcos F. Lusso, James E. Franke and James A. Strickland; Altria Client Services, Richmond, VA, USA

Although tobacco is an agricultural crop, little has been published on the variability in NNN levels in tobacco grown within a single crop year (intra-crop year variability) by multiple growers. Furthermore, the potential impact of intra-crop year variability of NNN levels in tobacco on NNN levels in smokeless tobacco products is unknown. In this work, NNN levels were measured in dark air-cured, dark fire-cured and burley tobaccos used to produce smokeless tobacco products over either a six or ten crop year period. Additionally, NNN levels were measured in nine smokeless tobacco products manufactured over seven years. Only results from smokeless tobacco products manufactured using a single crop year of tobacco were included. NNN measurements over ten crop years for dark air-cured and dark fire-cured tobacco and
over six years for burley tobacco demonstrated a 10 fold range (lowest concentration to highest concentration) within a single crop year. Mean NNN levels exceeded 1ppm in dark-fire cured and burley tobacco in every year tested and 6 of 10 years for dark-air cured tobacco. Depending on the specific smokeless tobacco product and year, the measured NNN levels generally varied from three to six fold within a single crop year. Over the seven years, mean NNN levels (dry weight basis) exceed 1ppm for the vast majority of smokeless tobacco product measurements. The demonstrated variability in measured levels of NNN in smokeless tobacco products, due to natural variability in levels of NNN in tobacco, provides significant insight about measured NNN levels among smokeless tobacco products.

74. THE ENANTIOMERIC COMPOSITION OF N'-NITROSONORNICOTINE IN AIR-CURED TOBACCO INFLUENCED BY NICOTINE DEMETHYLATION. CAI Bin1, Huihua Ji2, Franklin F. Fannin1, and Lowell P. Bush2; 1Guizhou Academy of Tobacco Science, Guiyang City, China; 2University of Kentucky, Lexington, KY, USA

N'-Nitrosonornicotine (NNN) is a potent and organ-specific carcinogen found in tobacco and tobacco smoke in substantial amounts. The aim of this study was to investigate the variations in NNN enantiomeric composition in nicotine demethylase mutants. A gas chromatography/thermal energy analyzer method using two columns in series was developed to separate the enantiomers of NNN, N'-nitrosoanabasine, and N'-nitrosoanatabine. Tobacco lines with different combinations of three nicotine demethylases inhibited were grown in the field. Air-cured leaves were analyzed for the concentration and enantiomeric composition of nicotine, nornicotine and NNN. In mutant lines, the concentration of NNN ranged from 0.44 μg g-1 to 13.63 μg g-1 and the percentage of (R)NNN of total NNN varied from 7% to 69%. The measured NNN had the same enantiomeric composition as nornicotine, rather than nicotine, even when the level of nornicotine was reduced to 0.6% of nicotine in a triple mutant line. The pattern of the enantiomeric composition of nicotine, nornicotine, and NNN demonstrated that the direct formation of NNN from nicotine, if it occurs, is negligible in air-cured tobacco. Since (S)NNN is dominate form in smokeless tobacco and more carcinogenic than its R form, the reduction of (S)-nornicotine should be a priority for the reduction of NNN.

76. THE POWER OF TACTILITY: TIPPING PAPER IN INTERACTION WITH THE CONSUMER. Michael LINDNER and Eike Schopper; Tannpapier, Traun, Austria

Tipping Paper plays a highly essential role for the manufacture of filter cigarettes as it connects the filter plug with the tobacco rod, controls the level of smoke yields via Tipping perforation and acts as design tool for the customized branding of cigarettes. However, besides these technical and visual aspects, Tipping is also the only component of a cigarette which is in direct contact with the lips of consumers. Since the human lips are rather sensitive to mechanical and physical surface properties of touching objects, Tipping Paper offers perfect opportunities to interact with smokers by stimulating the tactile senses of the labial areas. Hereby, the first idea is to use the lip-release effect of commercially applied Tipping Papers to generate a comfortable impression during the smoking process. While lip-release by means of transparent varnishes or color coatings is a state-of-the-art feature, the recently introduced Super Lip-Release Tipping comprises superior hydrophobic characteristics for an outstanding smoking experience for different smoking habits. In this context, the water-repellent strength of Super Lip-Release and standard Tipping Paper will be demonstrated and compared with a mathematical absorption model and a small survey amongst regular smokers. The second approach to communicate with the consumer is to activate the haptic perception on the lips and fingers. Based on a sophisticated embossing technology, Textured Tipping represents a smart way of realizing an extraordinary cigarette mouthpiece which comprises unique haptic surface patterns on its Tipping Paper. Using various Textured Tipping samples, potential application and design options as well as structural limits will be thoroughly discussed. As a conclusion, Super Lip-Release and Textured Tipping are powerful examples to indicate the fusion of technical functionality and appealing tactility.
AN APPROACH TO IDENTIFY FACTORS RELATING TO CHANGES IN CHEMICAL CONSTITUENTS IN CURED TOBACCO LEAF DURING AGING. Atsushi NAGAI and Daichi Mochizuki; Japan Tobacco, Yokohama, Japan

The aging process, the long-term preservation process which takes place before cigarette production, improves the aroma/taste compared with non-aged tobacco leaves. In attempts to understand the reaction mechanism of chemical changes in the aging process, many studies have been conducted. Three factors (chemical reaction, enzymatic reaction and microbial action) have been thought to be causative in the changes. However, it is still unclear which factor is dominant in the chemical changes, because of the difficulty in distinguishing between the effects of the three factors. This research intended to identify dominant factors relating to chemical changes during aging. We report on: (i) preparation of model samples that could distinguish between the three factors, (ii) an aging test using the model samples, and (iii) identification of dominant factors related to changes in each chemical constituent during aging.

Flue-cured Virginia, cultivated and cured in Japan, was used for this test. Samples of the leaves were pre-treated with super-heated steaming (SHS) and/or electron beam irradiation (EB) before the aging test. SHS preferentially deactivated enzyme rather than microbe. In contrast, EB successively sterilized, without enzyme deactivation. All of the samples were aged simultaneously at 22°C / 60% RH for 6 months, or at 33°C / 60% RH for 1 month. The pre-aging/post-aging changes in several chemicals including sugars, organic acids, amino acids, and alkaloids, were observed. The dominant factors relating to the changes in each constituent could be estimated from the differences in the pattern of change between model samples. Results indicated that most of the changes in the constituents analyzed in this experiment were produced mainly by chemical reaction, or by the combination of chemical reaction and enzymatic reaction.

DEVELOPMENT OF A MULTI-STAGE LIQUID EXTRACTION METHOD FOR THE QUANTITATIVE ANALYSIS OF FLAVORS IN TOBACCO FILLER. John A MATHIS, R. A. Stanelle and J.-M. D. Dimandja; U.S. Food and Drug Administration, Atlanta, GA, USA

A method to quantify flavor chemicals in the filler of combustible tobacco products was validated using GC-MS. The tobacco filler samples from cigarettes, little cigars, cigarillos, and cigars were prepared in a multi-step extraction procedure using ethyl acetate and water followed by dilution with hexane. The features of the extraction stages include: extraction of non-polar compounds into ethyl acetate, water addition for polar compounds, partitioning into two-layers with subsequent centrifugation, followed by the removal of the ethyl acetate layer and mixing with an equal volume of hexane containing the internal standard (IS), second centrifugation, yielding an extract for GC-MS analysis. The selected flavor chemicals are quantitated by GC-MS in full scan mode by relating the peak area ratios to the concentration using target compound extracted ion chromatograms (EIC) compared to the IS peak area.

The linearity, accuracy, precision, limit of quantitation (LOQ), and selectivity results were used to demonstrate the extraction and instrumental analysis was appropriate for the quantitative analysis. The linearity of the method was assessed across the range of 10 to 300 ppm (μg/g). The accuracy and precision was evaluated using replicate measurements of matrix spiked samples at 50, 100, and 150 ppm levels, in addition to the LOQ standard at 10 ppm. The LOQ was established by evaluating the precision of all compounds, < 10% relative standard deviation (%RSD), as well as the signal-to-noise ratio for the lowest absolute peak area EIC, S/N≥11. The accuracy ranged from 95-127% for individual replicates and the precision at each concentration level ranged from 3-8% RSD. The selectivity results, which required no interfering peaks in the blank matrix and sufficient resolution at the LOQ level, met the validation criteria by visual interpretation and illustrated for each EIC. In addition to the quantitative analysis of the selected flavor compounds, the use of the full scan mode can also provide identification and library searching capabilities for unknown compounds. The results from the validation support the use of the proposed method for quantitative analysis of flavor related compounds in unburned portion of combustible tobacco product filler.
79. PHYSICAL CHARACTERISTICS OF HANDMADE PREMIUM CIGARS: SPECIFICATIONS AND CONSEQUENCES OF SPECIFICATIONS. Beatrice TEILLET, Thomas Verron, Stephane Colard; SEITA, Fleury-les-Aubrais, France

Handmade premium cigars consist of natural and heterogeneous leaves rolled by hand to a given dimension. This traditional making process is performed by people (rollers) with considerable experience and training. The rollers adapt their technique, cigar to cigar, according to the leaves’ natural characteristics, such as volume or shape during bunching. This traditional manual process needs to be taken into account when assessing the physical characteristics of a cigar.

This study investigated diameter, length, weight and pressure drop of handmade premium cigars. A wide range of sizes were sampled at one point in time and analysed. In order to assess their respective variability, a distinction was made between those characteristics related to cigar specification and those related to the consequence of a specification.

Results showed low variability of diameter and length, below 10% and 2% respectively, whereas weight and pressure drop exhibited substantially higher variability up to 40% and 120% respectively. This confirms that diameter and length, i.e. specified parameters, are directly and well controlled conditions. In contrast, weight and pressure drop are not directly controlled but are simply a consequence of the cigar specifications and the manual making process.

80. WASTE REDUCTION IN THE PRIMARY BY USING MICROWAVE IN-LINE TECHNOLOGY FOR PROCESS CONTROL AND OUTGOING GOODS INSPECTION. Hendrik RICHTER; TEWS Elektronik, Hamburg, Germany

Meeting quality control objectives in the Primary encompasses the processing of tobaccos at client-target moisture levels within associated control ranges for each blend. To achieve these quality goals, it is crucial that process control be effectively managed and that final quality inspection can be verifiably executed. Currently, but certainly in the past, such verifications and control functions were usually performed by intermittent sample taking from both the conveyer line and the final inspection station. Typically, C48 boxes were sampled to provide validation or reference values from laboratory-based instrumentation. These procedures are time consuming and do not allow for automated process control at locations such as the DCC or the final dryer.

In this presentation we demonstrate that tobacco waste can be measurably decreased when deploying TEWS inline systems based on microwave resonance technology by significantly reducing in-process moisture variability. TEWS inline systems measure real-time and high-speed true core moisture while simultaneously making available associated outputs that allow for moisture control automation. TEWS microwave transmission technology provides high accuracies during the moisture inspection of bulk goods at the final inspection station.

81. A PRODUCT SURVEY OF 25 PIPE TOBACCOS PURCHASED FROM WEB-BASED MERCHANTS. John H. LAUTERBACH; Lauterbach & Associates, Macon, GA, USA

In May 2016, the FDA extended their regulatory authority to cover pipe tobaccos and other tobacco products, which were not originally included in the FSPTCA. Pipe smokers are a small percentage of US tobacco users, but they have a wide variety of products to smoke ranging from expensive traditional products to much lower cost products that can be found in many retail locations that sell cigarettes. However, there is little knowledge about blends, casings, flavors and the processing that are unique to pipe tobaccos, particularly the more expensive products that are sometimes in non-cut forms and often have special processing that is atypical for cigarette tobaccos. Consequently, we conducted a product survey of 25 pipe tobaccos that we purchased from web-based merchants. These products included those that were twists and pressed cakes and included two products having deer tongue in the blend. In addition to analytical results from commercial laboratories, we determined cocoa, coumarin, vanillin, ethyl vanillin, licorice, polyphenols, and sugars using a gradient HPLC system consisting of 3 Waters 510 pumps, Waters 680 gradient controller, Rheodyne 7125 injector, Waters 486 TAD, and HP 3396 Series II integrator.
Columns used were a C18, 10 µm 4.6 x 250 mm and a YMC Triart C18, 5 µm 250 x 4.6 mm. Methods used were adapted from those in the literature and/or publicly available. Much useful data for distinguishing among the samples came from application of scan techniques using a MeOH extract of the tobacco and a slow gradient from 93/5/2 H2O/MeOH/HOAc to 10/88/2 H2O/MeOH/HOAc and setting the TAD wavelength at 340 nm, 280 nm, or 254 nm.

83. THERMAL DEGRADATION STUDIES OF ELECTRONIC CIGARETTE LIQUIDS PART 1: A NOVEL ANALYTICAL METHOD TO STUDY α-DICARBONYL FORMATION. Matt S. MELVIN, Karen C. Avery, Regina M. Ballentine, William P. Gardner and Karl A. Wagner; Altria Client Services, Richmond, VA, USA

The formation of carbonyl compounds in electronic cigarette (e-cigarette) aerosols from thermal decomposition processes has been well established in the literature. These thermal decomposition products are thought to originate from the primary e-liquid components: propylene glycol (PG) and glycerin (GLY). The presence of specific α-dicarbonyl compounds has also been reported in e-cigarette aerosols. The α-dicarbonyl compounds of interest include glyoxal, methylglyoxal, 2,3-butanedione (diacetyl), and 2,3-pentanedione (acetyl propionyl). The formation of these compounds is not readily explainable through typical dehydration and auto-oxidation pathways. The objective of this work is to develop an understanding of the potential reaction pathways for the formation of these compounds. To facilitate this study, an analytical method specific for α-dicarbonyl compounds was developed utilizing o-phenylenediamine (OPD) as the derivatizing agent to produce the corresponding quinoxaline product. OPD has the advantage of forming a single, stable product instead of a mixture of isomers that are susceptible to the reversible reactions that are seen when derivatizing with 2,4-dinitrophenyl hydrazine. The derivatization reaction occurs rapidly in water and is amenable for the collection of aerosols. Sample preparation and aerosol collection procedures were optimized for e-liquids and e-cigarette aerosols. Prepared samples were analyzed by liquid chromatography-mass spectrometry. This method was validated and deemed fit for purpose for the determination of the analytes of interest in e-liquids and aerosols. The optimized method conditions and the results of the validation will be presented.

84. THERMAL DEGRADATION STUDIES OF ELECTRONIC CIGARETTE LIQUIDS PART 2: DEVELOPMENT OF A MODEL REACTION SYSTEM USED TO STUDY α-DICARBONYL FORMATION. Matt S. MELVIN, Karen C. Avery, Regina M. Ballentine, William P. Gardner and Karl A. Wagner; Altria Client Services, Richmond, VA, USA

The formation of carbonyl compounds in electronic cigarette (e-cigarette) aerosols from thermal decomposition processes has been well established in the literature. These thermal decomposition products are thought to originate from the primary e-liquid components: propylene glycol (PG) and glycerin (GLY). The presence of specific α-dicarbonyl compounds has also been reported in e-cigarette aerosols. The α-dicarbonyl compounds of interest include glyoxal, methylglyoxal, 2,3-butanedione (diacetyl), and 2,3-pentanedione (acetyl propionyl). The formation of these compounds is not readily explainable through typical dehydration and auto-oxidation pathways. The objective of this work is to develop an understanding of the potential reaction pathways for the formation of these compounds. A derivatization method using o-phenylenediamine was used to study the formation of the α-dicarbonyl compounds in e-liquids. To this end, a model reaction system that simulates a potential reaction environment of an e-cigarette atomizer was developed using a microwave reaction system. The effect of the e-liquid components (PG, GLY, nicotine, water, and flavors), reaction temperature, and time on α-dicarbonyl formation were determined. The implication of these results on potential reaction pathways for the formation of α-dicarbonyl compounds in the e-cigarette aerosolization process will be discussed.

85. THE DETERMINATION OF DIACETYL AND ACETYLPROPIONYL IN AEROSOLS FROM ELECTRONIC SMOKING DEVICES USING GAS CHROMATOGRAPHY TRIPLE QUAD MASS SPECTROMETRY. Serban MOLDOVEANU, Amanda G. Hudson and Andrew Harrison; R.J. Reynolds Tobacco, Winston-Salem, NC, USA

A reliable and sensitive method for the measurement of the levels of diacetyl (2,3-butanedione) and acetylpropionyl (2,3-pentanedione) in the aerosol (both the particles and the suspending gas) of electronic
smoking devices (e-cigarettes) has been developed. The method uses a gas chromatographic separation on a Carbowax type column with the measurement of the analytes on a triplequad mass spectrometer working in positive MRM mode. The method has been validated using standard requirements regarding selectivity, sensitivity, recovery, accuracy, and repeatability. The limit of quantitation (LOQ) for the method was determined to be 0.41 ng/mL for diacetyl and 0.21 ng/mL for acetylpropionyl as measured for standards. These values translate to an LOQ of 0.082 ng/puff for diacetyl and 0.042 ng/puff for acetylpropionyl as measured for 50 puffs from an e-cigarette placed in 10 mL acetone. The samples analyzed included collected aerosols from several e-cigarettes, and a number of liquids used in electronic cigarettes (e-liquids). 3R4F Kentucky reference cigarette was also analyzed for evaluating the accuracy of the procedure, with good agreement with data from the literature. Diacetyl and acetylpropionyl were distributed in both particulate phase and also in vapor phase. The levels of diacetyl and acetylpropionyl in particulate phase collected from 3R4F cigarettes were found to represent only about 22% for diacetyl and only 31% for acetylpropionyl, while the vapor phase for diacetyl represented 78% and for acetylpropionyl 69% of the total analyte. The levels of diacetyl and acetylpropionyl in the aerosols of most electronic smoking devices was found to be very low. The analysis of the two analytes in e-liquids showed a very large range of levels.

86. ANALYSIS OF (S)- AND (R)-NICOTINE IN VARIOUS NICOTINE SAMPLES AND IN E-LIQUIDS. Serban MOLDOVEANU1, Gary M. Dull2, Ross J. Oden1, Wayne A. Scott1; 1R.J. Reynolds Tobacco, Winston-Salem, NC USA; 2RAI Innovation, Winston-Salem, NC, USA

Present study measured the level of nicotine in 38 commercially available liquids used in commercial electronic smoking devices (e-liquids), estimated if the nicotine in these e-liquids is predominantly (S)-nicotine (obtained from tobacco), and measured the ratio of (S)-nicotine and (R)-nicotine in four different nicotine samples of USP purity (United States Pharmacopeia). The quantitative analysis of (total) nicotine in e-liquids was performed using a GC-FID technique. In addition to nicotine, the levels of propylene glycol (PG), glycerin, and menthol were determined. The analysis of the ratio of the (R)- and (S)-enantiomers was performed by two different techniques. One technique was based on a GC/MS with the separation on two columns in series RT-GammaDEXsa 30 m x 0.25 mm i.d., with 0.25 um film. The other technique was a HPLC-UV with the separation on a Chiracel OJ column 250 mm x 4.6 mm with 3 um size particle. Three of the analyzed nicotine samples were obtained from the tobacco plant (Nicotiana tabacum, Nicotiana rustica, etc.) and one was synthetic nicotine. The natural nicotine from all three samples was found to be made from about 0.67% (R)-nicotine and 99.33% (S)-nicotine. The synthetic nicotine sample contained predominantly (S)-nicotine indicating they contain natural nicotine.

87. ANALYSIS OF ORGANIC ACID CONTENT IN E-LIQUIDS AND E-CIGARETTE AEROSOL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY – ULTRAVIOLET DETECTION (HPLC-UV). Angel RODRIGUEZ-LAFUENTE, Romulo Lodevico, Mehran Sharifi, Peter Joza and William Rickert; Labstat International, Kitchener, ON, Canada

Weak organic acids can be used as preservative agents, potentially affecting the stability of e-cigarette solutions. However, they can also impact characteristics like the aroma and taste of aerosols. The objective of this study was to develop and validate a simple analytical method for the analysis of 6 organic acids (formic, acetic, glycolic, lactic, benzoic and oxalic) possibly found in e-liquids.

Initially, one method was developed for the determination of benzoic acid. The aerosol was collected onto a filter pad and extracted with isopropyl alcohol, then diluted with a 0.1% phosphoric acid solution. The samples analyzed included collected aerosols from several e-cigarettes, and a number of liquids used in electronic cigarettes (e-liquids). 3R4F Kentucky reference cigarette was also analyzed for evaluating the accuracy of the procedure, with good agreement with data from the literature. Diacetyl and acetylpropionyl were distributed in both particulate phase and also in vapor phase. The levels of diacetyl and acetylpropionyl in particulate phase collected from 3R4F cigarettes were found to represent only about 22% for diacetyl and only 31% for acetylpropionyl, while the vapor phase for diacetyl represented 78% and for acetylpropionyl 69% of the total analyte. The levels of diacetyl and acetylpropionyl in the aerosols of most electronic smoking devices was found to be very low. The analysis of the two analytes in e-liquids showed a very large range of levels.
Allure Organic Acids column (5 µm, 300 x 4.6 mm), able to utilize a mobile phase with up to 50% of acetonitrile (ACN), reducing the run time to 40 minutes.

88. NICOTINE PHARMACOKINETICS OF ELECTRONIC CIGARETTES: EXPERIMENTAL DATA AND A REVIEW OF THE LITERATURE. Jan M. FEARON1, Alison Eldridge1, Nathan Gale1, Mike McEwan2, Paul Nelson2, Elaine Round2 and Mitch Stiles2; 1British American Tobacco (Investments) Limited, Southampton, UK; 2RAI Services Company, Winston-Salem, NC USA

E-cigarettes are battery-powered electronic devices from which users can inhale nicotine following its aerosolisation from a heated liquid solution. Some regulators and public health bodies consider e-cigarettes as potentially playing a major role in tobacco harm reduction. The ability of e-cigarettes to deliver nicotine to smokers in a manner and form generally similar to cigarette smoking have been recognised as key factors in helping smokers reduce or cease the use of combustible cigarettes. Nicotine pharmacokinetic studies of e-cigarettes have been performed for several years and are beginning to show how nicotine delivery is evolving as the products themselves evolve. In this presentation, we provide a critical overview of the literature to describe what is known about nicotine delivery from e-cigarettes, by both presenting data from our own clinical studies and from what has been published in the literature. We will discuss how the progression of e-cigarette design, development, and user familiarity and subsequent use behaviour has allowed increases in nicotine delivery, in the context of how much and how rapidly nicotine is delivered during acute-use periods. This presentation will also provide insight into current research gaps, and highlight a potential need for standardisation of the methodologies used to assess nicotine uptake to facilitate comparisons between different products and between different sub-categories of e-cigarettes.

89. AN ALTERNATIVE APPROACH TO QUANTITATIVE METHODS: LIMIT TEST FOR ANALYSIS OF DEEMING ANALYTES IN ELECTRONIC CIGARETTES. Salem CHOUCHANE, Darius B. Grissom and Carl J. Adams; Eurofins Lancaster Laboratories, Winston-Salem, NC, USA

Electronic cigarettes generate an aerosol produced from a heating element powered with a battery and a liquid in a cartridge without combustion. The liquid typically consists of humectants, water, flavors and nicotine. Except for the main components of the electronic cigarette liquid and aerosol, the levels of HPHC analytes in the aerosol or liquid of electronic cigarettes are extremely low and in many cases not detectable. Performing routine and high throughput quantitative analysis for analytes that are consistently reported below the level of quantitation is costly and inefficient. Given the need to see very low levels of analytes in products where the analyte is not expected to be present, limit tests offers an opportunity to be more efficient and cost effective. Limit tests are also an acceptable testing convention of the FDA, ICH, and USP.

Eurofins Lancaster Labs developed and validated limit test methods. Our efforts have been focused primarily on modifying previously validated methods or developing new methods to include electronic cigarette liquid and aerosol matrices. This presentation will highlight the advantages of limit test methods versus quantitative methods. The limit test approach reduces solution preparation, chemical cost, and analysts experimental and review time. Several examples of validated limit test methods will be presented.

90. MARKET ENDS E-LIQUIDS AND OPEN DEVICE CHEMICAL EVALUATION. David T. SZABO1, Amanda G. Hudson2, and Courtney G. Culbert1; 1RAI Services Company, Winston Salem, NC USA; 2R. J. Reynolds Tobacco, Winston Salem, NC, USA

Refillable or open electronic nicotine delivery systems (ENDS) have grown in popularity within recent years. Evaluating the interaction between e-liquid ingredients and device structural materials during intended use of the product is a routine stewardship practice to mitigate any potential unexpected exposures to the consumer. Market evaluations indicate that fruit flavored e-liquids is a top selling category for e-liquids used in open ENDS. In this study, we measured several compounds in popular brands of fruit flavored e-liquids and generated aerosols from a popular open ENDS. Aerosolization used a common machine puffing regime with a 55 mL puff volume, 30 second interpuff and 3.3 second puff duration (0.3 seconds added to ensure push button actuation). E-liquid and aerosol measurements included carriers that are commonly used, as well as flavors, carbonyls, and metals. Results indicated that formation of carbonyls...
and presence of metals were dependent on e-liquid formulation interaction with the device. These findings support the need for investigation of the combinatorial response between flavoring compounds and ENDS device functional parameters, ultimately leading to the protection of public health.

91. INVESTIGATION OF TSNA FORMATION IN ELECTRONIC CIGARETTE LIQUIDS AND AEROSOL. Xiaohong Cathy JIN, Karen C. Avery, Georgios D. Karles, William P. Gardner, Matt S. Melvin and Karl A. Wagner; Altria Client Services, Richmond, VA USA

Electronic cigarette (e-cigarette) formulations typically contain tobacco derived nicotine and therefore, may contain other tobacco related components such as trace levels of tobacco specific nitrosamines (TSNAs), nitrite, and minor alkaloids. Previous reports including work in our laboratories, suggest that TSNAs are present in e-cigarette liquids (e-liquids) and aerosols. The objective of this work was to investigate the possible formation of TSNAs in e-liquids and aerosols. There are reports in the literature that nitrite can react with nicotine and minor alkaloids in tobacco and cigarette smoke to form TSNAs. However, there are no literature reports on the effect of tobacco related compounds on the formation of TSNAs in e-liquids and aerosols. These studies were conducted by fortifying (spiking) nicotine containing e-liquids (mixture of propylene glycol/glycerin/water/nicotine) with nitrite, nitrate, ammonia, and minor alkaloids. The fortified e-liquids were analyzed to assess if TSNAs were formed. These e-liquids were used to fill e-cigarettes to determine the transfer and potential formation of TSNAs during the aerosolization process. Model systems were used to provide an understanding of the TSNAs formation pathways. The experiments demonstrated that nitrite led to the formation of TSNAs in e-liquids and e-cigarette aerosol. This information should be useful for regulators and manufacturers when making science-based decisions on which HPHCs and constituents to monitor in e-vapor products.

92. COMPARISON OF RESULTS FROM THREE TECHNIQUES FOR THE ESTIMATION OF E-LIQUID pH-VALUES. John H. LAUTERBACH; Lauterbach & Associates, Macon, GA, USA

Several techniques have been reported for the estimation of the pH-values of undiluted e-liquids. Such pH-values may be useful for QA and regulatory purposes. However, they may not be relevant to the aerosol formed when the e-liquid is vaporized and the nicotine is absorbed by the vaper’s mouth and airways. Consequently, we compared the pH-values of two noncommercial e-liquids, using three techniques. The first involved the estimation of the pH-value after dilution of the e-liquid with water (Lisko et al., 2015). The second involved use of apparatus similar to that used for puff-by-puff smoke pH to measure the pH-values of the aerosol generated from the e-liquid. The third involved use of a glassmouth containing 10mL Pickering Laboratories 1700-0304 artificial saliva (pH ~6.8). The glassmouth was also fitted with a pH probe to measure aerosol pH. Aerosols were generated with standard V2 battery sections and blank cartomizers containing about 500mg of e-liquid. The first e-liquid was 50mg/mL nicotine in PG. The second was made by adding propionic acid (30mg, 0.4mmole) to 6mL of the 50mg/mL nicotine in PG (300mg, 1.85mmole total). The pH-values obtained after dilution of the e-liquids with water (two sample replicates per sample) were 9.56, 9.66, 6.34, and 6.26, respectively. The measured pH-values of the aerosol using the puff-by-puff apparatus were [pH-values at the 25th puff (55/3/30 puffing regimen)] were 7.99, 7.80, 6.61, and 6.48. The aerosol pH-values at the 50th puff (55/3/30) were 7.63 and 6.66 using the glassmouth. The saliva pH-values were 7.49 and 7.40; and there was little difference in the saliva nicotine concentrations. Additional e-liquids have been studied, and the results will be reported in the presentation.

93. IMPACT OF CIGAR PHYSICAL VARIABILITY ON CIGAR EXPOSURE USING PROBABILISTIC RISK ASSESSMENT. Felix Ayala-Fierro, Karshak KOSARAJU and Rob Stevens; ITG Brands, Greensboro, NC, USA

Cigars are unique tobacco products of a wide variety of sizes: length, diameter and weight, and include large cigars, small cigars (cigarette-like) and cigarillos. The weight of cigars can range widely which influences exposure to cigar constituents, either by inhalation or through direct contact with lips. Another variable is that little cigar and cigarillo smokers are typically dual users of cigars and conventional cigarette products. In addition, unlike cigarette smokers, cigarillo smokers commonly practice partial smoking and re-lighting. Cigarillo consumption also varies widely from as few as one per week to daily which...
determines the total smoking time (days/year). The objective of this study was to determine the impact of cigar physical variability on uncertainties associated with exposure.

Consumer exposure to cigars, displayed as lifetime average daily intake (LADI) was explored using probabilistic risk assessment (PRA). First, “input” variables for different exposure scenarios were established. These parameters were found to differ from traditional cigarette exposure. Second, the impact of cigar weight, cigar consumption (weekly) and smoking behavior (whole vs. partial), on the overall exposure was investigated by PRA sensitivity analysis. Results from PRA analysis indicates that the size of the cigar (weight) has the greatest impact (~50%) on the overall exposure to the analyte of concern followed by the exposure duration and cigars smoked per week. It appears that smoking behavior (whole vs. partial) has little impact on lifetime exposure from cigar exposure. For dual smokers the combined exposure from cigars and cigarettes determines the overall exposure to a given analyte of concern. Understanding the impact of cigar physical variability on the overall consumer exposure to cigars is critical to explain uncertainty associated with smoking.

94. CONCEPT OF CHARACTERIZATION AND CERTIFICATION OF THE 1R6F REFERENCE CIGARETTE. Socrates Jose P. CANETE; University of Kentucky, Lexington, KY, USA

The quality and reliability of data generated by validated methods are strongly reinforced by the use of reference materials (RMs) and, in particular, certified reference materials (CRMs). The 1R6F is designed to mimic a matrix that closely resembles currently marketed cigarette products in the United States. The planned production inventory is such that it will allow consistent distribution to stakeholders over five years or longer. This paper discusses the concept of characterization of the 1R6F discussing the sampling as well as the statistical treatment of analytical data from contracted laboratories. The statistical processing of analytical data involved a two-tier approach to better reflect the two-tier concept of characterization of the 1R6F. The two-tier approach reflects two uncertainties; one is the expected uncertainty that can be expected for single-laboratory use of the RM and the other is the expected uncertainty when two or more laboratories compare their data on the 1R6F. The certification of these two uncertainties allows for individual labs to set their upper and lower limits in their quality control monitoring while allowing for assessments of results (on the 1R6F) between labs.

95. ALTERNATE MATERIALS AND THEIR POTENTIAL IMPACT ON HPHC. Michael J. MORTON, Nripa T. Jain, Kathleen H. Fox, Raquel M. Olegario, and Timothy L. Danielson; Altria Client Services, Richmond, VA, USA

It is commonplace to use alternate materials from different suppliers interchangeably in the manufacture of cigarettes. To be used interchangeably, these alternate materials will often meet the same specification, perform the same in the finished product, and result in the same finished product specifications even though the materials may not be chemically identical. The objective of this study was to evaluate the impact, if any, on HPHC yields from the use of several different types of alternate non-tobacco materials in PM USA cigarette products.

The alternate materials used in this study are all commercially available materials and included two plug wraps, two base tipping papers, two cigarette seam adhesives, two tipping adhesives, and three filter tows. To evaluate the various combinations of alternate materials, the study employed an adaptation of a fractional factorial using 16 combinations of the alternate materials. Using a designed experiment allowed us to estimate the effects of the alternate materials with much greater precision than a “one factor at a time design.” Each of the cigarettes in the study used the same base design with matched tobacco filler and cigarette paper, so that the products were the same except for the alternate materials under study.

The cigarettes were tested for the smoke constituents listed in the FDA abbreviated HPHC list both under ISO and Health Canada Intense smoking regimes in ISO 17025 accredited laboratories. The results of this study demonstrate that the use of these alternate materials does not impact the HPHC yields of the cigarette product. The estimated differences in HPHC yields were all numerically small, and, after adjusting for testing multiplicity, none of the estimated differences were statistically significant.
96. AN EVALUATION OF THE VARIABILITY OF HPHCs IN CIGARS AS COMPARED TO CIGARETTES. Karl A. WAGNER, Tammy L. Blake, Matt S. Melvin, Michael J. Morton and Jennifer H. Smith; Altria Client Services, Richmond, VA, USA

In May 2016, the U.S. Food and Drug Administration (FDA) issued a final rule to deem cigars to be subject to the Federal Food, Drug, and Cosmetic Act (the FD&C Act), as amended by the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act). As part of this regulation, the FDA will require manufacturers to report the quantities of Harmful and Potentially Harmful Constituents (HPHCs) in cigar filler and smoke. The diversity of the cigar product category adds challenges to the measurement of HPHCs. CORESTA has developed recommended methods for nicotine and CO for cigars; however, standardized methods do not exist for other HPHCs. Consensus methods, with defined repeatability and reproducibility (r&R), do exist for many of the abbreviated list HPHCs in cigarettes. However, these methods have not been shown to be fit for purpose for the analysis of cigars and we found that some of the methods developed for cigarettes need modification to be suitable for cigars. We will present data that demonstrate that HPHC variability in cigar testing is greater than that observed for cigarette testing. This presentation will cover some of the factors that affect cigar testing variability including smoke collection, variation in physical properties, and tip styles. In order to effectively test cigars for HPHCs and define the uncertainty of the reported results, the three pillars of effective analytical testing need to be implemented: consensus standardized methods with defined r&R, proficiency testing, and reference products.

97. EVALUATION OF MAINSTREAM CIGARETTE SMOKE COMPONENTS YIELDS OF DIFFERENT CIGARETTE TYPES BASED ON PRINCIPAL COMPONENT ANALYSIS. LI Zhonghao, Yang Fei, Liu Shanshan, Fan Ziyian, Wang Ying, Bian Zhaoyang, Deng Huimin, Tang Gangling and Hu Quingyuan; China National Tobacco Quality Supervision & Test Center, Zhengzhou, China

The feasibility of using Principal Components Analysis (PCA) in order to analyze a complex cigarette smoke mixture, seven cigarette mainstream smoke components, including carbon monoxide (CO), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK), Benzo(a)pyrene (B[a]P), crotonaldehyde, phenol, hydrogen cyanide (HCN) and ammonia, were determined from 21 representative commercial cigarette samples. The test results were verified by Kaiser-Meyer-Olkin (KMO) measure of Sampling Adequacy and Bartlett`s Test of Sphericity initially, and PCA was used to identify yield patterns, clustering of different types of cigarettes and these clusters causes. The results demonstrated that the selected three Principal Components (PC) could explain about 81.5% of total data variability. Ammonia, NNK and phenol were the main contributors for PC1, crotonaldehyde, HCN and CO for PC2, and B[a]P for PC3. Based on the statistical results of the selected three PCs’ scores, the description and explanation on different types of cigarette specimens (tobacco blend, design tar yield, slim, charcoal filter) were achieved successfully. Clustering of lower mainstream smoke components emission were found for slim types, and yields of seven components in mainstream smoke for the non-menthol and menthol subpopulations showed few significant differences. The study demonstrates that application of PCA to describe and analyze the dataset of complex cigarette smoke mixtures can be feasible and practical.

98. OXIDATIVE STRESS AND OXIDATIVE DAMAGE IN HUMAN LUNG EPITHELIAL CELLS INDUCED BY TOTAL PARTICULATE MATTER EXTRACTIONS FROM THREE DIFFERENT TOBACCOCS. KANG Yu, Qiao Liangjun, Hu Qiao, Hua Chenfeng, Xie Fuwei, Shang Pingping, Li Xiang and Zhao Junwei; Zhengzhou Tobacco Research Institute, Zhengzhou, China

Global sales and popularity of e-cigarettes have been growing rapidly in the past few years, but there is still a big share of conventional cigarettes and other tobacco products, which had led to the concerns about the toxicities of different tobacco products. Four representational tobacco products, including two conventional cigarettes, one e-cigarette product and one snus pouch, were selected to investigate the oxidative stress and oxidative damage induced by total particulate matters (TPM) extractions. Mainstream smoke/aerosols of 3R4F, CM8 cigarettes and one e-cigarette were captured according to ISO regime with smoking machines and the extraction of CRP1 Swedish style snus pouch (mentioned as TPM) was prepared by extracting the soluble substances with DMSO. Cell viability, intracellular reactive oxygen species (ROS) and DNA oxidative damage (8-OHdG) were tested on Beas-ab cells.
Results showed that: Extractions of cigarette smoke led to lower cell viability as compared with snus pouch, and that of the e-cigarette almost showed no effect on the cell viability within the tested dose range; Within the 0-50 µg/mL TPM doses range of the present experiment, extractions of the cigarette smoke induced significant oxidative stress to Beas-2b cells, which also showed dose-response relationship, whereas that of the snus pouch showed significant oxidative stress when the doses were as high as 100-500 µg/mL. The extractions of e-cigarette did not show significant effects on oxidative stress induction; Under the given experimental conditions, extractions of cigarette smoke, e-cigarette and the snus pouch induced the highest 8-OHdG levels at 20, 40, 500 µg/mL exposure doses, respectively. The characteristics of oxidative damage to DNA of the four products are different from these characteristics of those to cell viability and oxidative stress to Beas-2b cells. The results had shown different in vitro toxicological characteristics of these tobacco products.

99. SIMULTANEOUS DETERMINATION OF 22 AMADORI COMPOUNDS IN TOBACCO LEAVES BY LC-MS/MS. WANG Xiaoyu, Liu Leiyu, Wang Sheng, Qin Yaqiong, Jia Yunzhen, Cui Huapeng, Pan Lining, Xie Fuwei and Liu Huimin; Zhengzhou Tobacco Research Institute, Zhengzhou, China

Amadori compounds are important aroma precursors. Therefore, rapid and corrective determination of Amadori compounds is meaningful for monitoring the quality of tobacco leaves. By the end of 2016, the published methods have analyzed no more than 6 Amadori compounds simultaneously in tobacco. And in 2017, our research group reported a HPLC-MS/MS method to simultaneously quantify 10 glucose derived Amadori compounds in tobacco. This paper is based on our previous study. With respect to another 12 Amadori compounds without commercial standards, model reactions were conducted. And by using HPLC-Q-TOF-MS with high mass resolution, the 12 Amadori compounds were identified in the model reaction systems based on being matched with accurate mass and confirmed with the fragmentation way. The quantitative and the qualitative ion pairs in MRM mode were also selected. Then using HPLC-MS/MS, we quantified the 10 Amadori compounds with standards using external standard quantitative method, and semi-quantified the 12 Amadori compounds without standards using the standard curves of others with similar retention time and abundance in tobacco. Through large sample test, except 4 compounds which were unstable or of abundance close to LOQ, the intra-day precisions of 18 Amadori compounds were lower than 10%. The inter-day precisions of 14 Amadori compounds were lower than 10%, and that of 4 compounds could be controlled between 10% and 15%. The developed method was used in testing tobacco leaves of different types. The results showed that the total weights of 22 Amadori compounds accounted for 2.4% in flue-cured tobacco, 1.7% in oriental tobacco, 0.14% in burley tobacco. And the content distributions of the 22 Amadori compounds were also different among different type of tobacco leaves.

100. ELECTRONIC CIGARETTE AEROSOL DYNAMICS IN A PHYSICAL MODEL OF THE ADULT HUMAN ORAL/PHARYNGEAL CAVITY. Weiling Li¹, Qiang Wang²; Nicolas Castro³, Jingjie Zhang⁴, Yezdi Pithawalla¹, Michael Oldham⁵, Peter Lipowicz⁶ and Ali Rostami⁷; ¹Altria Client Services Richmond, VA, USA; ²Lancaster Laboratories, c/o Altria Client Services, Richmond, VA, USA

The objective of this work is to generate experimental data to validate a computational fluid dynamic (CFD) model for e-cigarette aerosol deposition. An adult human oral/pharyngeal wet walled hollow physical model has been developed for this purpose. The physical model was generated using a 3D printer from the CT scan of a 28 year-old healthy male and had an internal volume of 69.8cc. The wall was covered with a layer of cotton cloth that can be saturated with water to replicate the high humidity conditions typically encountered in a human oral/pharyngeal cavity. The model was placed in an oven at 37 °C, and measurements were taken under both wet and dry wall conditions. Deposition efficiency from a MarkTen® product using a prototype formulation was determined by measuring cumulative aerosol mass from five puffs (gravimetric) and individual constituents from a single puff (GC/MS analysis) at the entrance and exit of the physical model. Humidity at the exit of the physical model was maintained at > 90% at a constant air flow rate of 0.66 L/min. A 37 °C dry wall condition with a constant flow of 0.66 L/min through the model resulted in a mean aerosol mass loss of 6.6 ± 0.9% due to the deposition to the wall. Under wet wall conditions, the aerosol mass increased by 38% for a 5s puff duration, with 55cc puff.
volume and 37 °C wall temperature. The increase is due to moisture uptake by the aerosol. The aerosol mass increased by 80% using a 3s puff duration, 55cc puff volume and 37 °C wet walled condition. The experimental data will be used to validate the CFD model.

103. THE MEASUREMENT OF ADDICTIVENESS AND ATTRACTIVENESS. Neil SHERWOOD; Neil Sherwood Consulting, Commugny, Switzerland

Despite a rapid increase in regulatory demands for information regarding the addictiveness and attractiveness of tobacco or consumer nicotine products, there is a notable lack of clarity on how these concepts should be assessed and which metrics should be employed. This presentation considers the core scientific constructs which underlie addictiveness and attractiveness and suggests an approach which may go some way to satisfying regulatory demands. An explanatory model is described, built around definitions and demonstrations of addictiveness and attractiveness which have been offered by various scientific bodies. The model provides a framework upon which to understand the relationship between attractiveness and addictiveness and how these may influence nicotine and tobacco use. Accordingly, addictiveness can be assessed by measures of “liking” whereas attractiveness can be assessed by measures of “wanting”. Finally existing metrics are placed within this model, their properties discussed and the opportunities for development of new metrics are considered.

104. ADAPTING TRADITIONAL HUMAN ABUSE LIABILITY TESTING TO TOBACCO PRODUCTS. Graham K. Wood, and Michael M.C. KONG; Altasciences Clinical Research, Laval, QC, Canada

Under the Family Smoking Prevention and Tobacco Control Act that grants the FDA with full regulatory jurisdiction over all tobacco products, companies pursuing market access of novel tobacco products, including electronic cigarettes and smokeless tobacco products, require a premarket tobacco application (PMTA). As detailed in the FDA’s draft guidance concerning PMTAs, one of the key issues that must be addressed is the risk for addiction and abuse potential. This includes the likelihood that a novel tobacco product leads non-smokers to adopt the new tobacco product and/or its effectiveness in curbing existing smokers from using combustible cigarettes. These additional regulatory demands on tobacco products draw parallels to the human abuse potential (HAP) issues that pharmaceutical companies face when developing drugs that target the central nervous system. The methods used to demonstrate HAP - for example, acute dose effect comparisons, physical dependence measures, and behavioural economic procedures, have evolved over the past few decades and are designed to estimate the liking and wanting of the product. While some experience has been gained applying some of these methods in preclinical tobacco research, there is significantly less research using laboratory based human models of tobacco use. Furthermore, the model of addiction for tobacco products is different than pharmaceuticals, due to a much higher contribution of wanting over liking to the addictiveness, requiring consideration of this in the HAP study designs. The purpose of this lecture will be to describe how some of the methods used to study HAP of drugs can be applied to tobacco products and to discuss some of the characteristics and challenges unique to tobacco.

105. A CLINICAL SITE PERSPECTIVE: THE USE OF E-SCALE VERSUS PAPER SCALE TO CAPTURE SUBJECTIVE MEASURES FROM TOBACCO/NICOTINE WITHDRAWAL AND PRODUCT EFFECT QUESTIONNAIRES. (Presented by Doug Copeland) Jonathan Austin, Lorraine Rusch, Mark Pearson and Margarita Nunez; High Point Clinical Trials Center, High Point, NC, USA

Capturing data in an accurate and timely fashion is important in clinical research trials. Visual analog scale (VAS) assessment is often used to evaluate participants' subjective responses to Potential Reduced Exposure Products (PREPs). Advances in technology are enabling researchers to move from traditional, paper-based data collection to an e-tablet based methodology.

Methods: Two clinical trials each involving three different tobacco products with 24 subjects used either paper forms or an e-tablet to investigate the abuse liability potential of given products with two questionnaires: VAS Tobacco/Nicotine Withdrawal Scale and the VAS Product Effects Questions. Both studies had similar protocol design, inclusion/exclusion criteria, and patient demographics. Data was
collected on the following: 1) time to complete the surveys and perform quality assurance 2) number of errors detected during quality assurance.

Results. With paper assessments, there was an average of 8.9 errors per subject involving measurement of the VAS line or documenting time/date. The e-tablet based surveys had four queries in total. The mean times to complete the paper VAS/ per subject were 40.1 seconds for the Tobacco/Nicotine Withdrawal Scale and 38.8 seconds for the Product Effects Questionnaire. The e-tablet questionnaires were completed in 38.2 seconds and 36.9 seconds respectively. Mean query time by staff on the paper surveys was 3 minutes and 18 seconds. The e-tablet did not require any query time from the site perspective.

106. A STUDY TO EXAMINE CHANGES IN EXPOSURE TO CIGARETTE SMOKE CHEMICALS WHEN A SMOKER SWITCHES TO USING A TOBACCO HEATING PRODUCT. PART I: STUDY DESIGN. Nathan GALE1, Mike McEwan1, Alison Eldridge1, Oscar M. Camacho1, John McAughey1, James Murphy1, Chuan Liu1, Christopher J. Proctor1, Ian M. Fearon1, Neil Sherwood2, Edward Bowen3, Simon McDermott1 and Emma Holmes3; 1British American Tobacco (Investments) Ltd, Southampton, UK; 2Neil Sherwood Consulting, Commugny, Switzerland; 3Covance, Leeds, UK

A tobacco heating product (THP), glo™, which heats rather than combusts tobacco and produces significantly reduced machine yields of chemical toxicants compared to combustible cigarettes, was assessed in this study. Biomarker of exposure (BoE) studies are an important tool in determining whether this reduction in toxicant emissions translates to actual reductions in human exposure to cigarette smoke toxicants when smokers switch to using glo™. Data from such studies may potentially be required as part of a regulatory package, particularly as one aspect of a modified-risk assessment of a novel product.

This presentation (Part I of II) will outline the design of a two-centre, in-clinic (confinement), forced-switching, randomised controlled clinical study, performed in Fukuoka, Japan (UMIN000024988, ISRCTN14301360) evaluating this novel THP. In this study, we compared baseline levels of selected BoE to cigarette smoke toxicants in the exhaled breath and urine of 180 smokers, to those seen when the smokers either remained smoking combustible cigarettes, switched to using a THP, or quit all tobacco use completely, for 5 days. We also assessed levels of two biomarkers of biological effect before and after switching, and determined nicotine pharmacokinetics for the study products. The study was approved by a local Institutional Review Board and was run in accordance with ICH-GCP. Subjects provided written informed consent prior to study participation and were deemed healthy following medical examination and clinical laboratory screening. Smoking status was verified by exhaled CO and urinary cotinine measurements.

107. A STUDY TO EXAMINE CHANGES IN EXPOSURE TO CIGARETTE SMOKE CHEMICALS WHEN A SMOKER SWITCHES TO USING A TOBACCO HEATING PRODUCT. PART II: STUDY FINDINGS. Nathan GALE1, Alison Eldridge1, Mike McEwan1, Graham Errington1, James Murphy1, Christopher J. Proctor1, Ian M. Fearon1, Simon McDermott2, James Glew2 and Andrew Hedge3; 1British American Tobacco (Investments) Ltd, Southampton, UK; 2Covance, Leeds, U.K.

A tobacco heating product (THP), glo™, which heats rather than combusts tobacco and produces significantly reduced machine yields of chemical toxicants compared to combustible cigarettes, was assessed in this study. Biomarker of exposure (BoE) studies are an important tool in determining whether this reduction in toxicant emissions translates to actual reductions in human exposure to cigarette smoke toxicants when smokers switch to using glo™. Data from such studies may potentially be required as part of a regulatory package, particularly as one aspect of a modified-risk assessment of a novel product. This presentation is the second of two and will describe the findings from this study, the design of which was outlined in the first presentation.

180 subjects completed the study. Subjects who switched from smoking to exclusive use of the glo™ device (n=30) for 5 days showed reductions in levels of exhaled carbon monoxide and a range of urinary BoEs, compared to their levels at baseline. In contrast, the continued smoking group (n=30) showed little change in BoE levels throughout the study. We also present data for the continued menthol smoking group.
A continuum of risk exists among tobacco products, whereby non-combusted products present less health risks than combusted products. Achieving tobacco harm reduction requires risk information be effectively communicated to consumers, while also communicating appropriate cautions. This study assessed consumers’ comprehension and perceptions of reduced risk information for Camel Snus. An online sample of 4,924 U.S. adult tobacco users and non-users viewed an advertisement, stating that smokers who switch completely from cigarettes to Camel Snus can greatly reduce their risk of lung cancer, oral cancer, respiratory disease and heart disease. Respondents answered questions about the risks of Camel Snus relative to cigarettes, absolute risks of Camel Snus, and other information in the advertisement. Across the four diseases mentioned, <10% of respondents indicated Camel Snus presented no risk at all; most (52–62% across diseases) indicated Camel Snus presented less risk than cigarettes, but still some risk. Some indicated Camel Snus had the same risk as cigarettes; this varied by disease, even though risk information did not indicate differences. Thirty-seven percent of respondents indicated the risk of oral cancer was the same for Camel Snus and cigarettes, compared to only 20% for lung cancer, suggesting that responses were shaped by intuitions and pre-existing beliefs, as well as the advertisement’s content. High proportions of respondents (>80%) indicated Camel Snus is addictive, quitting tobacco use is the best choice, and non-tobacco users should not use Camel Snus – all statements included in the advertisement. Only 4% believed smokers would receive a health benefit if they continued to smoke while using Camel Snus. These findings suggest that appropriate information regarding the relative risks of tobacco products can be effectively communicated to consumers.

109. REDUCED RISK INFORMATION FOR CAMEL SNUS: PROJECTING LIKELIHOODS OF USE AMONG CURRENT SMOKERS, FORMER SMOKERS AND NEVER TOBACCO USERS. Geoffrey CURTIN1, Karen Gerlach2 and Saul Shiffman3, 1RAI Services Company, Winston-Salem, NC, USA; 2Pinney Associates, Burlington, VT, USA; 3Pinney Associates, Pittsburgh, PA, USA.

A risk differential exists among tobacco products, whereby non-combusted products present less health risks than combusted products. Informing smokers of the lower risks associated with using a reduced risk product could encourage switching to that product, and thus benefit population health. This benefit must be weighed against the possibility that such information may increase tobacco use among non-users and/or reduce quitting among smokers. Using an online sample of 11,302 U.S. adults, likelihood of use for Camel Snus was assessed among current and former smokers, and never tobacco users. Respondents were randomized to view an advertisement stating that smokers who switch completely to Camel Snus can greatly reduce their risk of lung cancer, oral cancer, respiratory disease and heart disease (while also conveying cautionary information); or, a control advertisement that only described Camel Snus. Respondents indicated their intent to purchase Camel Snus for trial (1-10 scale); these ratings were then converted to projected likelihoods of use, via a predictive algorithm. Projected Camel Snus use was significantly higher among current smokers than former smokers or never tobacco users. Importantly, the reduced risk information differentially increased projected use among current smokers (8.2%), while having a minimal effect on former smokers (1.9%) and never tobacco users (0.5%). For never tobacco users, projected use was highest among those already susceptible to tobacco use. For current smokers, projected use was lower among those likely to quit (4.2%) than those not likely to quit (8.7%). These findings suggest that providing reduced risk information for Camel Snus may increase use among current smokers who are already susceptible to tobacco use. For current smokers, the reduced risk information might encourage switching to Camel Snus, potentially reducing their risk of tobacco-related health outcomes.
smokers not likely to quit, with minimal potential to increase use among groups who could be harmed by adopting the product.

110. MODELING THE POPULATION HEALTH EFFECTS OF CAMEL SNUS WITH REDUCED RISK INFORMATION. Annette Bachand\textsuperscript{1}, Sandra Sulsky\textsuperscript{1}, Saul Shiffman\textsuperscript{2} and Geoffrey CURTIN\textsuperscript{3}; \textsuperscript{1}Ramboll Environ U.S., Amherst, MA, USA; \textsuperscript{2}Pinney Associates, Pittsburgh, PA, USA; \textsuperscript{3}RAI Services Company, Winston-Salem, NC, USA

We present findings from a statistical model, used to assess the population health effects of Camel Snus with reduced risk information. Analyses followed a single cohort of 1 million males from age 13 to age 72, comparing the number of survivors in a base case (cigarette use only) to a counterfactual scenario (cigarette or Camel Snus use). Age-specific likelihoods that relevant groups – smokers who otherwise would quit, smokers who otherwise would continue smoking, non-tobacco users who otherwise would initiate smoking, and non-tobacco users who otherwise would remain non-users – would use Camel Snus with reduced risk information were derived from consumer testing that projected purchase probabilities; secondary transitions, such as potential gateway effects for Camel Snus, used conservative probabilities. Analyses that considered all beneficial and harmful transitions estimated that increased Camel Snus use would improve survival by ~7,000 individuals (assuming 89-92% risk reduction, versus smoking); and, a survival benefit would be retained if Camel Snus reduced risk by as little as 55%, compared to smoking. Extrapolation of the estimated survival benefit to a realistic U.S. cohort of 4.1 million (both genders), suggests survival to age 72 years would be increased by >25,000 individuals. Tipping point analyses that included all primary and secondary harmful transitions indicated that if ~1.5% of continuing smokers switched completely and persistently to Camel Snus at each age interval, there would be a survival benefit. These single cohort analyses demonstrate that the transition with the greatest effect on population health is complete switching among smokers who otherwise would continue smoking. Collectively, these analyses indicate that providing reduced risk information for Camel Snus is likely to have a beneficial effect on overall population health.

111. FLAVORED E-CIGARETTE USE AMONG U.S. ADULTS: RESULTS FROM TWO NATIONAL SURVEYS. Mimi KIM\textsuperscript{1}, Mark Sembower\textsuperscript{2}, Saul Shiffman\textsuperscript{2}; and Geoffrey Curtin\textsuperscript{1}; \textsuperscript{1}RAI Services Company, Winston-Salem, NC, USA; \textsuperscript{2}Pinney Associates, Pittsburgh, PA, USA

Flavored e-cigarette use among U.S. adults was examined, based on data from two national surveys. PATH provides a representative sample of 32,320 adults (1,575 current established e-cigarette users), surveyed in-person from September 2013 to December 2014; the National Tobacco Behavior Monitor (NTBM) provides a weighted sample of 46,637 adults (4,845 past-30-day e-cigarette users), surveyed online from January 2014 to June 2015. The surveys define current e-cigarette use differently, yet provided similar findings. Overall, two-thirds of e-cigarette users reported using flavored varieties (PATH: 67%/NTBM: 67%). Flavored e-cigarette use was lowest among non-Hispanic Caucasians (63%/62%). In NTBM, African Americans reported the highest rate of flavor use (86%), due to a high percentage using menthol e-cigarettes; 78% of African Americans in PATH reported using flavored varieties, but flavor was not specified. Flavor use declined steadily with age in both surveys, from 83%/85% among 18-24-year-olds to 50%/41% among those >65 years. The relationship between flavored e-cigarette use and frequency was modest (varying from 66% to 73% in PATH and 60% to 76% in NTBM, across a range of use frequencies), and was less consistent between surveys. E-cigarette users who were former smokers were more likely to use flavors (70%/74%), compared to current smokers (66%/66%); among current smokers, those who smoked less frequently were more likely to use flavors (80%/82% of <weekly smokers, 63%/58% of daily smokers). These patterns suggest adoption of flavored e-cigarettes increases with decreasing smoking (including cessation). The relatively close agreement between the two surveys - despite differences in definitions of use, sampling, and data collection methods - suggests these findings are robust, and that similar estimates can be obtained via different methods.
112. VARIATIONS IN INTENSITY OF E-CIGARETTE USE, SMOKING HISTORY, AND DEMOGRAPHICS AMONG PAST-30-DAY E-CIGARETTE USERS. Saul Shiffman¹, Mark Sembower², Mimi KIM² and Geoffrey Curtin⁵; ¹Pinney Associates, Pittsburgh, PA, USA; ²RAI Services Company, Winston-Salem, NC, USA

Analyses examining e-cigarette use often define "users" as those with any past-30-day use, ignoring substantial variations in use patterns and leading to incorrect perceptions of users. We used data from a national sample of 153,019 U.S. adults (16,987 e-cigarette users), surveyed from online research panels during 2013-2016, to define and describe levels of e-cigarette use based on frequency and amount. Among adults reporting any past-30-day e-cigarette use, 10% used on only one day/month, and 28% used less than weekly (≤4 days/month); conversely, 22% used daily/nearly daily (≥27 days/month). Amount of use also varied, with median use being one use/day; >75% of users reported ≤5 uses/day. Individual characteristics varied substantially among categories of past-30-day users. Daily users were older than non-daily users (43 versus 36 years; this and all comparisons cited, p<0.005). Young adults (18-24 years) were more likely to report having used e-cigarettes during the past 30 days (OR=1.8), but more likely to have used e-cigarettes on one day/month (OR=2.2) and to report low levels of use (<5 uses/day) (OR=1.6). Older adults (≥45 years) were more likely to use daily (OR=2.2), and to report ≥10 uses/day (OR=1.8). Although women were less likely to report past-30-day use (OR=0.7), they were more likely to use daily (OR=1.4). Overall, >89% of past-30-day e-cigarette users had been established smokers (100+ cigarettes), and those who used e-cigarettes more frequently were significantly more likely to have quit smoking; 56% of those reporting ≥10 uses/day were no longer smoking. These data document large variations in frequency and amount of e-cigarette use among past-30-day-users, suggesting that a more discriminating and detailed characterization of users is necessary to understand e-cigarette use.

113. COMPARISON OF BACKGROUND RISKS WITH RISKS ESTIMATED FROM CONSTITUENTS IN TOBACCO PRODUCTS. Charlene LIU and Kristin Marano, RAI Services Company, Winston-Salem, NC, USA

The U.S. Food and Drug Administration (FDA) has identified a list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke for reporting under the Family Smoking Prevention and Tobacco Control Act. The HPHCs include some chemicals approved by FDA as food flavorings (acetaldehyde, o-, m- and p-cresols) or ingredients used in food packaging (acrylonitrile), and active ingredients or excipients used in drugs and consumer products (formaldehyde and polycyclic aromatic hydrocarbons [PAHs]). Some HPHCs are found in the soil, water, and air as a ubiquitous presence in the human environment (arsenic, acrolein, cadmium, benzene, formaldehyde, PAHs, toluene). The objective of this study is to compare potential excess lifetime cancer risk (ELCR) associated with HPHCs from cigarette smoke and smokeless tobacco products with ELCR associated with exposure to the same chemicals from background ambient air, drinking water, and food sources. U.S. market survey data of HPHCs in machine-generated cigarette smoke and smokeless tobacco products, and chemical concentrations of HPHCs in the environment and food are obtained from the literature. Potential cancer risks are evaluated following standard quantitative risk assessment process, utilizing toxicity values from the U.S. Environmental Protection Agency (EPA) recommended hierarchy sources and standard default exposure factors from EPA and FDA. These were supplemented as necessary with assumptions to develop estimates of tobacco use and accompanying exposures. Results of the study indicate that estimated ELCR associated with HPHC from tobacco products are similar to, or in some cases less than, cancer risks attributed to the same chemicals from air, food, and drinking water sources. While no tobacco product is safe or without risks, these findings indicate that potential cancer risks from exposure to many HPHCs in tobacco products, as they are estimated by contemporary procedures, are minimal and do not increase cumulative ELCR in a meaningful way, when associated with background intake of the same chemical.

116. QUANTIFYING TOBACCO LEAF COMPOUNDS BY VISIBLE LIGHT ANALYSIS.
Makoto YOSHIZAKI; Japan Tobacco, Yokohama, Japan

The quality of tobacco leaf is related to its constituents. However, a simple and convenient method to characterize leaf compounds during cultivation, harvest, and curing has not been developed. Visible light analysis is a simple and fast method to measure leaf color, which is an important factor in the grading of
tobacco leaves. Previous research has shown that tobacco leaf color is mainly derived from carotenoids, flavonoids, and chlorophylls. Carotenoids are degraded during cultivation and curing processes, yielding the compounds responsible for tobacco’s aroma. In this study, we investigated degraded carotenoids using visible light analysis. Thirty-one flue-cured Virginia tobacco samples with different leaf positions from 13 countries were pulverized, and the color of the tobacco leaves was measured by spectrophotometry. The colors were represented by the L*a*b* color space (L*: Lightness; a*: Green-red chromaticity; b*: blue-yellow chromaticity). Twelve degraded carotenoids were selected as major compounds and analyzed by gas chromatography/mass spectrometry. Multiple regression analysis was performed to estimate the abundance of the selected compounds from color parameters, and the statistical significance of estimated partial regression coefficients was tested. Visible light analysis was effective in measuring five compounds. The L*a* degree and a* degree effectively estimated the levels of 3-hydroxy-7,8-dihydro-beta-ionol and blumenol C. However, the highest coefficient of determination was only 53%, implying that other pigments can interfere with visible light analysis.

117. INVESTIGATION ON THE ROLE OF COPPER (II) ON NITROSAMINE FORMATION FROM NICOTINE. Syed HAQUE and Socrates Jose P. Canete; University of Kentucky, Lexington, KY, USA

The role of Cu2+ in nicotine nitrosation reaction was investigated using High-Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR) and UV-vis spectroscopy. The nitrosation reaction of nicotine was carried out in both “clean” citrate phosphate buffer and in tobacco matrix. Titration with Cu2+ monitored by NMR and pH dependent UV-vis profiles strongly indicate that Cu2+ binds irreversibly to the pyridine nitrogen of nicotine. Studies on nitrosation kinetics reveal that in presence of Cu2+, the activation energy of the nitrosation reaction was increased suggesting unfavorable nitrosamine-formation kinetics. NMR and kinetics data strongly suggest that by binding irreversibly with the pyridine moiety of nicotine, Cu2+ imparts partial inhibition of nitrosamine formation from nicotine. This presentation will demonstrate the extent of nicotine nitrosation and role of copper on in the nitrosation reaction.

118. PON AND NNK CHANGES IN CURED BURLEY TOBACCO WITH STORAGE. Ying WU, Huihua Ji, Franklin Fannin and Lowell Bush; University of Kentucky, Lexington, Kentucky, USA

4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is considered to be the most potent carcinogen of the TSNAs that are a class of nitrosamines that occur in tobacco products. Previous kinetics studies have shown the generation of NNK is pH dependent and temperature can affect the reaction rate from pseudo-oxy nicot ine (PON) to NNK. A very rapid formation of NNK from PON was measured using 200 µmole ml-1 nitrite in pH 3 buffer at 37°C. Nitrate and nitrite enhancement for the increase in TSNAs during warm temperature storage and moisture content is not well understood. The objectives of this study were to determine the PON and NNK changes during the storage time at four different moisture conditions. Cured ground burley tobacco which contained 7, 12, 18 and 28% moisture were stored with excessive nitrate and nitrite in separated sealed containers at 60 °C. Nitrate level did not effect NNK accumulation in 15 days. Excessive nitrite increased NNK levels from 5.5-35X compared to controls in 15 days under different moisture levels. The greatest increase of NNK occurred with 12% moisture (~35X). PON levels were increased 4-6-fold with all moisture levels during 15 days of treatment. Measured PON increased rapidly during the first two days, especially at 7% moisture. The results indicate that NNK accumulation was altered by temperature and moisture content of tobacco.

123. THE REGULATION OF MICROORGANISM ON THE FORMATION OF TSNAS IN TOBACCO. ZOU Congming, Xia Zhenyuan, Lei Liping, Huang Wei, Zhao Gaokun, Jin Yan and Wu Qun; Yunnan Academy of Tobacco Agricultural Sciences, Kunming, China

TSNAs in tobacco are directly approved to be the carcinogens which is getting more and more attention than before. The regulation of TSNAs is becoming one of the most important topics of tobacco harm reduction. It has been shown that TSNAs formation is significantly interacted with the microbes in the flue-curing process. Therefore, the objective of this study is to explore the effects of microbial communities on the formation of tobacco TSNAs and further confirm the relationship between them. Three series of studies
were conducted to explore the effects of microbial communities on the TSNAs formation, including fresh
leave cultivation, air-curing process, cured leave cultivation. The sequencing method was used to identify
the type and function of each strain. This study found that a microorganism (J54) could significantly
increase the content of TSNAs in tobacco, while the other (05-5402) could significantly reduce the content
of TSNAs to a large extent and other microorganisms were less obvious. Sequencing results show that J54
belongs to Pseudomonas sp., and 05-5402 belongs to bacillus pumilus. This study clarifies the positive and
negative effects of several strains on tobacco TSNAs formation and would play an important role in
reducing carcinogens for tobacco. The reduction effect can be directly used in the development of
biological method to reduce harmful contents, at the same time, the microorganism (J54) could provide the
target for reducing harm study in tobacco.

124. ARE AVAILABLE TEST METHODS FOR THE DETERMINATION OF AMMONIA IN
MAINSTREAM CIGARETTE SMOKE FIT FOR THE ANALYSIS OF CIGARS? Yevgeniya
PREPELITSKAYA, Kathleen Spangler, Jennifer H. Smith, Karen Avery, James Wilkinson, Matt Melvin
and John H. Miller IV; Altria Client Services, Richmond, VA USA

In May 2016, the U.S. Food and Drug Administration (FDA) issued a final rule to deem cigars to be subject
to the Federal Food, Drug, and Cosmetic Act, as amended by the Family Smoking Prevention and Tobacco
Control Act. As part of this regulation, the FDA will require manufacturers to report the quantities of
Harmful and Potentially Harmful Constituents (HPHCs) in cigar filler and smoke. Standardized methods
do exist for the analysis of ammonia in cigarette smoke; however, these methods may not be fit for purpose
for the analysis of cigars. Cigars vary widely in blend composition and size compared to cigarettes, which
could further complicate the analysis of these products. CORESTA Recommended Method No. 83
Determination of Ammonia in Mainstream Cigarette Smoke by Ion Chromatography (CRM 83) is a
standardized method used for ammonia in cigarette mainstream smoke. The results of this work indicate
that the CORESTA Recommended Method may not be appropriate for all cigar products. In our evaluation
of the trapping efficiency, we observed higher yields of ammonia in mainstream smoke with increasing
concentration of acid in the trapping solution. In addition, the amount of ammonia detected in sample
extracts increased over time faster with higher concentrations of acid. These observations suggest that
under acidic conditions, another component in mainstream smoke may break down into ammonia. We also
observed differences in these effects depending on the cigar blend composition. These results indicate that
specialized methods need to be developed for the analysis of ammonia in cigar smoke.

125. DETERMINATION OF HARMFUL AND POTENTIALLY HARMFUL CONTAMINANTS IN
TOBACCO SMOKE USING STANDARD REFERENCE MATERIAL 3222. Walter B. WILSON and
Lane C. Sander, National Institute of Standards and Technology, Gaithersburg, MD, USA

Under the Food, Drug, and Cosmetic Act (FD&C) all tobacco manufacturers and importers are required to
report the levels of harmful and potentially harmful constituents (HPHCs) found in their tobacco products
and tobacco smoke. Studies have reported the detection of up to 7000 different chemical components that
can be inhaled through tobacco smoke such as nicotine, tar, nitrosamines, and polycyclic aromatic
hydrocarbons. In 2012, the Food and Drug Administration (FDA) included in the Federal Register a list of
93 HPCs suspected to be found in tobacco products and tobacco smoke. To support the Family Smoking
Prevention and Tobacco Control Act, the National Institute of Standards and Technology has recently
collaborated with the Center for Tobacco Products at the FDA to develop a new low nicotine tobacco filler
Standard Reference Material (SRM 3222) to support the analysis of tobacco products. This new tobacco
material has certified and reference mass fraction values for nicotine, N-nitrosonornicotine (NNN), 4-
(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and moisture. In the current study, approximately 10
g of SRM 3222 was burned to produce tobacco smoke. Several different smoke collection apparatuses and
organic solvents were evaluated to allow for the highest amount of tobacco smoke to be collected. After
filtration, the homogenous solution was screened via gas chromatography/mass spectrometry for the
identification of many HPHCs in the tobacco smoke.

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Note that the 71st TSRC had to be rescheduled due to a hurricane. As a result, several papers were withdrawn, and several had to be presented by the author’s colleague.
127. **FUMEX: LIGHT SCATTERING SENSOR FOR THE ANALYSIS OF ELECTRONIC CIGARETTE AEROSOLS.** Weiling Li¹, Qiang Wang²; Peter Lipowicz³, Wilhelm Dunkhorst⁴ and Wolfgang Koch⁵; ¹Altria Client Services, Richmond, VA USA; ²Lancaster Laboratories, c/o Altria Client Services, Richmond, VA USA; ³Fraunhofer ITEM, Hannover, Germany

A new sensor based on light scattering has been designed to perform highly time resolved measurements of the mass concentration and the mass median diameter (MMD) of aerosols formed in e-cigarettes. Aerosols generated in e-cigarettes are spherical, submicron particles with well-defined constant optical properties. The Fumex sensor measures scattered light from the e-cigarette aerosol at two polarizations. The mass concentration and mass median diameter of the aerosol is calculated from the light scattering signals assuming a fixed geometric standard deviation and refractive index equal to that of the bulk e-liquid. The light source used in Fumex is a laser diode emitting polarized light at 680 nm. The scattered light is detected at 90° using a semi conductor photodetector. The validation of this sensor was carried out with an impactor for MMD and with filter gravimetric measurement for mass concentration. Good correlation for both parameters was observed, R-squared value 0.97 for MMD and 0.95 for mass concentration. Application ranges for Fumex are: mass concentration range of 1-50 g/m³, MMD of 0.2-1.5 µm, 100ms time resolution, and 0.2-3 l/min flow rate. This sensor has been used to measure particle size of e-cigarettes with different carriers, and a variety of commercial e-cigarettes. Results were compared to measurement done with Spraytec and impactors. Measurement with different commercial e-cigarettes using this sensor will also be discussed in the presentation. Fumex can provide fast measurement of mass concentration and real-time monitoring of aerosol generation during a single puff, which allows better understanding of aerosol formation in the e-cigarette.

129. **A COMPUTATIONAL MODEL TO CHARACTERIZE THE VITROCELL® CELL EXPOSURE SYSTEM FOR EVALUATION OF AEROSOLS.** Nicolas Castro¹, Ali A. Rostami¹, Michael J. Oldham¹, Yezdi B. Pithawalla¹; Francesco Lucci⁵ and Arkadiusz Kuczaj²; ¹Altria Client Services, Richmond, VA, USA; ²Philip Morris International, Neuchâtel, Switzerland

In vitro exposure systems can be used as tools for toxicological assessment of e-cigarette aerosols. The VITROCELL® exposure system is designed such that exposure of cell cultures to the aerosol occurs at the air/liquid interface, which is relevant to e-cigarette use. It is difficult to experimentally quantify the actual cell dose applied in the VITROCELL® system, as it depends on multiple parameters such as system geometry, particle size and distribution, air flow-rate, exposure level and duration, etc. A computational fluid dynamics aerosol tracking and deposition model that employs Lagrangian particle tracking method has been developed to quantify deposition rates of particles on the air/liquid interface in a VITROCELL® 24/48 system. The system consists of a 6 mm diameter main line carrying the aerosol, with six smaller 3 mm diameter tubes (trumpets) branching down to the cell exposure plates. Results of simulations for a main line airflow rate of 1 L/min and trumpet airflow rates of 1-4 mL/min will be discussed. Simulations were performed for inert non-reacting solid particles with a range of diameters between 0.5 and 4.5 µm and densities of 1050 kg/m³. The impact of particle size and airflow rate on deposition efficiency on the cell plates was explored. Results show that for a trumpet to main airflow ratio of 2/1000, the fraction of inlet particles deposited on the cell exposure plates is less than 0.001. This can be attributed to (1) lower particle concentration near the wall in the main line and (2) carryover of particles by the trumpet airflow out of the system. Once validated, the model will be used to quantify cell exposure from different e-cigarette aerosol streams.