2017 CORESTA JOINT STUDY GROUPS MEETING

SMOKE SCIENCE and PRODUCT TECHNOLOGY

ABSTRACTS

ORAL PRESENTATIONS

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

IG 01

Comparison of intra-crop year variability in NNN in tobacco and NNN levels in smokeless tobacco products

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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 year crop 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 1 ppm in dark fire-cured and Burley tobacco in every year tested and six of ten 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, NNN levels exceed 1 ppm (dry weight basis) 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.

IG 02

Delivering positive change through the Sustainable Tobacco Programme

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AB Sustain has a wealth of tobacco farming and processing sustainability data gathered over the last 16 years on a global scale. The latest iteration of our sustainability service, the Sustainable Tobacco Programme (STP), was launched in 2016. This collaborative, industry wide initiative is supported and operated in the supply chains of six leading tobacco brand owners. The programme operates in over 52 countries and gathers data on 180+ suppliers of tobacco across 5 million smallholders. Our specialist assessors work with field technicians and supply chain business owners to verify the data collected on farms. We gather data on over 1000 sustainability indicators across five pillars; Governance, Crop, People, Facilities and Environment, to give a holistic and verified report into practices at both tobacco processing sites and their supplying farms. At the programme's core is continuous improvement, with a strong focus on providing supply chains the information/data needed to help drive positive change.

The next step for STP is to further validate this approach, and provide ongoing tangible evidence that it is delivering change for the industry in a sustainable way. There is also the opportunity to add value to the wider sustainability landscape through leveraging the dataset that we have amassed to provide further insight into global practices and the impact of change.

ST 02

An evaluation of the variability of HPHCs in cigars as compared to cigarettes

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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. 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.

ST 03

Comparison of select analytes in tobacco and smoke for cigar products across a range of design features

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On August 8, 2016, the FDA finalized a rule that extended its regulatory authority to all tobacco products, including e-cigarettes, cigars, and hookah and pipe tobacco, as part of its goal to improve public health. While the analytical testing of conventional cigarette products benefits from both standardized methods and the availability of multiple reference cigarette products, cigar testing experience is more limited. The objective of this study was to compare various cigar products with a range of design features for chemical analysis of tobacco analytes and smoke constituents. Design features such as size, flavour, making technique (machine versus hand), and tipping (untipped, filtered, mouth-piece designs) were included in the study design. Products were tested at multiple laboratories for key physical characteristics. Tobacco and smoke were tested for harmful and potentially harmful constituents (HPHC) typically applied to cigarette testing: carbon monoxide, smoke nicotine, select carbonyls, volatile organic compounds, tobacco nicotine, tobacco ammonia, tobacco-specific nitrosamines, polyaromatic amines, and polyaromatic hydrocarbons. For smoke measurements, CORESTA recommended puffing regimes as described in CORESTA Recommended Methods (CRMs) 64 and 65 were employed. Details from these analyses along with information regarding challenges associated with testing such a range of cigar products will be provided.

ST 04

Challenges and considerations for methods development and testing of cigar products for smoke constituents beyond TNCO

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While testing for smoke constituents for conventional cigarettes is challenging, there is a rich body of literature, inter-laboratory studies, and significant hands-on experience for this area of work. On the other hand experience and standardization with cigar testing is more limited. In addition, cigar testing offers many challenges that are not of concern, or have been previously addressed, for cigarette testing. While there is a standardized puffing regime and handling requirements (described in CORESTA Recommended Method [CRM] Nos. 46, 64, and 65), application of that regime to smoking for analytes beyond tar, nicotine, carbon monoxide (TNCO) methods presents challenges for both method development and testing consistency. The objective of this presentation is to review some of the challenges encountered during in-house method development for cigar methods and challenges encountered during an inter-lab study for various cigar products. Topics discussed will include the impact of conditioning on flavoured products, the effects of lighting technique and ash removal on burn quality and analyte yield, and the relative yields of analyte versus matrix. These challenges will be discussed along with issues for consideration for methods development for a range of smoke constituents and data analysis and reporting considerations.

ST 05

Are available test methods for the determination of ammonia in mainstream cigarette smoke fit for the analysis of cigars?

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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 (CRM) 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.

ST 06

Development, validation and routine use of a method for the determination of carbonyl compounds in cigar smoke

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The U.S. Food and Drug Administration recently finalized a rule that extends its regulatory authority to all tobacco products including cigars. This change could require the routine reporting of compounds of concern in cigar smoke including carbonyl compounds. There is currently no accepted method for the analysis of carbonyl compounds in mainstream cigar smoke. CORESTA Recommended Method (CRM) No. 74 has been found to be suitable for the analysis of carbonyl compounds in mainstream cigarette smoke and we investigated this method to determine if it could be expanded to include carbonyl compounds in mainstream cigar smoke. In the development of this method, we evaluated sample collection using the CORESTA cigar smoking conditions given in CRM 64. Method performance was verified for the stability of the trapped hydrazine during sample collection, trapping efficiency and accuracy of the method. With a few modifications to CRM 74, we were able to develop a fit for purpose method to determine carbonyl compounds in mainstream cigar smoke. This included a range of cigars including both flavored and unflavored products. We will report a summary of the method development, method validation and long term method process control samples.

ST 07

Physical characteristics of handmade premium cigars: specifications and consequences of specifications

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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.

ST 08

Investigating reuse of coil/wick sub-assemblies (atomisers) in electronic nicotine delivery systems (ENDS)

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Certain ENDS are reusable, tanks are refilled and atomisers changed when required. Although there are many published studies describing emissions of ENDS, the majority do not make assessments following multiple refills and it is not known if user detectable signs of failing atomizers relate to increases in potentially harmful carbonyls. This study aims to provide insight into flavour changes and emissions over multiple refills whilst reusing the atomizer.

Two tobacco flavoured formulations have been assessed; one contains sugars (A) the other does not (B). An unflavoured control is being assessed at the time of writing. Tasting using a flavour wheel as a guide was carried out, whilst aerosol mass, carbonyls, and nicotine emissions were also assessed. Puffs to waste were carried out to drain the tank in between refills. Tasting and emissions measurement was then repeated. Aerosols were generated using CORESTA Recommended Method (CRM) No. 81 modified with a five second puff duration. Seven tank fills were completed for both flavours. No trends were observed for nicotine and aerosol mass measures. Mean formaldehyde increased for both flavours, 1.4 to 4.4 µg/10 puffs and 1.3 to 25 µg/10 puffs in A and B respectively. Acetaldehyde, acrolein, and propionaldehyde showed similar trends, no crotonaldehyde or butyraldehyde were detected. When tasted over the course of numerous refills, flavour A changed, with more incidences of “burnt” taste occurring at refill 5 onwards; the burnt description was accompanied by a majority of tasters indicating atomizer replacement at this point. Flavour B changed subtly throughout seven refills however fewer “burnt” tastes were recorded compared to A. Also, tasters did not record a clear indication to replace atomizers compared to A. Changes in ENDS flavour profile are detectable by users; this flavour change is not linked to threshold levels of carbonyls.

ST 09

Oxidation dynamics of e-liquids in electronic nicotine delivery systems with atomisers of various types

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The processes of the thermal oxidative destruction of e-liquid components play a key role in the appearance of undesirable products in aerosols generated by electronic nicotine delivery systems (ENDS). The objective of the present studies is to obtain insight into understanding the key factors, that govern the oxidation processes in e-liquids upon the ENDS operation. The basic methods we used pertained to the kinetic approaches of the oxidation chemistry, including measuring the chemiluminescence emission derived from interactions of reactive oxygen species (ROS) and peroxide reactions and monitoring the formation of oxidation products. The main results of the reported experiments are the following. We have shown for the first time that the temperature-dependent formation of carbonyl compounds in the ENDS aerosols proceeds through the free-radical chain mechanism with the intervention of hydroperoxides. However, the temperature, being the most important parameter, is not the only factor accounting for the occurrence of the pertinent oxidation products in the ENDS emissions, and materials of evaporators, contents of e-liquids and an aeration of atomizers influence significantly the oxidative status of the ENDS aerosols. In the present work, we examined the diversity of factors controlling the oxidative developments in e-liquids upon vaping with a particular emphasis on comparing the oxidation dynamics in cases of ceramic and metal atomizers. In this context, the advantages and disadvantages of each atomizer type are revealed. Besides, the direct microscopy of condensates of the ENDS aerosols enabled us to elucidate the structure of microparticles formed as a result of the thermal destruction of heating coils and fibrous materials of evaporators. The above-mentioned findings are of interest for improving the ENDS design and for optimizing their functioning and handling.

ST 10

Investigation of TSNA formation in electronic cigarette liquids and aerosols

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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 harmful and potentially harmful constituents (HPHCs) and constituents to monitor in e-vapor products.

ST 11

Thermal degradation studies of electronic cigarette liquids Part 1: A novel analytical method to study α-dicarbonyl formation

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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.

ST 12

Thermal degradation studies of electronic cigarette liquids Part 2: Development of a model reaction system used to study α-dicarbonyl formation

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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.

ST 13

New aspects of cellulose acetate biodegradation

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Cellulose acetate (CA) as it is used for cigarette filters is known to be biodegradable, although more slowly than fast degrading materials like cellulose. Nevertheless, there is still controversial communication about the biodegradability performance of cellulose acetate filter tow.

In order to get a more comprehensive picture of CA degradability and the possible mechanisms behind it, studies in different environments were performed like on soil or sealed surfaces, in water, in marine water, in soil, in home compost, in industrial compost and under biogasification conditions.

On surfaces without significant microbial activity, degradation can take several years but can be accelerated by improving photo degradability. CA degrades best in anaerobic conditions (without presence of oxygen) like during biogasification or in landfills, with only minor shortfall compared to cellulose. In the presence of oxygen (aerobic), e.g. in water or soil, CA shows a distinct lag phase resulting in slower degradation compared to cellulose. This observation can be explained by a probably slower biofilm formation of deacetylating microbes.

Due to the lag phase, CA degrades too slowly to fulfil the requirements for biodegradability certifications, but it degrades in all microbially active model environments. In contrast e.g. to the likewise biobased polylactic acid, CA degrades considerably at temperatures below 50 °C.

To meet certification demands, the lag phase of CA can be significantly reduced by the addition of specific biodegradation promoters.

ST 15

Crush strength of aged capsules in cigarettes

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A capsule is a widely used flavour carrier in cigarettes. It is a very effective tool to deliver menthol and other flavours to mainstream tobacco smoke. Even though menthol will be banned in EU from 2020, capsule cigarettes are still very popular in other markets like Asia, Middle East and Africa.

The structure of this study is divided into three sections.

- Influence of triacetin and menthol on the capsule crush strength. Capsule filters were produced applying 0 %, 5 %, 10 % and 15 % of triacetin with 0.3 mg/mm menthol and without menthol. Filters were stored within polyethylene/propylene bags and outside for several weeks in ambient conditions. The capsule crush strength and colour change was recorded each week.
- Open pack test – change in crush strength of commercial capsules when cigarettes are stored in an opened pack in ambient conditions for several weeks. It was observed that some capsules dried out. The crush strength was measured each week, dried capsules were quantified and a statistical analysis performed.
- Capsule crush strength in half-smoked cigarettes. Some smokers like to crush the capsule after smoking half or more of a cigarette. It was observed that during smoking capsules lose the “click” effect, crush strength and leak the flavour. Flavour analyses were made using Agilent 5973N GC-MS.

This study provides practical and insightful data about shelf life and crush strength changes of capsules in cigarettes under various storage conditions.

ST 16

Computational fluid dynamic (CFD) simulation of distribution characteristics of cigarette smoke flow field in grooved filter

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A CFD model for the smoke flow in grooved filter was developed to study the flow field distribution in grooved filter and the ratio of cigarette smoke outflowing from the grooves (the proportion of amount of smoke outflowing from grooves in relation to the amount of smoke entering the grooved filter). By applying the established model, the smoke flow in the grooved filter of cigarette smoked under ISO smoking regime was simulated at an average velocity of 0.38 m/s. The effects of groove shape, groove number and groove depth on the ratio of cigarette smoke outflowing from grooves were investigated. The distribution and variation characteristics of cigarette smoke velocity and pressure in the acetate fibre filled portion and grooves of grooved filter were calculated. Furthermore, the ratio of smoke outflowing from grooves was calculated, too. Whilst keeping all other conditions and parameters of the model unchanged, the amount of smoke outflowing from grooves increased depending on the groove shape: rectangular groove > triangle groove > trapezoidal groove. The ratio of smoke outflowing from grooves increased slowly first and then decreased gradually with the increase of groove depth, however it increased linearly with the increase of groove number. The simulation results are well in-line with experimental results and literature reports. The developed CFD model could simulate the smoke flow in grooved filter and is suitable for the optimization and design of filter.

ST 17

Determination of eight carbonyl compounds in e-liquids for electronic cigarettes by LC-MS/MS

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A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to simultaneously determine eight carbonyl compounds (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone, butyraldehyde) in e-liquids for electronic cigarettes. The carbonyl compounds in e-liquids reacted with 2,4-dinitrophenylhydrazine (DNPH) under acidic conditions to generate hydrazone compounds, then the derivatives were determined by LC-MS/MS. Acetonitrile was chosen to prepare DNPH solution after investigating the influences of solvent background of methanol and acetonitrile on test results. In order to ensure the applicability of the analysis method, dispersant was mixed into the reaction system to make the e-liquid matrix of high glycerol/propylene glycol ratio more miscible with the DNPH solution. The parameters, such as DNPH concentration, acidic solution concentration and reaction time, were optimized. The contents of the eight carbonyl compounds in e-liquids determined by LC-MS/MS were compared to those by liquid chromatography. The results showed that the eight carbonyl compounds had good correlation coefficients (R^2 more than 0.999) in corresponding concentration ranges, the limit of detection was in the range of 5-28 ng/g, the average spiked recoveries ranged from 92.5 % to 106.4 % with relative standard deviation between 3.5 % and 6.8 %. The method possesses high sensitivity, good linearity, strong anti-interference ability, good repeatability, simple and fast pre-treatment, and it is suitable for determining e-liquid samples of large quantities.

ST 18

A simple sample preparation method for the determination of elements in e-liquids and aerosols of e-cigarettes

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Samples analysed by ICP-MS are typically subjected to a mineralisation/oxidation process to completely dissolve all elements. Therefore, samples are digested using an acid matrix in a block digestor or microwave. During this process, the organic sample content is decomposed into carbon dioxide, which is subsequently removed from the sample. However, if residual carbon is present, the instrument sensitivity can increase significantly. Especially elements with high ionization potential such as selenium (Se), arsenic (As) or beryllium (Be), are susceptible to this phenomenon known as signal enhancement or carbon effect. As a result, quantities of some elements in e-liquids or aerosols of e-cigarettes and recoveries in fortified samples might be overestimated and vary according to the carbon content of the sample.

The decomposition process of reactive samples (e.g. fats, oils) is exothermic and the reaction kinetics are to be monitored carefully. Slow heating rates can help to decompose a sample safely but result in longer sample handling time. Hence, avoiding a digestion step is saving time, reducing the risk of contaminations and eliminating a potential safety hazard.

Herewith, we present a novel approach to overcome matrix effects while analysing e-liquids or aerosols of e-cigarettes without prior digestion. By adding a significant amount of methanol to all standards as well as the sample solution, the signal enhancement effect is matched and does not influence quantification. Introduction of such high amounts of organic solvent is made possible by aerosol dilution systems which are present in modern ICP-MS systems. Variable carbon contents in the samples do not have to be taken into account and recoveries of, for example, a range from 94.9-105.1 %. As a result, a simple, reliable and robust method for the determination of numerous elements in e-liquids as well as aerosols of e-cigarettes has been developed and validated.

ST 19

An alternative strategy for the determination of pH values of aerosol from electronic cigarettes

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Any pH determination to measure the effective acid-base properties of an aerosol is dependent on the measurement technique. Determinations of aerosol pH using puff-by-puff collections are influenced by the buffer used to condition the electrode, while measurements of aqueous extracts of aerosol collected on glass-fibre filter pads are influenced by the background from the pad. Utilizing the water soluble properties of the e-aerosol matrix, the pH of aerosol trapped directly into an aqueous solution can be measured, with the measured pH being more consistent with that determined for the e-liquid.

In this study, aerosol generated from the puffing of electronic cigarettes was passed through a liquid trap (70 mL fritted impinger) containing 25 mL of a 1 % (w/v) NaCl degassed aqueous solution. The pH of the resulting solution was measured using a pH meter and combination electrode. Multiple products, anticipated to contain e-liquid of different pH, were puffed using two regimes for three different collection segments.

Accuracy for aerosol pH could not be determined. At best, a comparison between the measured pH of the aerosol and the pH determined for the e-liquid, in combination with precision of the measurements, was used as some measure of the 'pseudo-accuracy' of the method. The method collection system demonstrated excellent repeatability and reproducibility (<5 %) independent of the puffing regime. The measured aerosol pH was influenced by both the aerosol amount, measured by device mass loss, and the total collection volume.

This technique was determined to be suitable for comparing the effective acid-base properties of the aerosol generated from the puffing of electronic cigarette devices.

ST 20

Single and resonance enhanced multi photon ionization mass spectrometry for the investigation of product use behaviour of e-cigarettes and heat-not-burn products

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Over the last few years, e-cigarettes and other innovative smoking/vaping products have become more and more commonly used. Whereas most manufacturers increase the safety and reliability of their products, there are consumers modifying devices in an unsafe way or directly misusing devices for generating an aerosol with an elevated level of drug. Soft photoionization (PI) of the aerosols released from those products provides an effective tool for investigating the chemical composition of the respective aerosols on a puff-by-puff resolved time basis. The most prominent unintended e-cigarette use-behaviour is the so called 'dry burn' or 'dry puff'. This phenomenon occurs, when either the e-liquid reservoir is empty or the e-liquid cannot be transferred fast enough to the heated coil, especially, if the coil is not working temperature controlled. Consequently, a wide range of pyrolysis products could be released by the respective products. Applying online PI-mass spectrometry (MS) could potentially identify respective puffs and conditions, when the 'dry burn' appears. Besides the unintended e-cigarette use-behaviour caused by user modification or cheap, unsafe devices, PI-MS allows also to investigate intentional misuse of devices, e.g. for drug vaporization.

In general, there are two PI techniques that can be performed depending on the substance spectrum of interest: single-photon ionization (SPI@118nm), which ionizes a broad range of organic compounds, and resonance-enhanced multi-photon ionization (REMPI@266nm), which selectively ionizes aromatic compounds. Both techniques allow to effectively suppress analytical interferences originating from the smoke matrix, such as oxygen or nitrogen and even water vapour. Furthermore, mathematical/statistical methods such as non-negative-matrix-factorization can improve method performance whilst distinguishing between potential harmful compounds in the vapour/smoke of e-cigarettes as well as heat-not-burn products and measurement related artifacts.

ST 21

Saliva pH after exposure to e-cigarette aerosols in a glassmouth with and without stirring of the saliva during exposure

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After we presented our initial work at the 2016 CORESTA Congress (ST46) on the use of a glassmouth to determine change in saliva pH after exposure to an aerosol generated by an e-cigarette, a member of the audience suggested that the results of our experiments might be different if the saliva were circulated during the exposure period. A satisfactory system for circulating the saliva during exposure was not available. Instead, we built a magnetic stirrer that would fit under the bottom of the glassmouth. This stirring rate was approximately 30 rpm. We ran several sets of experiments where the same e-liquid was evaluated with and without stirring during the aerosol exposure period (50 puffs, 55/3/30 puffing regimen). Results obtained were not as expected. For example, when V2 Menthol 2.4 % Nicotine cartomizers were used with V2 battery sections, the pH rise in the saliva (Pickering 1700-0304 artificial saliva, 10 mL) was about 0.1 pH unit. When blank V2 cartomizers, which were filled with about 500 mg of either 50 mg/mL nicotine in PG or 50 mg/mL nicotine in PG (5200 mg) to which propionic acid (110 mg) was added, anomalous results were obtained. Aerosol pH at the 50^{th} puff was higher (0.3 pH unit) when the saliva was stirred than not stirred. Addition of propionic acid reduced the aerosol pH from 7.63 to 6.41 (no stir) and from 7.97 to 6.67 (stir). Final saliva pH-values for after exposure to aerosols from the 50 mg/mL nicotine in PG were 7.43 and 6.91 (no stir/stir) and were 6.41 and 6.96 (no stir/stir) for the acidified e-liquid. Saliva samples to be analysed for nicotine and propionic acid to provide additional understanding of the results as well as additional analytes such as menthol and added basic constituents to determine if they will raise aerosol pH.

ST 22

Estimation of e-cigarette aerosol yields based on puff duration

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There are regulatory requirements in quantifying and comparing the emission levels of major and minor aerosol constituents from e-cigarettes. Up until the recent publication in 2015 of CORESTA Recommended Method (CRM) No. 81 - "*Routine Analytical Machine for e-Cigarette Aerosol Generation and Collection - Definitions and Standard Conditions*", no internationally recognised standard was in place to describe how these products should be tested and a variety of different puffing regimes have been reported in the literature.

In May 2016, the U.S. Food and Drug Administration published guidance for Industry entitled '*Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems*' which requires new tobacco products, including vaping products, to be tested under non-intense and intense conditions. Therefore, it was important to understand the impact of different vaping regimes on e-cigarette emissions.

Our study investigated two closed and two open system devices using two different e-liquid compositions (50/50 and 70/30 PG/VG) containing 1.2 % Nicotine (w/w). All combinations were tested under four vaping regimes with puff duration ranging from 2 to 6 s and puff volume from 27.5 to 82.5 mL. Weight loss, nicotine, PG and VG were analysed.

The data obtained in this study showed that there is a strong linear correlation between weight loss and puff duration as well as weight loss and main component emissions. The observed correlations between puff duration and aerosol yield showed that changes can be explained mainly by puff duration, independent of device types and e-liquid used. Puff volume and air flow showed minor influence on aerosol yields. Based on these findings, a model is described allowing weight loss and the main compound yields to be estimated according to the puff duration regardless of the puff volume.

ST 23

Biomarkers of exposure specific to e-vapor products based on stable-isotope labelled ingredients – clinical study design

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E-vapor products (EVPs) are becoming an accepted alternative to conventional cigarettes among smokers. Despite the increasing popularity of EVPs, little information exists on the fate of the main ingredients glycerol (G), propylene glycol (PG) and nicotine (Nic). Since exposure to G and PG can occur from other sources, stable-isotope labelled e-liquid ingredients can directly assess exposure. Accordingly, we developed and applied methods that allow the unequivocal determination of EVP use-related uptake of e-liquids containing stable isotope-labeled Nic, G and PG. This approach assesses biomarkers to measure the absorption, metabolism and further fate of PG, G, and Nic as well as compounds formed from these precursors in the vapor, or formed endogenously. A diet-controlled clinical study was conducted with 25 healthy male subjects. The subjects were divided into 3 groups: Group I (N = 10): experienced EVP users, vaping under low wattage conditions; Group II (N = 10): experienced EVP users, vaping under high wattage conditions; Group III (N = 5): smokers, smoking conventional non-filter cigarettes spiked with stable isotope-labelled Nic, G and PG (positive control). The study was divided into two settings: confinement (Part A) and ambulatory (Part B). Periodic samples of various biofluids (plasma, urine, saliva and sputum) were collected. The presentation will include the detailed study design. To the best of our knowledge, this is the first study dealing with the absorption, metabolism and formation of degradation products in the human body solely derived from the consumption of EVPs. These data are important for providing foundational knowledge regarding EVPs.

ST 24

Biomarkers of exposure specific to e-vapour products based on stable-isotope labelled ingredients – results

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E-vapor products (EVPs) consumption has steadily increased worldwide over the past decade. Ever since their introduction, there have been discussions about the potential health risks of EVPs in the scientific and public health community. These discussions are complicated by contrary findings, especially with respect to aldehydes, which may be formed in the aerosol generated from EVPs. In order to assess the internal dose of the major ingredients in EVP users and to investigate their metabolism and potential decomposition products, we conducted a clinical study with 20 EVP users and 5 smokers of conventional non-filter cigarettes. We used stable-isotope labelled e-liquid constituents and mass spectrometry based detection in various body fluids (plasma, urine, saliva, sputum). We developed and modified several bioanalytical methods regarding the quantification of the labelled main ingredients of e-liquids, namely propylene glycol (PG), glycerol (G) and nicotine (Nic) as well as their metabolites and potential degradation products which may be formed from PG and G under pyrolysis conditions. No interferences due to the diet or exposure to other consumer products were observed for labelled PG, G, and Nic in plasma and urine. Stable-isotope labelled mercapturic acids (MA) formed from acrolein (3-HPMA) and propylene oxide (2-HPMA) were not detectable in urine of EVP users. In contrast, labelled 3-HPMA and 2-HPMA were observed in the smokers smoking non-filter cigarettes spiked with stable-isotope labelled PG, G, and Nic. Hence, inclusion of a smoker positive control group allowed us to reveal pyrolysis products specifically derived from PG and G. In conclusion, our data proved the applicability of the stable-isotope labelling concept to unequivocally assess EVP-specific internal dose of the major ingredients PG, G, and Nic as well as the presence of potential degradation products in the vapour and their further metabolism in the human body.

ST 25

A clinical study in Japanese smokers investigating changes in exposure to cigarette smoke chemicals in participants who switch to using a tobacco heating product for a five day period

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Tobacco heating products (THP) are electronic devices that heat tobacco, rather than combusting it. Due to this lack of combustion, significantly fewer toxicants are formed but nicotine is still released into the inhaled aerosol. Clinical studies investigating biomarkers of exposure (BoE) are an important method to determine if this translates into a reduction in exposure to cigarette smoke toxicants when smokers switch to using a THP.

A clinical study was conducted in a confined environment to investigate changes in BoE to smoke toxicants when smokers switched to using glo™, a novel THP. This study was conducted in Fukuoka, Japan (UMIN000024988, ISRCTN14301360), approved by a local Institutional Review Board and run in accordance with ICH-GCP. 180 subjects completed the study.

The study included a baseline phase and an exposure phase. The baseline phase consisted of two days where the subjects smoked combustible cigarettes. This was followed by an exposure phase with the subjects randomised into groups where they either continued to smoke the combustible cigarettes, switched to using glo™, or abstained from any tobacco product use, for five days. In both phases of the study 24 hour urines were collected for analyses of a range of BoE and exhaled CO was also measured daily.

Results from the urinary BoE and exhaled CO showed reductions in levels in subjects who switched to the glo™ device for five days, as well as reductions in the levels in subjects who abstained from any tobacco use for five days.

These data show that smokers experience reductions in levels of exposure to smoke toxicants when switched to the glo™ THP. Longer term BoE and Biomarker of Biological Effect studies may demonstrate that these reductions in exposure are sustained, and whether this translates into reductions in smoking-related health risks in subjects who switch to glo™.

ST 26

Dynamic determination of nicotine and 11 metabolites in brain and blood of rat by simultaneous microdialysis coupled with UHPLC-HRMS

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Nicotine is the most specific cigarette smoke component and plays a key role in tobacco addiction. Nicotine dependence based on neuropharmacology effects is closely associated with nicotine metabolism. Although peripheral metabolism of nicotine has been studied extensively, nicotine disposition and metabolism in the central nervous system has been given little attention. In order to accurately determine nicotine and its metabolites in brain and blood of rat, a simultaneous microdialysis method coupled with ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) was established to characterise the pharmacokinetics of nicotine metabolites. Microdialysis probes were inserted into the right striatum and jugular vein/right atrium of SD rats simultaneously, and dialysates were collected every 15 min after nicotine administration (2.0 mg/kg, i.p.). Target analytes were separated on a hydrophilic interaction liquid chromatography column (HILIC 3.0 × 150 mm, 2.7 µm) and detected by Q-Exactive under Full MS/Targeted-MS^2 mode. The results showed that 11 metabolites (cotinine, nornicotine, norcotinine, nicotine-*N*-oxide, cotinine-*N*-oxide, *trans*-3'-hydroxycotinine, nicotine-*N*-glucuronide, cotinine-*N*-glucuronide, *trans*-3'-hydroxycotinine-O-glucuronide, 4-oxo-4-(3-pyridyl)-butanoic acid and 4-hydroxy-4-(3-pyridyl)-butanoic acid) were generated from nicotine in blood and brain tissue of rat. The concentration-time profiles of nicotine metabolites and pharmacokinetic results indicated that cotinine was the main metabolite, *trans*-3'-hydroxycotinine and norcotinine were the second most abundant metabolites, and the other eight metabolites were present in minor amounts. Both blood and brain levels of nicotine declined rapidly, while nicotine was predominately diffused into brain tissue. It should be noted that the metabolic characteristics of nicotine in the brain were relatively different from blood, although the detected nicotine metabolites were the same in brain and blood of rat in this study. The method is suitable for the simultaneous analysis of nicotine and its metabolites in rat for pharmacokinetic applications.

ST 27

Pharmacokinetics of nicotine following the controlled use of a prototype novel tobacco vapour product

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The objective of this clinical study was to investigate the pharmacokinetics of nicotine following the use of a prototype novel tobacco vapour (PNTV) product in comparison to a conventional cigarette (CC1). The study was conducted in Japanese healthy adult male smokers, using an open-label, randomised, two-period crossover design, to assess the pharmacokinetics of nicotine after controlled use of a PNTV product or CC1. During the study period, blood samples were drawn from subjects for the measurement of plasma nicotine concentrations and nicotine intake was estimated from the mouth level exposure (MLE). The Cmax and AUClast following the use of PNTV product were 45.7 % and 68.3 %, respectively, of those obtained with CC1 and there were no significant differences in the Tmax and T1/2 between PNTV product and CC1. The estimated MLE following the use of PNTV product was approximately two-thirds of that obtained following the smoking of CC1, but the relative bioavailability of PNTV product to CC1 was approximately 104 %. The differences in Cmax and AUClast between the PNTV product and CC1 therefore are explained by differences in nicotine intake. These results suggest that the PNTV product shows a similar pharmacokinetic profile to CC1, while delivering less nicotine following controlled use.

ST 28

Nicotine pharmacokinetics of electronic cigarettes: experimental data and a review of the literature

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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.

ST 29

Impact of cigar physical variability on cigar exposure using probabilistic risk assessment

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Cigars are unique tobacco products of 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, 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 work was to determine the impact of cigar 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. 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 variability on the overall consumer exposure to cigars is critical to explain uncertainty associated with smoking.

ST 30

Comparison of probability of risk associated with cigar exposure

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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, PRA analysis indicated the change was too small and may not be considered significant. In fact, PRA showed >90 % overlap between probability distributions of two products. Sensitivity analysis suggests that the smoking behaviour and other parameters contributed to >90 % of the observed variability associated with the analyte of concern between the products. PRA is a useful tool when comparing the probability of risk of cigar products.

ST 31

Comparison of background risks with risks estimated from constituents of tobacco products

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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.

ST 32

Consumers' responses to communication materials of a novel heat-not-burn tobacco product

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Modified risk tobacco products (MRTPs) are defined by the U.S. Food and Drug Administration (FDA) as tobacco products that are sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products. Clear communication is important to ensure adult consumers understand MRTPs features, benefits and risk profile. We report an analysis of three studies (1510, 1509, and 1534 participants, respectively), in which adult smokers and non-smokers were exposed to communication materials for a candidate MRTP, the Tobacco Heating System (THS). In these studies, participants answered global comprehension questions and assessed risk perceptions of THS based on different combinations of THS communication materials (brochure or pack) and warnings (THS-tailored PMI warning or US Surgeon General's warnings), along with study-specific benefit claims. Multiple linear regression analyses were conducted to assess the covariate-adjusted influence of communication-based factors on levels of comprehension and risk perception. In all three studies, the PMI warning was overall associated with higher levels of comprehension of the information about the THS risks compared to currently mandated cigarette warnings. Compared to the pack, the brochure was associated with higher levels of risk perception in all three studies and lower levels of comprehension in one of the studies. Comprehension and risk perception of THS communication materials were also influenced by sociodemographic variables, including race and education. Pooling of the results across the three studies showed a higher level of comprehension in the two studies with reduced risk claims while levels of perceived risk was not influenced by the type of claims. These findings could help to develop effective communications on MRTPs.

ST 33

Air quality assessment during indoor use of the Tobacco Heating System 2.2

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PMI's heat-not-burn Tobacco Heating System 2.2 (THS 2.2) generates tobacco aerosol containing mainly water, glycerine and nicotine. To address public health concerns about the possible presence of polluting substances during indoor use of THS 2.2, a study was conducted using an environmentally controlled room to simulate a residential environment with a low ventilation rate (0.5 air changes/h) and design occupancy of 8 m^2/person (two panellists, one PMI representative). Twelve sticks were consumed over the two-hour air collection period.

Twenty-three constituents (nicotine, 3-ethenylpyridine, solanesol, respirable suspended particles by gravimetric measurement, particulate matter by UV and fluorescence, total volatile organic compounds (TVOCs), acetaldehyde, acrolein, crotonaldehyde, formaldehyde, acrylonitrile, benzene, 1,3-butadiene, isoprene, toluene, glycerine, propylene glycol, *N*-nitrosonornicotine (NNN), nicotine-derived nitrosamine ketone (NNK), carbon monoxide, nitrogen oxide and combined oxides of nitrogen) were quantified by validated and ISO17025 accredited methods. These analytes cover environmental tobacco smoke (ETS), indoor air quality (IAQ) and product-specific markers (e.g. glycerine).

In comparison to background air, only three compounds can be attributed to the use of THS 2.2: nicotine (1.15 µg/m^3), acetaldehyde (3.44 µg/m^3) and glycerine (10.5 µg/m^3). Their levels are far below the maximum exposure levels as defined in existing air quality guidelines. Moreover, based on Total Volatile Organic Compounds data analyses, the chemical composition of the background air and air during use of THS 2.2 were remarkably similar. Evaluation of the concentrations of particulate matter constituents, volatiles and semi-volatile compounds confirm that THS 2.2 is not a source of ETS. In conclusion, using THS 2.2 does not have a negative impact on the overall air quality.

ST 34

Influence of storage conditions on smoke yields and optical appearance of cigarettes

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Chemical and physical changes of the cigarette paper can occur during the storage of cigarettes. These changes may have an influence on the smoke yields, the taste of a cigarette and also on the optical properties of the cigarette and the ash appearance during smoking.

The aim of this study is to investigate the influence of storage conditions on cigarette paper parameters, smoke yields and ash appearance. In a first step cigarettes were stored under different conditions of temperatures from 22-50 °C at 60 % RH for defined time periods between 11 and 33 days, followed by an analysis of cigarette paper parameters, smoke yields and ash as well as optical appearance (e.g. spotting)

With respect to cigarette paper parameters, the storage conditions show only a minor effect on the basis weight and the air permeability but a statistically significant impact on opacity and brightness. Opacity increased from 73 % to 85 %, while brightness decreased from 87 % to 64 % and the chalk content decreased from 29.5 % to 27 %. The reduction of chalk is a consequence of the migration of ions during storage, which has been reported in other publications. In these publications migration effects were observed for the cations and anions between the tobacco and the paper and may explain the observed change in cigarette paper parameters.

Further the smoke yields of the cigarettes before and after storage were measured. A statistically significant effect on the CO values was observed, which decreased by approximately 10 %, all other smoke yields were substantially unchanged. Furthermore, an influence on the ash and optical appearance was found.

The results show that the main influencing factor is temperature, while the storage time does not affect the yields significantly.

ST 35

Pyrolysis experiments to assess cigarette paper design contribution to thermal degradation without and with tobacco

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With worldwide emerging regulations on tobacco products and their ingredients, pyrolysis studies seem to be inescapable when product health risks under a design modification should be assessed.

The study objective was to evaluate the effects of the cigarette paper design in combination with tobacco on thermal degradation components.

Firstly, the pyrolysis conditions (temperature, oxygen level) and their influence on thermal degradation components were examined. For cigarette paper, thermal degradation occurred at very low levels below 350 °C. The oxygenated species decreased with temperature. The pyrolysis profile was not strongly impacted by the oxygen level (2-20 %). The observed changes were mainly pronounced in the intensity.

Secondly, the cigarette paper ingredient effects were evaluated in comparison to cellulose. At 450 °C, higher analytical readings for levoglucosan and furfural were observed with cellulose, whilst for cigarette paper higher analytical readings for propanal, pentanol, furanone and cyclopentadienone derivatives were found. The main thermal degradation pathway for cellulose is a depolymerisation, whilst for the cigarette paper the dehydration pathway was identified.

Thirdly, the effect of cigarette paper with and without tobacco through different cigarette paper design parameters including porosity, basis weight, filler and salt was examined. At 450 °C only basis weight and citrate levels affected the thermal degradation and impacted the formation of acetone, diacetyl, 1,3-cyclopentadiene. At 700 °C, an effect of basis weight on benzene, toluene, phenol was observed. Additionally, an effect of citrate on crotonaldehyde was found. The variation induced by the cigarette paper design was very low. The analytical results suggest that the effects seen, are mainly driven by the tobacco.

In conclusion, pyrolysis experiments coupled with analytical methods are a useful tool to identify harmful by-products components generated by thermal degradation of ingredients. Pyrolysis studies could help to identify the level of the effect of each ingredient and its potential interaction with paper or tobacco.

ST 36

Alternate materials and their potential impact on HPHCs

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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 tow materials. 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.

ST 37

Nicotine reduction: use of a modelling approach to evaluate unintended consequences, a focus on illicit trade

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In 2015, the WHO TobReg issued an advisory note recommending a strategy of reducing nicotine in tobacco to substantially lower levels. The authors considered that regulation of nicotine levels would lead to a decrease of smoking prevalence. In their review, they assessed the feasibility and relevance of the strategy using a range of different research activities, from tobacco plant genetics to consumer sensorial perception. However, what is also readily apparent is a number of unintended consequences that arise from such a strategy. For example, a reducing nicotine raises multiple issues from an agronomy perspective, which would threaten the livelihood of millions of farmers. Additionally, market disruption would foreseeably benefit and increase illicit trade. Although the use of simulation models to predict the impact of a new policy is recognised as extremely valuable, only a few publications have simulated the impact to a population. The use of simulations would allow regulators to evaluate options and make informed decisions that do not have unintended consequences that undermine the original policy aim.

The objective of our study was to develop a simulation model enabling the assessment of the impact of nicotine reduction policy on illicit trade. Both the baseline and counterfactual case were considered. The baseline corresponds to the status-quo and predicts population status in future if regulation does not change. The counterfactual case predicts the population status if current conventional cigarette (CC) becomes illicit once replaced by reduced nicotine cigarette (RNC). Each individual of the population was classified in one of four groups: non-smoker, smoker of licit products (RNC), smoker of illicit products (CC) and former smoker. For the baseline, status transitions were derived from published data. For the counterfactual case, scenarios were tested considering the reported negative impact of RNC on smoker's satisfaction and on the probability to switch to more satisfying illicit products. Trends were assessed under various scenarios and demonstrate in some conditions the risk associated with a nicotine reduction strategy.

ST 38

Assessing the likelihood and magnitude of a population health benefit following market introduction of a modified risk tobacco product (MRTP)

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The evaluation and implementation of tobacco policies intended to reduce harm to the population as a whole, including tobacco users and non-users, must assess the potential for both intended and unintended consequences associated with those policies. These assessments should be based on the combined dimensions of magnitude, and thus likelihood, of shifts in exposure patterns needed to produce a population benefit or harm, and magnitude of the expected population benefit or harm. To provide researchers and policymakers with a means of conducting such assessments, we developed a dynamic population modeler, DPM(+1), to estimate differences in survival if exposure patterns in the population shift from a higher risk product (e.g. cigarettes) to a modified risk tobacco product (MRTP) in specified ways. Statistical analyses that estimate the effects on all-cause mortality following market introduction of an MRTP that presents 8 % of the risk associated with cigarette smoking indicate that, within a single birth cohort, switching completely from cigarettes to the MRTP is more likely to lead to a population health benefit than initiating tobacco use with the MRTP instead of cigarettes. This is because tobacco initiation rarely occurs beyond young adulthood, whereas continuing smokers exist in all age categories, leading to a greater cumulative effect. In addition, complete and persistent switching to MRTP use among a small proportion of cigarette smokers in each age category offsets the survival deficit likely to occur with unintended shifts in exposure patterns, such as MRTP initiation among never tobacco users followed by transitioning to cigarette smoking and/or cigarette smokers switching to MRTP use instead of quitting. This is because the magnitude of risk reduction among smokers who switch completely from cigarettes to MRTP use far exceeds the risk associated with MRTP use among tobacco non-users, even when employing conservative estimates for unintended exposure patterns.

ST 39

Addressing U.S. FDA's population health standard for Camel Snus with modified risk messaging

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R.J. Reynolds Tobacco Company has submitted Modified Risk Tobacco Product applications to the U.S. Food and Drug Administration seeking risk modification orders for Camel Snus advertising, which includes information that smokers who switch completely to Camel Snus can greatly reduce their risk of lung cancer, oral cancer, respiratory disease and heart disease. These applications provide results from scientific studies that assess the risks and benefits of the proposed advertising to the population as a whole. Studies examining consumers' comprehension and perceptions of the advertising indicated that vast majorities understood and applied the risk information, as well as appropriate cautions. Only 4 % of consumers indicated smokers would receive a health benefit if they continued to smoke while using Camel Snus, while >80 % indicated Camel Snus is addictive, quitting tobacco use is the best choice, and non-tobacco users should not use Camel Snus. After viewing the proposed advertising for Camel Snus, consumers indicated their intent to purchase the product for trial, and intent ratings were converted to projected likelihoods of use by using a predictive algorithm. Modified risk advertising for Camel Snus differentially increased projected use among current smokers (8.2 %), while having minimal effect on former smokers (1.9 %) and never tobacco users (0.5 %). Projected use was lower among current smokers likely to quit (4.2 %) versus not likely to quit (8.7 %). Finally, statistical modeling was used to assess the overall effects on population mortality based on likely use of Camel Snus with modified risk advertising. Assuming 89-92 % risk reductions for Camel Snus, analyses that included age-specific primary and conservative secondary (e.g. gateway) transitions estimated a survival benefit of >25,000 individuals in a U.S. birth cohort of 4.1 million. Tipping point analyses indicated that if 1.5 % of continuing smokers switched completely to Camel Snus at each age interval, there would be a population health benefit.

ST 40

FDA's proposed NNN product standard: review of the scientific evidence

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The U.S. Food and Drug Administration (FDA) has published a proposed standard of 1 µg/g (dry weight) N-nitrosonornicotine (NNN) in finished smokeless tobacco 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 smokeless tobacco 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 results, and likely effect on population health, the proposed limit of NNN in smokeless tobacco products would not benefit, or otherwise be protective of, public health.

ST 41

Biomarker assay validation: the gold, silver, bronze approach

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Biomarkers are an important tool for helping the tobacco industry understand their products and to help register new products. Biomarker data are the most common tool used to support “scientific evidence of substantial overall reductions in exposure to the harmful substance(s)” in the FDA guidance for an modified risk tobacco product (MRTP) application. The pharmaceutical industry has held many workshops on biomarker validation and the tobacco industry can learn from those workshops. The information obtained from most biomarkers can be classified as “nice-to-have” versus “need-to-have”. Obviously, the amount of validation effort will be much different between “nice-to-have” versus “need-to-have” biomarkers. Problems arise however when it is desired to include data validated at a “nice-to-have” level in a regulatory submission. Therefore, it is important to understand the life cycle of biomarker assays. This presentation will define the uses and the fit-for-purpose validation for the following phases of a biomarkers life:

- Regulatory need → “Gold”
- Key-decision making → “Silver”
- Exploratory → “Bronze”

This presentation will offer a strategy for all stakeholders of a project (medical directors, clinical pharmacologists, statisticians and the bioanalytical group) to have a common simple terminology for the life cycle of biomarkers.

ST 43

Feasibility study of simultaneous determination of particulate and volatile compounds in mainstream cigarette smoke

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This study demonstrates the feasibility of simultaneous determination of tobacco-specific nitrosamines (TSNAs) and volatile organic compounds (VOCs) in mainstream cigarette smoke.

WHO TobReg has proposed mandated lowering of nine toxicants in mainstream cigarette smoke. Four toxicants (NNN, NNK, 1,3-butadiene, benzene) are classified together as TSNAs and VOCs. CORESTA has developed CORESTA Recommended Method (CRM) No. 70 for VOC analysis and CRM 75 for TSNA analysis.

According to CRM 70, VOCs are collected by passing mainstream smoke through a Cambridge filter pad (CFP) and into a cryogenic impinger containing methanol. The CFP is used as a quality control measure. TSNAs are collected with a CFP based on CRM 75. By combining these two methods, TSNAs and VOCs can be collected simultaneously to reduce the total time of the combined method compared with that of the standard CRMs. However, pressure loss caused by the connecting impingers may change the puff-profile and affect the generation of particulate compounds. To date there have been no reports on the effects of connecting impingers on the measured values of particular compounds. The objective of this study was to investigate the above effects and assess the feasibility of simultaneous determination of TSNAs and VOCs.

The shape of puff-profiles and the values of TSNAs and VOCs were measured when the pressure loss was varied by changing the number of impingers (0–3) and the pressure drops of the frits (low/middle/high).

In this presentation, effects of the number of impingers and the pressure drop of the frits on the measurement values, repeatability, and reproducibility will be discussed.

ST 44

Investigation of tetrahydrocannabinol (THC) in smoke by application of an online photo ionisation mass spectrometry

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Photoionisation time of flight mass spectrometry (PI-TOFMS) is well suited for online characterisation of tobacco smoke. Depending on the photoionisation method (single photon ionisation, SPI or resonance-enhanced multiphoton ionisation, REMPI) smoke constituents such as butadiene, acetaldehyde, naphthalene, phenol or polycyclic aromatic hydrocarbons (PAH, by REMPI) can be detected with high time resolution (puff-resolved). With the increased (legal) availability of marihuana/cannabis and THC containing smoking products not only for medical purposes, the interest in understanding the release processes of the active smoke constituents is increasing as well. Puff-by-puff emissions analysis of different products ('joints') filled with tobacco mixtures containing dried marihuana flowers, leafs or hashish were performed to investigate the release profile of THC and related smoke constituents in comparison to nicotine as the main active compound of the added tobacco. Within this study, a Laser PI-TOF system (Photonion GmbH, Schwerin/Germany) in REMPI mode was used coupled to a LM1 smoking machine (Borgwaldt KC, Hamburg/Germany). The REMPI methodology enables focusing on aromatic structures primarily relevant for this investigation. Smaller molecules being present in higher concentrations in smoke (e.g. aldehydes), which could lead to a suppression of the target smoke constituents during the measurement, are suppressed effectively compared to SPI (single photon ionisation). Environmental gases such as oxygen or nitrogen are suppressed by photoionisation anyway. Furthermore, the present study evaluates the influence of activated carbon filters, which are becoming more and more popular to reduce certain smoke constituents, such as PAHs (polycyclic aromatic hydrocarbons), in mainstream smoke of any combustible product.

ST 45

A multiple heart-cutting two-dimensional liquid chromatography coupled to quadrupole-orbitrap high resolution mass spectrometry for simultaneous determination of Aflatoxin B1, B2, G1, G2 and Ochratoxin A in snus

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A multiple heart-cutting two-dimensional liquid chromatography (MHC-LC-LC) was coupled to quadrupole-orbitrap high resolution mass spectrometry (HRMS) to simultaneously determinate the aflatoxins (AFs) and ochratoxin A (OTA) in snus. A C18 capillary column was used as the first dimension (1D) column to isolate the AFs and OTA from the complex matrices, then a 2-position/10-port high pressure valve equipped with two 60 µL loops was employed to transfer the heart-cuts of 1D-LC into a pentafluorphenyl (PFP) column, where the second dimension separation was performed. Results of use of the MHC-LC-LC system, the ionisation suppression was noticeably reduced while the method's sensitivity was enhanced, which is essential for trace mycotoxins analysis. With excellent capability for qualitative analyses compared to triple quadrupole mass spectrometry, the HRMS was employed to eliminate the false positives and avoid the unnecessary clean-up pre-treatment. A dynamic range of 0.2 µg/kg to 20 µg/kg was achieved with the quantification limits of 0.05 µg/kg for AFB1 and 1.0 µg/kg for OTA in dry mass of product. The results revealed that the established method exhibited good repeatabilities and recoveries, and could be used as a rapid and reliable approach for routine analysis of AFs and OTA in snus.

ST 46

Analysis of PAHs in smokeless tobacco by GC-MS/MS

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Polycyclic aromatic hydrocarbons (PAHs) are a large group of compounds; several of which are classified as potentially carcinogenic. Sixteen PAHs are listed on the U.S. Food and Drug Administration (FDA) "*Established List for Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke*". Therefore, a sensitive and reliable analysis method for PAHs is important for many tobacco industry laboratories.

In this presentation, a gas chromatography-tandem mass spectrometry (GC-MS/MS) method for the analysis of 19 PAHs, including naphthalene, in smokeless tobacco will be presented. Additionally, results from a collaborative pre-study will be presented (naphthalene not included).

PAHs are extracted for 30 min from the sample using methanol after the addition of deuterium-labelled internal standards. The extract is centrifuged and a small aliquot is analysed for napthalene using headspace GC-MS/MS. The rest of the extract is cleaned using SPE and analysed for 18 PAHs using GC-MS/MS. The method is a modification and expansion of CRM 82 that is used for the analysis of B[a]P. The modifications include the removal of the laborious filtration and evaporation stages. The limit of quantification was determined to range from 0.6 ng/g to 5 ng/g.

The method has been validated for snus, moist snuff and chewing tobacco and a CORESTA collaborative study is currently ongoing to establish the performance characteristics and includes a wider application area (e.g. cigarette and cigar fillers).

To conclude; this is a sensitive and non-laborious method that includes all the PAHs on the “FDA-list”.

ST 47

Adapting traditional human abuse liability testing to tobacco products

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Under the Family Smoking Prevention and Tobacco Control Act that grants the U.S. Food and Drug Administration (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 presentation 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.

ST 48

Compulsive tobacco use - an alternative hypothesis

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The compulsive use of tobacco has led to the common belief that nicotine is addictive and this is the core feature underlying the abuse liability of tobacco. However nicotine or tobacco fail to meet most criteria for abuse liability and even under the key addictiveness criterion of “liking”, nicotine or tobacco are not greatly liked compared to other substances. Convoluted theoretical models have been developed to explain these discrepancies but none has proved adequate in fully explaining tobacco use or in developing more effective treatments for tobacco dependence.

The liking response is associated with enjoyment and satisfaction after consumption. However there is a second major driver of motivated behaviour, a “wanting” response associated with anticipation and desire before consumption. To date models of tobacco use have subsumed the wanting response under the liking response but there are a number of reasons why they should be regarded as distinct systems. Most notably wanting (the incentive system) and liking (the hedonic system) can be dissociated by manipulation of different neurochemical systems (Berridge 1995) and by hedonic hotspots in the brain separable from incentive hotspots (Berridge et al. 2009).

This presentation will present evidence in support of a hypothesis that nicotine may influence the incentive (wanting) system more than the hedonic (liking) system. Not only would this explain the apparent lack of addictiveness of nicotine and tobacco but also suggest that compulsive tobacco use reflects a form of excess wanting. This could lead to new targets for the treatment of tobacco dependence and highlights the importance of monitoring both the hedonic (liking) and incentive (wanting) systems during the development of next generation nicotine and tobacco products.

ST 49

Mouth level exposure estimation of nicotine and NFDPM by applying part-filter analysis from smokers of super-slim and regular size cigarettes

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In recent years the super-slim cigarette market of low and medium tar products steadily increased in East Asian countries. According to statistical data in Korea, the market share of super-slim products in Korea steadily increased from 20 % in 2012, up to 27 % in 2016. However, studies on smoking behaviour such as smokers' exposure to nicotine have not been conducted. It is known that mainstream smoke yields determined by machine-smoking cannot adequately predict the exposure to nicotine as well as to NFDPM for human consumers.

In this study, the part-filter analysis, CORESTA Recommended Method No. 80 (CRM 80) was applied to estimate smokers' exposure to nicotine. The objectives of this study were to estimate and compare the mouth level exposure to nicotine and NFDPM for consumers smoking super-slim or regular size cigarettes. Eleven commercially available cigarette brands (ISO tar yields: 1~6 mg) from the Korea market were used to undertake this study. Nicotine, NFDPM and solanesol were analysed in filter tips through applying CRM 80. Machine smoking under five different smoking conditions were conducted to derive the calibrations for further calculation of the equations in the context of CRM 80. A linear calibration between the analytical readings out of CRM 80 (filter tips) and machine smoking (filter pads) was established for nicotine as well as NFDPM. Subsequently, a linear regression equation was calculated. With this linear regression, the mouth level exposure to nicotine and NFDPM for smokers of super-slim and regular size cigarette products were estimated.

ST 50

Flavored e-cigarette use among U.S. adults: results from two national surveys

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Flavored e-cigarettes have generated much controversy and interest. To examine flavored e-cigarette use among U.S. adults, we performed analyses of two national surveys. Population Assessment of Tobacco and Health (PATH) provides a representative, weighted 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) provided a weighted sample of 46,637 adults (4,845 past-30-day e-cigarette users), surveyed online from January 2014 to June 2015. The two surveys define current e-cigarette use differently, yet provided similar findings. Across surveys, 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 the flavor was not identified. Flavor use declined steadily with age in both surveys, from 83 % / 85 % for 18-24-year-olds to 50 % / 41 % after age 65. 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 less-than-weekly smokers, 63 % / 58 % of daily smokers). These patterns suggest adoption of flavored e-cigarettes increases with decreasing smoking (including cessation), consistent with prior reports that shifts toward flavored e-cigarettes are often part of a transition away from smoking. 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.

ST 51

Variations in intensity of e-cigarette use, smoking history, and demographics among past-30-day e-cigarette users

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Analyses that examine e-cigarette use often define "users" as those with any past-30-day use; this ignores substantial variations in use patterns, and leads to incorrect perceptions of e-cigarette 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 (days/month) and amount (uses/day). 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); conversely, 22 % used daily or 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 years 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 just one day/month (OR=2.2) and to report light 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 than men 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 - associated with different subject characteristics - among past-30-day-users, suggesting that a more discriminating and detailed characterization of users is necessary to understand e-cigarette use.

ST 52

Investigation of nicotine release behaviour of tobacco chewing gum in oral cavity by a novel simulated oral dissolution device

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For studying the nicotine release behaviours of smokeless tobacco products in the oral cavity, a controllable and reliable method was established and a novel simulated oral dissolution device was designed. Taking the *in vivo* nicotine dissolution amount of a commercially available tobacco chewing gum (containing 2 mg nicotine per pill) as a reference, the dissolution parameters, including chewing frequency, chewing force and saliva amount, of the simulated device were determined. Then the *in vitro* nicotine release behaviors of tobacco chewing gums were investigated. The fitting results of *in vivo* and *in vitro* dissolution curves indicated that the device simulated the *in vivo* nicotine dissolution rules of tobacco chewing gums relatively well. The nicotine dissolution rates of several typical tobacco chewing gums with different formula designs and manufacture technologies were compared. The results showed that: (1) The tendency of nicotine dissolution rates of three kinds of chewing gums was first fast and then slow; (2) The nicotine dissolution rates of three kinds of chewing gums reached about 80 % within 15 min; (3) The coating of chewing gum obviously postponed the *in vitro* dissolution of nicotine at initial chewing stage; (4) The *in vitro* nicotine dissolution rate of nicotine tartrate salt was much lower than that of ultrafine tobacco powder in previous 8 min, and the former was lower than the latter in the whole chewing process; the slow-release effect of nicotine tartrate salt was obvious. This simulated dissolution method provides a technical support for the manufacture and quality control of tobacco chewing gum.

ST 53

Fumex: light scattering sensor for the analysis of electronic cigarette aerosols

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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 polarisations. 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/m^3, MMD of 0.2 -1.5 µm, 100 ms 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 measurements 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.

ST 54

A low flow cascade impactor system for measurement of e-cigarette aerosol particle size

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

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

ST 55

Electronic cigarette aerosol dynamics in a physical model of the adult human oral/pharyngeal cavity

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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 computed tomography (CT) scan of a 28 year-old healthy male and had an internal volume of 69.8 cubic centimetres (cc). 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 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 due to the wet wall conditions. 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 43 % for a 5 s puff duration, with 55 cc puff volume and 37 °C wall temperature. The increase is due to moisture uptake by the aerosol. The aerosol mass increased by 110 % using a 3 s puff duration, 55 cc puff volume and 37 °C wet walled condition. The experimental data will be used to validate the CFD model.

ST 56

Spirometry as an effective tool for screening eligibility in e-cigarette clinical trials

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Emerging claims with regards to electronic cigarette's usefulness to defer COPD (chronic obstructive pulmonary disease) and other smoking related diseases has launched a wave of clinical research. In the commercial research clinic, spirometry has become the dominant tool for pulmonary function assessment. In controlled research, subjects with severe/advanced lung disease are excluded from participation to ensure that the outcome of "switchability" to an e-cigarette is not unduly impacted by the inclusion of subjects with advanced pulmonary small airway destruction.

Method: Screening was performed on subjects potentially enrolling in two clinical research trials. Forced Vital Capacity (FVC), Forced Expiratory Volume (FEV)1 and FEV1/FVC ratios were used to rule out reversible airway disease and obstructive patterns by evaluation of pre- and post-bronchodilator measurements. Both studies included a pre-bronchodilator FEV1/FVC ratio <0.7 and a post-bronchodilator FEV1/FVC ratio <0.75; FEV1 increases were set to <12 % or 200 ml. FEV1 <50 % was used to screen Study I subjects with a longer history of smoking; whereas, in Study II, which included a higher number of younger smokers, a more restrictive FEV1 <80 % was used.

Results: Subjects found to have airway disease that might have altered the study conclusions. In Study I there were 150 subjects screened with eight spirometry failures and in Study II 94 subjects were screened with four failures. These failures were directly attributed to COPD.

Conclusion: Obstructive lung disease indicates a loss of elastic recoil and airway radial support resulting in pressure-dependent collapse and is a common exclusion criteria for clinical research volunteers. Approximately 5 % of subjects that underwent spirometry during screening for two enrolling trials were detected to have COPD. Spirometry is a useful tool as a rapid functional test to eliminate inappropriate study subjects from participating in clinical trials, avoiding potential bias.

ST 57

13-week nose-only inhalation study of aerosolized propylene glycol in rats

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1,2-propylene glycol (propylene glycol, PG) is a colourless, tasteless viscous liquid. Because of its good atomisation effect, it is commonly used as a primary ingredient in electronic cigarette e-liquids and thus can present scenarios of potential long-term inhalation in humans. Its safety for chronic and high-dose inhalation exposures has therefore been of wide interest.

A 13-week nose-only inhalation experiment of aerosolised PG followed by 4-week recovery period in Wistar rats was conducted. A self-made capillary aerosol generator (CAG) was used to produce PG aerosol based on the electronic cigarette principle. Male and female Wistar rats were assigned into four groups (Sham, low-, middle- and high-dose). All exposure groups were exposed to PG aerosol at a concentration of 28 mg/L air and targeted doses of 100 mg/kg, 500 mg/kg, and 1500 m/kg (for low-, middle- and high-dose groups, respectively) were achieved by adjusting the exposure duration based on body weight. Biological endpoints after 13-week exposure and 4-week recovery period included hematology, coagulation, serum biochemistry, urinalysis, bronchoalveolar fluid (BALF) analysis, necropsy and histopathology.

The non-observed effect level (NOEL) values were determined to be 100 mg/kg based on body weight and food consumption data. All other statistically significant differences in hematology, coagulation, serum biochemistry, urinalysis, BALF analysis and organ weight data were considered to be attributed to biological variation and not regarded as adverse effects because all parameters with statistically significant changes were within normal ranges and there were no microscopic correlates. Histopathological examination of both sexes in all groups revealed the Sham group and high-dose group exhibited similar incidences and degrees of pulmonary pathological changes. No abnormal morphologic variations were observed in the nasal passage, trachea and larynx across groups.

In summary, exposure to PG aerosol for 13 weeks in rats results in relatively low toxicity.

ST 58

Contribution of gas-phase combustion to total heat generation from a burning cigarette

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Heat release is an important factor in understanding a burning cigarette. Heat releasing reactions are assumed to consist of three categories: pyrolysis, generating char and pyrolysis gas; combustion, generating combustion gas and residue; and gas-phase combustion of the pyrolysis gas, also generating combustion gas. Some studies involving numerical simulation of burning cigarettes have included these reactions in the mathematical model. However, there are few experimental reports referring to the heat released during these reactions.

The purpose of this study is to experimentally evaluate the amount of heat generated from each of these reactions in a burning cigarette.

The amount of heat generation from each reaction is evaluated from the balance of enthalpies between reactants and products. Combustion and pyrolysis testing is conducted to collect the reactants and products. A cigarette is fixed vertically in a tube and its top is ignited so that smouldering progresses downward. The enthalpy of the residue is measured after the cigarette has extinguished. The exhaust gas is collected, and the volume fractions of oxygen, carbon monoxide, and methane are measured. The total particulate matter in the exhaust gas is collected by a Cambridge filter. The pyrolysis gas is represented by a gas that is generated from pyrolysed cut tobacco leaves that are heated from room temperature to 400 °C in dry air.

The heat generated in the gas phase is estimated to be 812 J/g, which is about 12 % of the total heat generation of 6340 J/g from a static smouldering cigarette, and almost all of the rest of the heat is generated by char combustion. In the case of cigarette combustion during puffing, heat generated by gas-phase oxidation is estimated to be relatively insignificant, less than 5 % of total heat generation.

ST 59

Estimating the filling capacity of cut-rolled expanded stem (CRES) from Visible (Vis)–Near infrared (NIR) spectrum of stem

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Filling capacity is an important physical characteristic for evaluating cut-rolled expanded stem (CRES) quality and for designing cigarettes. As presented at the CORESTA Congress 2012, we developed a calibration model between Vis-NIR spectra and filling capacity of processed laminas. Nevertheless, no convenient measurement method exists as an economical and cost-effective approach for estimating the CRES filling capacity. A practical, rapid, and low-cost substitute for conventional methods via inefficient pre-production trials is eagerly anticipated to maintain appropriate CRES blending.

This study was conducted to establish a calibration model to estimate the filling capacity of CRES from Vis-NIR spectrum of the stem.

After 47 single-grade stem samples were ground, they were measured using Vis-NIR spectroscopy (400–2500 nm). The filling capacity of CRES produced from each stem sample was measured using a conventional method. The obtained values were indexed, with 100 representing the filling capacity of a certain grade. Partial least squares regression was adopted to develop a calibration model between the Vis-NIR spectra and filling capacity indexes.

The multiple correlation coefficient, the standard error of calibration, and the standard error of cross validation of the model were, respectively, 0.71, 6.2, and 9.1. Loading and regression coefficient plots showed that absorption around 670 nm and 2360 nm had an important role in the model. This agreed with results of multivariate analysis including the filling capacity, chemical constituents, and physical properties. Results show that a* denoting the red/green values in the CIE 1976 L*a*b* colour space and of cell wall components had higher correlation with the filling capacity.

These results suggest that the calibration model we established is a useful and rapid evaluation tool for filling capacity estimation and for subsequently CRES blending assessment.

ST 60

Development and application of an online sliver content detection device

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In order to determine the sliver content in tobacco shreds in a timely fashion, an online detection device was developed based on a multistage winnower. The main components of the device are an air fan, an air velocity measurement system and a gas-solid fluidized chamber. The fluidised chamber is partitioned into two section: one with a cross-sectional area of 220 mm × 220 mm and the other with a cross sectional area of 35 mm × 35 mm: alternatively forming air passages wherein the air velocities are 4.0 - 4.5 m/s at the cross-sectional area of 35 mm × 35 mm and 0.5 - 1.0 m/s at 220 mm × 220 mm. Slivers drop down in the lower air velocity area due to their critical fluidisation velocity of 1.15 m/s, while tobacco shreds continuously rise up in the whole gas-solid fluidised chamber, thereby separating sliver from the tobacco shreds. The accuracy, precision, optimal sampling volume, and sampling frequency of the device were tested. The results showed that: (1) The measured levels well agreed with theoretical estimations with absolute errors ranging from 0.0079 % to 0.1881 % and relative errors from 0.2950 % to 0.8623 %. (2) The optimal sampling volume was 150 g and optimal number of repeated tests was 30. (3) The measured levels were accurate at different sliver content in tobacco shreds whereas an increase of sliver content did not influence the accuracy and precision when the sliver content was more than 3 %.

ST 61

Application of Gibbs free energy change to evaluation of moisture retention of tobacco

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In order to evaluate the water retention of tobacco quickly and accurately, a model was developed based on water activity. Gibbs free energy change (GFEC) of tobacco was calculated according to thermodynamic correlation formula, and then the moisture migration of tobacco was predicted on the basis of GFEC. For a multi-component system, the GFECs of the variable component, invariable components and the whole sample were calculated separately to investigate the moisture variation of the cigarette blend in a test environment and the effects of different component proportions on water loss or absorption. The model was calibrated and checked by water detection and scanning electron microscopy. The results showed that: 1) GFEC could be used as a criterion of water loss or absorption of tobacco. 2) For the sample of cigarettes, the spontaneous reaction at room temperature is water loss if its GFEC is less than zero. The lower the GFEC is and the higher water dissipation rate from tobacco is. When GFEC is close to zero, water in tobacco remained broadly unchanged. When GFEC is positive, tobacco absorbs water, and the absorbing rate increases as the GFEC increases. 3) GFEC model is adaptive for single component systems, multi-component systems, variable systems and solid-liquid mixing systems. The results of tobacco blending experiments at different proportions and type of humectant validated the accuracy of the GFEC model. The model has the advantages of being simple and rapid; it is suitable for evaluating the water retention of tobacco blends.

ST 62

Overview of *in vitro* methods used to assess e-cigarettes based on "Toxicity Testing in the 21st Century" principles

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When the National Academies of Sciences released "Toxicity Testing in the 21st Century: A Vision and a Strategy" a new toxicological paradigm was created, focusing on the use of human cell lines and the disruption of key cellular pathways. In keeping with these principles we have sought novel assays for the biological assessment of our products. Due to the evolving regulatory landscape and dynamic nature of innovation with e-cigarettes, new assays are required to quickly determine the subtle biological response of these products for stewardship purposes. The published literature reveals that e-cigarette aerosols display a lack of significant cytotoxic and genotoxic responses in the CORESTA *in vitro* test battery.

For the stewardship of novel e-liquid ingredients, we screen all ingredients for carcinogenic, mutagenic and reproductive (CMR) properties and respiratory sensitising potential, from the scientific literature or using *in silico* predictions. If no major alerts are detected, the ingredients are assessed in a panel of biologically relevant assays. Examples of these assays include High Content Screening (HCS), *in vitro* human cell biomarkers, dermal sensitisation and irritation assays. Should assessment of the e-cigarette aerosol be required, 3D lung cell models can be exposed at the air-liquid interface to understand cytotoxic, inflammatory and oxidative response of the aerosol.

This presentation will discuss the various methods described above and will present some of the data generated so far for e-liquids with or without nicotine and the impact of flavours. Briefly, we have observed that lung cells employed in HCS and in *in vitro* human cell biomarker assay can detect increases in nicotine concentration in an e-liquid formulation. Moreover, characteristic fingerprint responses have been detected for certain e-liquid flavours, suggesting that flavours can play a role in the *in vitro* biological responses. These assays can greatly contribute to our current knowledge of e-liquid ingredients and aerosols and should form part of a weight of evidence approach for the assessment of this category of products.

ST 63

A computational model to characterize the Vitrocell® Cell Exposure System for evaluation of aerosols

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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 the 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 air flow rate of 1 L/min and trumpet flow 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/m^3. The impact of particle size and air flow rate on deposition efficiency on the cell plates was explored. Results show that for a trumpet to main air flow 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 air out of the system. Once validated, the model will be used to quantify cell exposure from different e-cigarette aerosol streams.

ST 64

A novel approach for the screening of e-cigarette aerosols using an Ames whole aerosol assay

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The *in vitro* mutagenic potential of the aerosol from Vype ePen e-cigarette was assessed using the Ames assay. E-cigarette aerosol was trapped on a Cambridge filter pad, eluted in DMSO and compared to cigarette smoke total particulate matter (TPM), generated in the same manner. E-cigarette and cigarette smoke aerosols were generated on a Vitrocell® VC 10 smoking robot and compared using a modified scaled-down 35 mm air agar interface (AAI) methodology.

E-cigarette aerosol collected matter (ACM) was found to be negative in the 85 mm Ames assay in strains TA98 and TA100 when conducted to OECD TG471, at concentrations up to 2400 µg/plate. E-cigarette aerosol was also found to be negative in both strains after an AAI aerosol exposure, when tested between 1-12 L/min dilution for up to 3-hours (360 puffs). In contrast, cigarette smoke TPM and aerosol from 3R4F reference cigarettes were found to be mutagenic in both tester strains, under comparable test conditions to that of e-cigarettes. To confirm these negative findings with e-cigarette exposure, further studies investigated extreme exposures up to 900 puffs. Some evidence of thinning of the background lawn and a marked reduction in revertants in TA98 and TA100 was observed following 900 puffs of undiluted e-cigarette aerosols. The data demonstrates that e-cigarette aerosols remained non-mutagenic under extreme testing conditions.

This novel whole aerosol approach could be used in a targeted manner to support e-cigarette assessments, and may even be useful in understanding the nature of the product in its extremes. The next key step is to contextualise against human consumption data, to ensure that these products are being tested within the constraints of their manufacturers' specifications and intended use. Furthermore, *in vitro* dosimetry approaches should be considered to draw more accurate comparisons between cigarette smoke, e-cigarette aerosol exposures and human use.

2017 CORESTA JOINT STUDY GROUPS MEETING

WORKSHOP PRESENTATIONS

WORKSHOP *IN VITRO* TOXICOLOGY

STW 01

Guidances related to the use of genetic toxicology assays for international regulation of tobacco products

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Tobacco products are subject to regulatory authorities in different geographic regions. Some countries/geographies require tobacco companies to apply for approval to sell their products. For instance, in the United States, the Food and Drug Administration (FDA) has regulatory authority and grants a market order for new tobacco products via three application pathways (substantially equivalent (SE), SE exemption, and pre-market tobacco products). In addition, companies can apply to have a market order for a modified risk tobacco product (MRTP) if it can be shown to have less risk than marketed products. These new regulations in which marketing applications are required increases the importance of *in vitro* toxicology testing and some of the guidances specifically list genetic toxicology and cytotoxicity tests as a part of the suite of evaluations that are recommended to be conducted and submitted. There is a CORESTA recommended *in vitro* genetic toxicology/cytotoxicity test battery that has been successfully used for several years to assess tobacco products. This battery has been effectively used as a part of product stewardship where the goal has been to assure that product changes do not significantly increase the overall toxicity of the final product, or to determine if different types of products (e.g. combustible cigarettes versus heat-not-burn products) have different degrees of toxicity. Whether the newly issued guidances impact the *in vitro* genotoxicity/cytotoxicity testing strategies that have been historically used will be considered with a view to prompting discussion at the workshop.

STW 02

The application of genetic toxicology assays to tobacco products

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Historically, *in vitro* genetic toxicology assays have been used to test additives and tobacco condensates (particulate matter) in a fairly routine way. The study design may need careful consideration depending on the question being asked – does this product induce an effect (yes/no) or is comparative testing of products required? The advent of a vast array of new products (ENDS) combined with a desire for the most relevant exposure in the test systems has prompted significant research in many laboratories. There are various approaches ranging from testing the gas vapour phase (GVP) and total particulate matter (TPM or eTPM) separately or combined, bubbling into medium or direct exposure to whole smoke or aerosol. The latter may be the gold standard but comes with many technical and practical challenges. Aerosol generation itself is technically challenging, along with exposure of cells at the air liquid interface. For example, a sufficient number of cells need to be exposed and recovered, monolayer cultures lend themselves more easily than suspension cultures, and a range of doses are required spanning an appropriate toxicity range. What determines the top dose (in the absence of toxicity) and are standard exposure times acceptable? From a regulatory compliance perspective, how should the test material be characterised and what considerations need to be given to software validation? Some of these challenges will be considered with a view to prompting discussion at the workshop.

WORKSHOP CIGARS

STW 03

Cigar tobacco and its required quality

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Some believe tobacco is tobacco, however there are significant genetic, physiological, and phenotypic differences across the tobacco types, as well as how and where the respective tobacco types are grown, managed, and ultimately used. For cigar tobacco the goal is perfection: create the “perfect” field; cure the “perfect” leaf; and deliver a final product that is “perfect.” Therefore the utmost care is given to most cigar tobacco from field to packed bale. This presentation will give a brief introduction of the following: cigar tobacco definitions; major differences in production practices; impact of quality; challenges in crop protection and quality; differences between cigars and cigarettes; and differences among cigars.

STW 04

Building blocks for regulated tobacco products and the challenges with cigars

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The EU and Canada have basic regulatory reporting requirements for cigars, however, with Deeming, the U.S. Food and Drug Administration (FDA) has pushed the cigar regulatory framework into an entirely new level of intensive rigor. However, in July, the FDA announced their new strategy which aims to start "striking the right balance between smart regulation and encouraging innovation of satisfying, less harmful products" while making sure any product standard is "steeped in science" and is made with "robust" participation from stakeholders" - *Mitch Zeller, CTP, NATO Conference, Denver, CO, DDAug2017*, but is the new FDA strategy appropriate for cigars?

The right balance is indeed difficult to achieve when a regulator's mission is to decrease combustible tobacco use while encouraging adult consumers to transition to satisfying, less harmful products. The FDA's Family Smoking Prevention and Tobacco Control Act states it cannot ban an entire category of tobacco products, such as cigars. So how can a regulator best navigate this terrain while ensuring that regulations do not negatively impact customer satisfaction by means of less desirable products, higher costs and/or less access?

This presentation will look at the building blocks of a regulated product and briefly cover why cigars pose a unique challenge to the regulators and to tobacco product registration applicants seeking a license to sell high quality and cost appropriate products to informed adult consumers.

STW 05

Challenges associated with the testing of cigars

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A series of CORESTA Recommended Methods (CRM) have been developed for the analysis of nicotine-free dry particulate matter (NFDM), nicotine, water and carbon monoxide in the mainstream emissions of cigars. Two of these (CRM 64 and 65), predominantly set the foundation for the additional testing of mainstream constituents in cigar smoke. However, the cigar product segment embodies a diverse range of cigar shapes and sizes with additional challenges to accommodate products like premium cigars.

Using the current guidance for conditioning (CRM 46), conditioning time is not restricted by a time limit. The impact of this is not fully understood. The process of lighting and re-lighting of cigars during the smoking process can vary amongst products, and can be influenced by airflow and the amount of ash built up during this process. For products with a diameter exceeding ≥12.1 mm, a different puffing regimen can result with every 0.2 to 0.3 mm change in diameter. However, the diameter within a product can vary by as much as 2.6 mm. Premium products can also produce a large amount of total particulate matter (TPM). This routinely requires the collection from a single cigar, often exceeding the defined 200 mg capacity of a 55 mm collection pad.

It is recognized cigars may not be uniform in shape, have a lack of homogeneity in the filler, and have variability in porosity and combustibility. When testing cigar emissions, it is important to attempt to differentiate the variability associated with the product from the variability that may be associated with the analysis. This is further complicated as no cigar reference product or monitor test piece, similar to that of cigarettes, exists. Additional strategies to recognize when repeat testing may be required will be further discussed.

2017 CORESTA JOINT STUDY GROUPS MEETING

SMOKE SCIENCE and PRODUCT TECHNOLOGY

ABSTRACTS

POSTER PRESENTATIONS

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

STPOST 01

The power of tactility: tipping paper in interaction with the consumer

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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 a design tool for the customised 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 colour 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 realising 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.

STPOST 02

Analysis of 20 elements in aerosols by inductively coupled plasma mass spectrometry (ICP-MS)

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Metals are present in the aerosol of tobacco products and are known as harmful or potentially harmful compounds.

The purpose of this study was to improve and re-validate an existing method for the determination of elements in smoke from cigarettes and in aerosol from potential reduced-risk products (RRPs)[1]. In addition, the scope of the method was extended to additional elements in order to cover additional needs related to RRPs and electronic cigarettes.

The method comprises the determination of 20 elements (Aluminium, Arsenic, Beryllium, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, Strontium, Tin, Titanium, Tungsten, Zinc) using an inductively coupled plasma with a mass spectrometer detection (ICP-MS).

Kentucky reference cigarettes 3R4F mainstream smoke and RRPs (tobacco heating systems/THS) mainstream aerosol are generated under Health Canada and ISO smoking regimes and e-cigarettes mainstream aerosol under CORESTA smoking regimes on a rotary machine. The mainstream smoke or aerosol is collected in a quartz electrostatic precipitation tube placed in an electrostatic precipitation unit followed by a micro impinger containing nitric acid 65 %. After aerosol collection, the electrostatic precipitation tube is extracted with nitric acid, which is combined with the nitric acid from the micro impinger and mineralised by microwave. Then, samples are diluted and analysed by ICP-MS.

The addition of a micro impinger allows the collection of elements in gas phase such as arsenic and selenium. For cigarettes, the gas phase represents approximatively 29 % of the total concentration of arsenic and 47 % of the total concentration of selenium.

The method was validated according to ICH guidelines and validation results showed the selectivity, precision, accuracy and linearity over different ranges of concentration depending of the element (in pg/mL).

The calculation of the limit of quantifications (LOQs) for the 20 elements was also reviewed and calculated based on smoked blank variability.

[1] Reduced-Risk Products (RRPs) is the term used to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking.

Kitzbühel - Austria 2017
CORESTA SSPT

STPOST 03

The analysis of harmful and potentially harmful constituents (HPHCs) in waterpipe tobacco products

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Released in 2009, the Family Smoking Prevention and Tobacco Control (FSP&TC) Act gave the U.S. Food and Drug Administration (FDA) under the Centre for Tobacco Products (CTP), the authority to regulate the manufacture, distribution, and marketing of cigarettes, roll-your-own and smokeless tobacco.

On 10 May 2016, the FDA published its final "Deeming Rule" which brought additional tobacco products, including waterpipe tobacco, under the jurisdiction of the FDA. The "Deeming Rule" subjects waterpipe tobacco products to the existing Food, Drug and Cosmetics Act (FD&CA) requirements, including the provision of data regarding the relative quantities of HPHCs under sections 904(a)(3), 905(j), and 910.

In the absence of validated and standardised methods, the analysis of HPHCs in waterpipe aerosol would be of limited value, since the data will be inconsistent and therefore unsuitable for product comparison purposes, as required under FD&CA sections 905(j) and 910.

The present study focused on the determination of the relative quantities of selected HPHCs in waterpipe tobacco from a range of products commercially available in the United States of America.

Whilst the determination of certain HPHC constituent levels in waterpipe tobacco (e.g. heavy metals, nitrosamines, and polycyclic aromatic hydrocarbons [PAHs]) remains a challenge when levels are consistently below the Limit of Quantification (LOQ) of the analytical technique, tobacco analysis may represent a more robust approach when compared to aerosol analysis, in meeting the requirements of FD&CA where product comparisons have been mandated.

STPOST 04

Development of a method for the estimation of mouth level exposure to nicotine from electronic cigarettes

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Mouth level exposure (MLE) assessments are valuable in helping interpret, as well as potentially predict, the results from clinical investigations, such as biomarkers of exposure studies. To date, methods to estimate nicotine-MLE from electronic cigarette (e-cig) have not been established.

Previously, we have shown a positive correlation between the nicotine yield from a tobacco vapour product and the weight loss (WL) from its device and its cartridge. In this study, we investigated whether WL can also be used to estimate the nicotine-MLE from e-cigs.

We investigated the correlations between nicotine yield and WL in two types of e-cigs, closed tank type and cig-a-like type, vaped under several regimes by machine vaping. To assess the influence of the puff profile on the correlations under several vaping regimes, two puff profiles were compared: square and bell-shaped. For both types of e-cigs, positive correlations were observed between nicotine yield and WL (closed tank; R^2>0.97, cig-a-like; R^2>0.93). When tested at the same liquid nicotine concentration (2.4 % w/w), the correlation between vapor nicotine yield and WL was similar between the two types of e-cigs (closed tank; nicotine (mg) = -0.028 + 17.819*WL (g), cig-a-like; nicotine = -0.125 + 18.151*WL). These results indicate that the liquid nicotine concentration, rather than device, is a primary determinant of the vapor nicotine yield. In this study, the nicotine yields and WLs measured under bell puff profiles lay within the 95 % confidence intervals for regression lines obtained with square puff profiles. This result suggests that puff profiles have a limited influence on the correlations between nicotine yields and WLs. In conclusion, the results from this study support the hypothesis that WL can be used to estimate nicotine-MLE from different types of e-cigs, irrespective of puff profile.

STPOST 05

Differences in plasma nicotine pharmacokinetic profiles for various e-vapor products used by adult smokers under *ad libitum* vs. controlled use conditions

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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 ($Cmax_{0\text{-}2hrs}$) and area under the curve ($AUC_{0\text{-}2hrs}$) 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 $Cmax_{0\text{-}2hrs}$ 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.

STPOST 06

The challenges of machine smoking the diverse cigar product category

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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 Sub-Group has been the main driver for the development of cigar smoking methods. This Sub-Group 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 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 nine commercial cigar products will be presented.

STPOST 07

Consistency of blu™ e-cigarette nicotine delivery according to the AFNOR standard

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Demonstrating the consistency of nicotine delivery from an e-cigarette is a requirement per EU Tobacco Product Directive (TPD) 2, Article 20, 3(f) (The European Council and the Council of the European Union, 2014). The Directive states that e-cigarettes placed on the European Union (EU) market should "…*deliver the nicotine doses at consistent levels under normal conditions of use.*" This means that the dose of nicotine should be within a specified acceptable range around a predefined target value (this can be either the mean average of the nicotine levels or the labelled claim for nicotine delivery per puff). However, the EU TPD2 does not provide definitions of a "consistent level", "dose" or "normal conditions of use." Therefore different interpretation can be made to define nicotine consistency.

In 2016 the French National Organization for Standardization (AFNOR) published the standard "Electronic cigarettes and e-liquids - Part 3", which will form the basis of European standards projects. This standard requires the nicotine concentration for the first, third and fifth series of 20 puffs to be within ±25 % of the mean value. The objective of this study was to assess whether the acceptance criteria was achievable using blu™ e-cigarettes marketed in the EU. Our study investigated nicotine delivery from several e-cigarette types according to AFNOR standard.

The results of our study show that the ±25 % as proposed by AFNOR is achievable with the products tested.

STPOST 08

Indoor air quality and surface deposition assessment following use of an open system e-cigarette

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Electronic cigarette (e-cigarette) nicotine delivery devices are growing in popularity worldwide. Both regulators and public health organisations are beginning to examine potential implications that exposure to exhaled e-cigarette aerosol may have on non-users in workplaces and enclosed public spaces. To our knowledge, no study assessing the potential impact of exhaled e-cigarette aerosols on indoor air quality and surface deposition following use of open system e-cigarettes has been reported. In the present study we aimed to understand the contribution of exhaled aerosols to the pre-existing load with chemicals in ambient indoor air and the potential deposition of nicotine to indoor surfaces before, during and after unrestricted use of a blu™ open system e-cigarette.

Our results indicate that use of the blu™ open system e-cigarette when used *ad libitum* by three experienced vapers for almost two hours did not negatively impact the indoor ambient air for all chemicals analysed when compared to regulatory indoor air quality guidelines. Moreover, the use of the open system e-cigarette in this study did not lead to a measurable increase in nicotine levels or the subsequent formation of TSNAs on the indoor surfaces. Our investigations suggest that the use of the blu™ open system e-cigarette is unlikely to pose a concern to bystanders in this regard.

STPOST 09

Analysis of metals in e-liquids - reduction of matrix effects

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An analytical method for simultaneous determination of various metals in e-cigarette aerosol using inductively coupled plasma mass spectrometry (ICP-MS) was reported at the CORESTA Congress in 2016. In the presence of propylene glycol and glycerol excessive recovery rates for specific metals, including arsenic (As) and selenium (Se), were found, most likely caused by matrix effects.

Different techniques could be applied to reduce the observed matrix effects whilst analysing As and Se in e-liquids. The purpose of this study is to show different approaches towards the reduction of matrix effects for the determination of As and Se in e-liquids.

Two different approaches to reduce the beforehand described matrix effect were investigated. The first approach was to use tellurium (Te) with a very similar ionisation potential like As and Se as an internal standard to quantify As and Se. The second approach was to add a low concentration of methanol to the calibration standards.

E-liquid samples prepared with propylene glycol and/or glycerol and containing small amounts of metals were diluted with 5 % nitric acid. Scandium (Sc), thallium (Tl) and either yttrium or Te as internal standards were added to both e-liquid samples and calibration standards to investigate the first approach.

To investigate the second approach, calibration standards each containing a small amount of methanol were prepared. In this approach, Sc, Tl and Te as internal standards were added to both e-liquid samples and calibration standards.

The samples and standards were analysed by ICP-MS equipped with a collision/reaction cell, and the recovery rates of the metals were calculated. Both approaches showed significantly improved As and Se recovery rates.

STPOST 10

Methods for characterising morphological and structural variations of cut tobacco in cigarettes during smoking

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The morphological and structural variations of cut tobacco significantly influence the state of a burning cigarette and the formation of smoke constituents. A series of new methods for characterising the micro-structure of cut tobacco, planar structure of the cone of a burning cigarette and the three-dimensional structure of the cigarette rod were developed. Additionally, the impact of the morphological and structural variations of cut tobacco on the CO delivery into mainstream smoke were investigated.

The morphological variations of cut tobacco in the simulated heating process were measured by scanning electron microscope (SEM). The transformation temperature was derived based on the morphological variations of cut tobacco heated at different temperatures.

For further investigations, the burning cigarettes were cut into slices to conduct optical microscope analysis. The length and planar porosity of cut tobacco in the burning cone were measured based on the micro-images from optical analysis.

The three-dimensional (3D) images of the burning cigarette were determined by Computer Tomography (CT) and a volume scanning technique. The morphology and structure of cut tobacco in the burning cone and remaining tobacco rod were measured on-line, whilst the porosity and apparent volume shrinkage ratio of cut tobacco in the burning cone were calculated based on the 3D-Scan images.

With the decrease of length and the increase of width of the cut tobacco (in the tobacco rod), the length of the burning cone decreased, whilst the planar porosity of the cut tobacco in the burning cone increased and the CO delivery in mainstream smoke decreased.

STPOST 11

Investigation of gene expression profile of cells after e-cigarette and cigarette smoke exposure

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To evaluate the cellular damage caused by e-cigarettes (e-cigs) and combustible cigarettes with respect to inflammatory expression, comparative analysis between two types of e-cigs and three types of combustible cigarettes were conducted. The aerosol of all investigated products was exposed to A549 cell lines. In parallel, the damages of A549 cell lines exposed to the aerosol collected under three different smoking/vaping regimes (ISO, Canadian Intense and CORESTA Recommended Method [CRM] No. 81) were investigated as well. The cytokine and chemokine gene expressions of IL-6, IL-8, IL-12, IL-1β, TNF-α, ICAM-1, MCP-1 and IFN-γ were determined by reverse transcription quantitative PCR (RT-qPCR). The results showed that the exposure to the aerosol of the two types of e-cig samples induced inflammatory gene expression in A549 cell lines. With respect to the induced inflammatory factor expression level, the aerosol collected under different smoking regimes caused different responses: CORESTA > Canada intense > ISO. After the exposure to the aerosol collected under the three regimes, the IFN-γ gene was significantly expressed, which indicates that it is a sensitive biomarker to tobacco smoke. On the contrary, the expression of MCP-1 and IL-8 did not change significantly, which suggests that they did not interfere with the mechanism of inflammatory gene expression induced by tobacco smoke. Under the three regimes, the inflammatory gene expression levels induced by the aerosol of e-cigs were all lower when compared to combustible cigarettes.

STPOST 12

Study on vaporisation temperature of electronic cigarette

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The vaporisation temperature of electronic cigarettes (e-cigarettes) affects the release and migration of harmful components in e-cigarettes greatly. Therefore a method for measuring the vaporisation temperature was established based on a thermocouple to investigate the influencing factors of the vaporisation temperature and their influence rules. The results showed that the vaporisation temperature of e-cigarettes was in the range of 160 °C - 250 °C, which correlated to the type, heating power, puff number and puff duration of e-cigarettes and the solvent composition of the electronic liquid (e-liquid) for e-cigarettes. During a single puff, the variation of vaporisation temperature could be divided into two stages, rapid rise stage and slow rise stage. In the whole smoking process of an e-cigarette, the vaporisation temperature of a disposable e-cigarette rose, while that of a refillable e-cigarette did not vary with the proceeding of smoking; which was probably due to the difference of e-liquid suction means between the two types of e-cigarettes. The higher ratio of glycerol/propylene glycol in e-liquid resulted in higher vaporisation temperature of e-cigarettes. Moreover, the vaporisation temperature rose with the increase of heating power of e-cigarette atomisers and the prolongation of puff duration. The results of this work provide a reference for the researches on pyrolytic harmful components in aerosol of e-cigarette and the migration of harmful ingredients in the material of the e-cigarette set.

STPOST 13

The comparative assessment of e-cigarettes and cigarettes on cytotoxicity and proinflammatory cytokine secretion level using an air-liquid interface system

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Electronic cigarettes (e-cigarettes), as an alternative to combustible cigarettes, have become more and more popular. In this study, the cytotoxic and proinflammatory cytokine secretion response of e-cigarette vapour (e-vapour) and traditional cigarette smoke (CS) were comparatively assessed. A commercially available e-cigarette (disposable) and two reference cigarettes Kentucky 3R4F (3R4F) and CORESTA Monitor 8 (CM8) were investigated. The whole e-vapour/CS exposure to human bronchial epithelial cells was conducted in the VITROCELL® system. The nicotine content of e-vapour and CS were quantitatively determined by GC-MS, and the cytotoxicity of e-vapour and CS and the secretion levels of proinflammatory cytokines, Interleukine-6 (IL-6), Interleukine-8 (IL-8) and tumour necrosis factor-α (TNF-α), were detected by neutral red uptake assay and enzyme-linked immunosorbent assay.

The results showed that: 1) The aerosol of 3R4F (IC_{50}: 0.89 % CS, 6.52 µg of nicotine per cigarette (µg NPC)) and CM8 (IC_{50}: 0.52 % CS, 8.33 µg NPC) significantly decreased cell viability; however the aerosol of 200 puffs (93.18 µg NPC) of the investigated e-cigarette only induced very low cytotoxicity, thus the IC_{50} could not even been calculated. 2) IL-6 secretion level was significantly decreased by the aerosol of 3R4F (0.27 % CS, 1.95 µg NPC) and CM8 (0.27 % CS, 4.27 µg NPC) and was significantly increased by the aerosol of the investigated e-cigarette (50 puff, 23.29 µg NPC; 100 puff, 46.59 µg NPC). 3) IL-8 secretion level was significantly increased by the aerosol of 3R4F (0.12 % CS, 0.88 µg NPC; 0.27 % CS, 1.95 µg NPC), CM8 (0.12 % CS, 1.94 µg NPC; 0.27 % CS, 4.27 µg NPC), e-cigarette (100 puff, 46.59 µg NPC). 4) The TNF-α secretion level was significantly increased by aerosol of 3R4F (0.27 % CS) or CM8 (0.12 % CS; 0.27 % CS), whilst it was not impacted after e-cigarette aerosol exposure. Compared to the aerosol of a combustible cigarette, the aerosol from the investigated e-cigarette showed much lower cytotoxicity and different proinflammatory response.

STPOST 14

New smoking machine approach for cigarette smoking under intense and ISO 3308 conditions

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

In 2007, on demand of the WHO, ISO TC 126 started the preparation of a more intensive smoking regime based on a 55 ml bell shape puff with a 2 s duration taken every 30 s 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 the first validation data for both smoking regimes compared to the yields delivered from standard linear and rotary smoking machines.

STPOST 15

“Track and Trace” in the smoking laboratory

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“Track and Traceability” 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 thoroughly tracked. But within the smoking laboratory this is more problematic. The products have to be linked carefully to complex smoking plans including multiple machines and different parameter settings. And finally the products are converted physically into loaded smoke traps continuing their way to the chemical analysis. Along this way there are plenty of possibilities to mix up samples, or data, or to lose the link between the traps and the smoked product.

This poster describes a technical solution for consistent tracking during the whole procedure. It is based on the automated identification of uniquely id-coded key components used in the smoking machine like cigarette storage cassettes and filter pad holder and extraction flasks communicating with a database. For example, all product relevant data including conditioned weight and all smoke parameters are linked to the cassette allowing the machine to set up automatically in regards. These parameters plus all additional smoke run parameters as well as the unloaded and loaded weight of the filter are then linked to the ID of the used CFP holder and furthermore to the coded extraction flask used in the chemical laboratory. This procedure helps to avoid or even eliminate errors and to gain online information about the current status of the product during the whole process which is an essential/important topic for simplified laboratory accreditation. This will also simplify the laboratory processes and offer not only the possibility for higher efficiency and higher process automation, but also ensures traceability from smoked product to chemical analysis results.

STPOST 16

Degradation of phytosterols in tobacco extract by bacteria to produce low benzo[a]pyrene reconstituted tobacco

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Pyrolysis of phytosterols during tobacco combustion is closely related to the yield of PAHs, such as the carcinogen benzo[a]pyrene. There are five types of phytosterols in tobacco, accounting for 0.2-0.5 % by weight. Most of the phytosterols are hardly removed during the production of tobacco products, which could cause a potential risk to smokers. Here, we try to reduce the contents of phytosterols in reconstituted tobacco by adding phytosterol degradation bacteria in the tobacco extract (TE). Therefore, the benzo[a]pyrene content was reduced in the mainstream smoke during pyrolysis of reconstituted tobacco. A novel bacteria *Paenibacillus* sp. isolated from the surface of tobacco leaf was used in this study. *Paenibacillus* sp. cells were cultivated under the optimal conditions (37 °C, pH 7.0) and then collected and added to the TE directly during the production of reconstituted tobacco for phytosterols degradation. Phytosterol degradation in TE was demonstrated by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Then, the contents of benzo[a]pyrene in reconstituted tobacco mainstream smoke was determined by gas chromatography/mass spectrometry (GC–MS/MS). Under optimal conditions (37 °C, pH 7.0, with the exponential-phase cells), the total degradation ratio of phytosterols reached 39.5 % in TE, including 44.2 % of stigmasterol (45.2 μg/ml to 25.2 μg/ml), 38.1 % of β-sitosterol (184.5 μg/ml to 114.2 μg/ml), 35.7 % of campesterol (4.82 μg/ml to 3.1 μg/ml) and 52.0 % of cholesterol (5.21 μg/ml to 2.5 μg/ml). Further analysis showed that the final contents of phytosterols in reconstituted tobacco were also reduced by 34.1 % (252.6 μg/g to 166.5 μg/g) compared with the control experiment (without adding bacteria in TE). The delivery of benzo[a]pyrene in mainstream smoke was reduced from 4.22 ng/cig to 2.88 ng/cig. These results indicated that the novel *Paenibacillus* sp. can potentially be used to produce low benzo[a]pyrene content reconstituted tobacco.

STPOST 17

Collection of carbonyl compounds in e-cigarette aerosols

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The CORESTA Recommended Method No. 74 (CRM 74) for the determination of carbonyls in cigarette smoke requires the collection of smoke with two impingers. Other literature references report that carbonyls present in e-cigarette aerosols should be collected on a Cambridge filter pad (CFP) in conjunction with only one impinger. As a vaping regimen for e-cigarettes, the CORESTA E-Vapour Sub-Group developed CRM 81. At the 2016 CORESTA Congress it was reported that an alternative vaping regimen is being developed at Sub-Group level. However, the effects of alternative smoking regimens on the carbonyls recovery rate have not yet been reported. The objective of the present study was to evaluate the recovery rate of carbonyls from e-cigarette aerosols under the several vaping regimens.

Carbonyls were intentionally generated by applying an excessively high voltage to an e-cigarette and collected using a CFP connected with two impingers containing 25 ml of a 2,4-dinitrophenylhydrazones solution. Different puff volumes (55 ml, 70 ml, 110 ml and 165 ml per 3 seconds) and puff durations (3 seconds, 6 seconds and 9 seconds at 18.3 ml/sec of flow rate) were applied to generate the aerosol. The total amount of carbonyls was calculated by summing the quantities collected on the CFP and the two impingers up. The recovery of the single impinger system was then determined by subtracting the carbonyl amount in the second impinger from the total amount. It was confirmed that carbonyls were sufficiently collected by the CFP and one impinger at all investigated puff volumes and puff duration levels. Furthermore, it was found that as the puff volume and puff duration increased, the proportion of formaldehyde passing through the CFP increased.

STPOST 18

Fibre geometry analysis of tobacco, additives and recon materials

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Present cigarette tobaccos are a blend of several components. The classical sieve method generates only limited information about individual components, particularly with materials, which do not match the typical fibre shape. Only a few classes are built by the different sieve sizes. Different sieve sizes, sieving times, oscillating frequencies and amplitudes lead to different results. In addition, the sieving degrades fragile materials.

To improve our understanding of the tobacco dimensional properties, we separated the fibres and measured them by means of a camera. In combination with the constant particle velocity under the camera a two-dimensional picture of each particle was captured. The image processing system collected the data for all fibres and classified them on the basis of geometrical parameters. We adopted methods from the particle analysis and fitted them by using the skeletonised length, the average width and the area of each particle. Additionally an area-weighted classification was calculated using the geometrical properties.

We found a good correlation between the video analysis and the sieving method. For recon material, a different distribution function of the particle sizes in the material than for tobacco fibres was observed. The analyses provided much more information about the shape of the particles for all materials. All measurements showed a good reproducibility.

The video analysis of the particle sizes and shapes of materials for cigarettes therefore provides useful information for detailed analyses of tobacco components, additives and recon material. Changes in the tobacco blend, their reasons and impacts can be then investigated more accurately.

STPOST 19

Combustible and smokeless tobacco product preparations differentially regulate calcium mobilization in the human leukemic HL60 cell line

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Increased calcium (Ca^{2+}) mobilization is central to leukocyte signaling and for regulation of immune responses. Although evidence suggests that cigarette smoking affects several biological functions including inflammatory responses, how the use of combustible (cigarette) and smokeless tobacco regulates Ca^{2+} mobilization and consequently inflammation is unclear. We evaluated the effects of several Tobacco Product Preparations (TPPs) including total particulate matter (TPM) from 3R4F reference cigarettes, phosphate buffer extracts of 2S3 moist snuff (as a representative of smokeless tobacco [ST]) and nicotine on Ca^{2+} mobilization in Human Leukemic HL60 cells. HL60 cells were initially loaded with Flow-3, a Ca^{2+} indicator dye and then treated with different equi-nicotine units of TPM (1, 5, 10, and 20 µg/mL) or ST (100, 150, 200 and 250 µg/mL), or nicotine (0, 10, 50, and 100 µg/mL). Intracellular Ca^{2+} mobilization $(Ca^{2+})^{I}$ was measured using flow cytometry. TPM treatment significantly increased $(Ca^{2+})^{I}$ in a concentration dependent manner, but not treatment with ST or nicotine. The induction of TPM-mediated $(Ca^{2+})^{I}$ occurred at lower nicotine equivalent units compared to ST or nicotine. Experiments using Thapsigargin (TG) for depletion of Endoplasmic Reticulum (ER) Ca^{2+} stores suggested that TPM-mediated $(Ca^{2+})^{I}$ induction occurs through multiple Ca^{2+} stores including ER stores. Assessment of extracellular Ca^{2+} mobilization $(Ca^{2+})^{E}$ using $CaCl^{2}$ in intact or TG treated cells for depletion of ER Ca^{2+} stores indicated that TPM-can mobilize $CaCl^{2}$ as an extracellular source of Ca^{2+} and induce $(Ca^{2+})^{E}$. These results, for the first time, demonstrate that combustible TPPs, such as TPM, trigger both intracellular Ca^{2+} release and also elicit $(Ca^{2+})^{E}$ by capacitative (in depleted ER stores) and non-capacitative (in intact ER stores) Ca^{2+} mobilization. On the other hand, Ca^{2+} mobilization was unaffected by ST or nicotine treatment under the experimental conditions. In conclusion, our results suggest that TPM drives intra- and extracellular Ca^{2+} mobilization that may activate signaling pathways potentially leading to altered inflammatory responses in leukocytes.

STPOST 20

Identification of gelatin in flavour capsules used in cigarette filters

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In the recent past, preference for cigarettes with capsules in filters has increased. This is probably due to the long awaited innovation to control the flavour release. A majority of cigarettes with capsules have shells made from gelatin; however, there are also capsules from plant origin. Currently, there is no method available to discriminate whether the capsules are derived from animal or plant sources and this is an essential need due to obvious religious beliefs and convictions. The objective of this study is to develop an identification tool for the presence or absence of animal-based gelatin in capsules.

The United States Pharmacopeia (USP) describes two identification tests; however, the tests are for pure gelatin. Therefore, the methods were suitably modified including sample preparation steps for the new matrix. The flavour from the capsule is removed using hexane and the dried shell is subjected to two identification tests. The first test includes development of a violet colour using copper sulphate and sodium hydroxide for the presence of gelatin, whereas the second test involves acid hydrolysis, oxidation and treatment with p-Dimethylaminobenzaldehyde to develop an intense red colour. Interference from the capsule shell delays violet colour development during the first test method, whereas in the second test method, the colour development is instantaneous and clearly distinctive for the presence of gelatin. Therefore, precise identification tests for the presence of gelatin have been developed for cigarette manufacturers to use to avoid any conflict by offering a known choice to consumers.

STPOST 21

The use of *in vitro* human biomarkers from relevant primary cell lines, to assess the effects of increasing nicotine concentration in e-liquids

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As part of the ongoing stewardship of electronic cigarettes, and in line with the National Academies of Sciences "*Toxicity Testing in the 21st Century: A Vision and a Strategy*", Fontem Ventures B.V. have investigated the utility of a series of endpoints in human primary cells. In the first instance, base e-liquids were used to determine the suitability of the test system.

DiscoverX Corporation, is a leading supplier of cell-based assays and services for drug discovery and development. The BioMAP® product was chosen, consisting of 12 primary human cell-based systems from multiple tissues. Cells are cultured either alone or as co-cultures and stimulated with a combination of biological proprietary factors (e.g. cytokines, growth factors, mediators, etc.) to recapitulate the multi-component signalling networks associated with disease states. The Diversity PLUS panel consists of 148 biomarker readouts and has been used as a tool for phenotypic drug discovery, competitive analysis and comparison to clinical standards of care.

Initial results of a base e-liquid (BL) containing only 50:50 propylene glycol and vegetable glycerine; BL containing 2.5 % nicotine and BL with a content of 4.5 % nicotine were tested at eight concentrations ranging between 0.031 to 4 % added directly to the cell media. BL with nicotine added to cell media at concentrations above 0.5 % lead to a characteristic fingerprint of biomarkers and showed a good dose response relationship with increasing concentration. This lead to an exaggeration of the fingerprint profile in selected cell panels, above that for the base e-liquid itself. The most sensitive cell panel in all cases was found to be the BT cell line composed of B cell and peripheral blood mono nuclear cells.

The results demonstrate that the use of BioMAP® is a sensitive and powerful tool, capable of detecting the addition of nicotine to e-liquids in a panel of human relevant primary cell lines. The next step is to assess the e-liquid aerosol, following stability trials of aerosol trapped in phosphate buffered saline.

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STPOST 22

Dosimetry: TPM and nicotine delivery of combusted tobacco products to the 24 and 96 Multi Well Plate and inserts on Smoke Aerosol Exposure *In vitro* System (SAEIVS)

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In vitro exposure systems are key tools for the risk assessment of emerging electronic nicotine delivery systems as well as for conventional tobacco products. The Smoke Aerosol Exposure *in vitro* System (SAEIVS) used at Imperial Tobacco Limited is designed to expose cells in multiwell plates (MWP) under Air Liquid Interface (ALI) conditions[1]. This procedure allows direct contact of both aerosol particles and gas phase components with the apical humid cell surface, mimicking the lung surface *in vivo*.

The exposure to the undiluted or diluted smoke can be performed in cells cultivated either on a collagen I matrix in 96 MWP or on inserts / transwells in 24 MWP format. Up to five tobacco products can be puffed simultaneously. This study presents the initial efforts to determine total particulate matter (TPM) and nicotine delivery from cigarettes to the wells of MWPs and inserts by physico-chemical analysis. The analytical determinations (LC/MS-MS) of nicotine were compared to optical density measurements of TPM at 400 nm. This method[2] allows an evaluation of water free deposited condensate for calculation of more reliable dose response effects caused by toxicologically relevant substances.

At first the influence of the exposed surface texture on deposition efficiency without cells was tested. As expected significant differences were found between dry and moistened plastic surfaces. In the next step, cell coated surfaces were exposed to smoke in order to mimic more realistically the deposition under ALI conditions. The amounts of TPM and nicotine deposited on the coated surface were compared to that found with the exposed living cells. Optical density measurement of tobacco smoke condensate provided reliable results which were comparable to those obtained by the standard LC/MS-MS and GC chemical analysis. Tobacco blend specific calibration curves will enable the estimation of the particle and nicotine concentration delivered to the wells in future experiments.

[1] Wieczorek R.; Trelles Sticken E. CORESTA Meeting, Smoke Science/Product Technology, 2015, Jeju, ST 44
[2] Wieczorek R.; Röper W.; Burghart H. CORESTA Meeting, Smoke Science/Product Technology, 2005, Stratford-upon-Avon, SSPT 24

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STPOST 23

Dosimetry: the effects of cigarette smoke dilution on nicotine delivery in a 24 and 96 well format using Smoke Aerosol Exposure *In vitro* System (SAEIVS)

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In vitro aerosol exposure systems are useful tools for screening of potential toxic effects of freshly generated smoke of conventional cigarettes[1]. An in house platform "Smoke Aerosol Exposure *In Vitro* System (SAEIVS)" (assembled by Burghart TABAKTECHNIK) was used to generate fresh whole smoke to simulate exposure of cells at the air liquid interface (ALI)[2].

The aim of this study was to increase our knowledge of the effects of smoke dilution on delivery of nicotine to an *in vitro* system. A CORESTA monitor test piece, CM7, was smoked under ISO 3308 smoking conditions to deliver smoke to 24 or 96 well multi titer plate (MTP) formats. The surface of wells in MTP contained (300/50 µl) of PBS to simulate the wet surface of the ALI exposure model.

A puff gradient was used every time in 2 puff-step-wise. All experiments were performed in three replicates. The method used to measure nicotine was validated for selectivity and sensitivity LC-MS/MS (AB Sciex Qtrap 2000). Nicotine was quantified by two specific mass transitions (Quantifier and Qualifier) with an internal Standard (Nicotine-d_4).

In this poster presentation, the validation process and necessary validation parameters, e.g. precision, repeatability, recovery will be discussed in relation to the effects of smoke dilution on nicotine deposition in 24 and 96 well plates.

[1] Thorne D.; CORESTA Congress, Berlin, 2016, Smoke Science/Product Technology Groups, STPOST 26
[2] Wieczorek R.; CORESTA Meeting, Smoke Science/Product Technology, 2015, Jeju, ST 44

STPOST 24

FDA's proposed N-nitrosonornicotine (NNN) Standard: epidemiological evidence

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The U.S. Food and Drug Administration (FDA) has advanced a proposed product standard of 1 µg/g N-nitrosonornicotine (NNN) content in finished smokeless tobacco (ST) products, citing selected findings from epidemiology to support the proposed limit. The purpose of this work was to evaluate FDA's application of epidemiological literature. An independent review was undertaken, and 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 U.S. 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.

STPOST 25

Comparison of WHO SOPs, CORESTA CRMs and ISO Standards for tobacco product testing

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The WHO has previously requested that TobLabNet develop analytical methods and Standard Operating Procedures (SOP) for the analysis of tobacco constituents and smoke emissions of cigarettes. The main aim is to establish methods that will enable comparison of results from testing laboratories worldwide. The need for harmonised methods is also one of the key reasons why, historically, CORESTA initiated the development of recommended methods (CRM). CRMs are based on robust and comprehensively validated methodologies, which are periodically reviewed through collaborative studies which also provide precision data with known repeatability and reproducibility. Over decades, CRMs have formed the basis of several ISO standards.

We undertook an in-depth comparison of existing CRM or ISO methods with TobLabNet SOPs to assess similarities and differences between analytical procedures. The comparison included investigations of processes, workup of samples, general principles, equipment set-up, analysis time and efficiency.

It was observed that the analytical principles of SOPs and ISO/CRMs are broadly similar, however, there are major differences in some of the procedural details. For example, sample preparation, internal standards, and apparatus set ups often differ. It is also notable that the SOP requirements restrict their scope of application by excluding a wider range of tobacco products. Consequently, there may be a need for additional method development for several product categories to enable effective regulatory testing.

STPOST 26

How do consumers use the candidate modified risk tobacco product (MRTP) tobacco heating system (THS): analysis of data from six countries

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PMI conducted consumer studies in several countries for a candidate MRTP, the THS. THS is composed of a tobacco heating device which heats specially designed tobacco sticks. The purpose of these studies was to investigate how adult daily smokers actually used THS in near to real-world conditions. The studies were conducted in Japan, Italy, Germany, Switzerland, South Korea and the US.

Each study was a single group, observational study, involving an assessment of subject-reported stick-by-stick consumption of Tobacco Sticks and cigarettes (CC). Participants received Tobacco Sticks free of charge on a requested basis and were able to consume Tobacco Sticks and CC *ad libitum*. The length of the observational period was at minimum four weeks.

To ensure a good representation of the country adult smoker population, each sample approximated the distribution of such population according to several key characteristics such as age, sex, race, income, social status and main brand of cigarettes (when appropriate). Participants were recruited using databases maintained by local market research agencies and enrolment was done through interviews in between two and eight central study locations, depending on the country. The number of enrolled participants ranged from 581 in Switzerland to 1,336 in the US. A passive surveillance mechanism was also put in place to collect spontaneously reported events (e.g. adverse events).

Across those six countries, the data shows that a sizeable proportion of participants adopted a usage behaviour involving predominant or exclusive use of Tobacco Sticks with THS, (i.e. ≥70 % of tobacco products [tobacco sticks and CC] used were tobacco sticks). It was observed that between 9.9 % in Switzerland and 37.1 % in South Korea demonstrated this level of use of THS at the end of the observational period. Moreover, the data suggests that these patterns of use were overall stable across the entire observational period.

STPOST 27

The application of microwave expanded cut tobacco stem in cigarette filter

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Microwave expanded tobacco stem (METS) is a type of cigarette material with good filling and adsorption capacity and many aromatic constituents. At present, METS is mainly used to make cut stem or granules and added into the tobacco blends. METS can reduce the consumption of tobacco and reduce tar delivery in the cigarette. Microwave expanded cut tobacco stem (MECTS) was prepared into a filter and then used to manufacture a dual filter with cellulose acetate. The objective of the study was to provide a new type of cigarette filter, and then to evaluate the effect of this filter on the mainstream smoke and sensory quality. The MECTS filter was manufactured by a self-developed filter rod maker and the filling content was 13.5 mg/mm. The dual filter was composed from a MECTS filter and cellulose acetate filter produced on a KR-4 filter rod maker. The specification of the dual filter was as follows: length (120±0.5 mm), circumference (24.0±0.2 mm) and pressure drop (3400±300 Pa). The combination ratios of MECTS and cellulose acetate were 1:2, 1:1 and 2:1 respectively. The results showed that the ability of MECTS to absorb the particle phase composition was stronger than that of cellulose acetate. The content of total particulate matter (TPM), water, nicotine and tar all decreased in the sample of the ratio of 2: 1, but the change of CO was not obvious. The delivery of NNK decreased by 12.6 %, and phenol increased by 34.6 %. The volatile components in mainstream smoke increased in different degrees. The sensory quality such as aroma, richness, coordination and comfort all improved significantly.

STPOST 29

Handmade premium cigars smoke emissions - limitations related to TNCO determination variability

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To date, few studies have been published for handmade premium cigars. The assembly process of a premium cigar involves combining natural leaves that 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 (CRMs). 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.

STPOST 30

Tobacco product comparison: extension of critical difference to account for manufacturing variability

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In the context of tobacco products comparison, it has been shown previously that the critical difference (ISO 5725) was the best method to consider the variability of testing and laboratories. However as mentioned in several publications, some additional sources of variability need to be considered when products are not manufactured during the same period of time or in the same factory. In such cases, the manufacturing process variability (e.g. batch, factory…) has to be included in the expression of critical difference. If the manufacturing process variability is ignored when comparisons are made, the risk increases of concluding that two products made to the same specification are significantly different.

To address this, two important steps have to be investigated: first, the evaluation of the manufacturing process variability and second, the extension of the critical difference formula. The first step is currently under investigation by the CORESTA Cigarette Variability Task Force.

Based on our findings, we propose an approach to extend the critical difference expression to include the manufacturing process variability according to the number of samples and batches collected for each product. The general formula will be presented and several specific cases including one vs two labs, short vs long period of time, and one vs several batches, will be detailed.

STPOST 31

Psychometric evaluation of the mCEQ applied to cigarettes and heat-not-burn products in the U.S. and Japan

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The modified Cigarette Evaluation Questionnaire (mCEQ) is a self-report instrument that assesses the reinforcing effects of smoking cigarettes from a smoker's perspective. Conceptually, the mCEQ consists of three multi-item and two single-item domains. The increasing availability of alternative products to cigarettes raises the question whether the mCEQ can also be used to assess the reinforcing effects of other tobacco and nicotine containing products. The study aimed at a psychometric evaluation of the mCEQ applied to cigarettes and a heat-not-burn tobacco product, the tobacco heating system (THS). Furthermore, the potential to integrate items from two other instruments (Minnesota Withdrawal Scale-Revised, MNWS-R; Questionnaire on smoking urges – brief version, QSUbrief) was investigated. The analysis was based on classical test theory (CTT) and Rasch Measurement Theory (RMT). The data set consisted of a sample collected in two 3-month reduced-exposure studies, one in the U.S. and one in Japan. Within CTT, factor analysis widely confirmed the structure of the mCEQ for both cigarettes and THS. While for two multi-item domains (Smoking Satisfaction and Psychological Reward) the items formed proper scales allowing for exploiting the psychometric benefits of RMT, the third multi-item domain (Aversion) showed extremely poor targeting (resulting in strong floor effects) as a fundamental psychometric problem regardless of the model used to estimate respondent measures. The two single-item domains remain to be interpreted as such, and attempts to extend these to multi-item domains revealed no real potential to improve the mCEQ. In its current form, it is recommended to administer the full mCEQ, which can be applied equally to cigarettes and THS in the U.S. and in Japan. The domains of Smoking Satisfaction and Psychological Reward (after excluding one item) qualify for being analyzed by the Rasch model, while additional items should be added to the domain of Aversion to mitigate the poor targeting.

STPOST 32

Assessment of the total volatile organic compounds in indoor air during the use of a new heat-not-burn product

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PMI is developing products that have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. The tobacco heating system (THS) is one of these products based on a technology that heats the tobacco instead of burning it. To address public health concerns about possible presence of polluting substances during indoor use of THS, a portfolio of methods has been established and currently covers 23 compounds. One of the methods consists of the determination of total volatile organic compounds (TVOC) based on ISO 16000-6. Due to the intrinsic nature of the matrices under investigation (high variety of volatile target compounds present at relatively low concentrations), the implementation/adaptation and validation of such a method is challenging. Indeed, although the ISO 16000-6 standard details sampling and some instrumental parameters, several additional aspects were critical to limit contaminations and improve overall method robustness. Examples of method improvements include preparation and storage of sampling tubes, implementation of monitors and use of parallel detectors. In addition, several approaches for chromatographic signals integration, compounds identification and semi-quantification have been investigated.

The validated method was applied in the context of a study reflecting the real-life use of THS. The determination of TVOC values, expressed in toluene equivalents, was achieved for three matrices (background air with human presence, air combined with the environmental aerosol of THS and air combined with environmental tobacco smoke [ETS] of a *Marlboro Gold* cigarette) as well as the identification and semi-quantification of their major constituents. This study was conducted under controlled conditions and proved that the variety of volatile compounds released in indoor air and their respective measured concentrations were higher in ETS compared to environmental aerosol of THS. The TVOC patterns during use of THS and the background were similar.

STPOST 33

Evaluation of available test methods for the determination of carbonyls in mainstream cigar smoke

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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 (CRM) 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 the DNPH derivatives may not be stable 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 smoke.

STPOST 34

Optical method for estimating mouth level exposure from conventional cigarettes and next generation tobacco and nicotine products

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Novel tobacco and nicotine products like the emerging devices that heat tobacco instead of burning it (THPs) and electronic nicotine delivery systems (ENDS), generate significantly fewer and lower amounts of toxicants compared with conventional cigarettes (CCs). Although the chemistry of these products appears safer than cigarettes, there is little information on the mouth level exposure received by users of these products. Previous methodologies that relied on collecting spent filters are generally not suitable due to the absence of filters in these products. THPs which have filters do not exhibit a robust linear relationship between what is retained in the filters and that exiting the filter.

A simple optical obscuration based methodology to estimate users' mouth level exposure of products across the risk spectrum is presented. A range of smoking regimes were used to calibrate optical tar (OT) or optical aerosol mass (OAM) against NFDPM or aerosol collected mass (ACM) using smoking machines in the laboratory dependent on the product type. This relationship was then used to estimate aerosol exposure using real time OT and OAM. These relationships were further validated by duplicating a selection of human topography records and comparing the measured OT with NFDPM or OAM with ACM.

There was a strong positive correlation observed between OT and NFDPM determined from duplication smoking of participant records with R^2 values of 96.7 and 97.9 % for cigarettes and 82.7 to 88.0 % for THPs. The mean difference between OT and NFDPM for the study products ranged from -1.9 to 0.4 mg/stick for THPs and 2.7 to 3.4 mg/cig for cigarettes. Data for ENDS - OAM with ACM will also be presented.

The OT data for THPs and CCs suggests that this method provides a strong basis for estimating MLE for products across the risk spectrum.

STPOST 35

Use of formalin in e-vapor products to monitor formaldehyde delivery

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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 Sub-Group 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. 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.

STPOST 36

Chemical and physical properties of FSC cigarette papers and physical properties of the cigarettes they were taken from: results from a small product survey of cigarette brand-styles sold in the USA

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By 2009, and before passage of the Federal Family Smoking Prevention and Tobacco Control Act, the use of FSC (Fire Standard Compliant) cigarette paper on cigarettes sold in the US had been mandated by all 50 states. New York State required all cigarettes to be FSC compliant by July 1, 2004, and at least three banding technologies were used by the major companies and the smaller companies that sold products in New York State. Companies that did not sell in New York State and/or only sold selected brand-styles in New York State continued to use non-FSC paper on their products sold in other states. Thus, on February 15, 2007, the FDA "Grandfather" date, which was unknown at that time, only a few companies had all of their products in the U.S. market with FSC paper. However, when the FDA Substantial Equivalence rules were implemented in 2011, companies with non-grandfathered products had problems even if the only change in their cigarettes was the change to FSC paper. FSC paper changed the burning properties of the cigarettes, sometimes resulting in increased puff counts and TNCO deliveries, particularly with 100-mm "Gold" and "Silver" brand-styles. Consequently, we undertook a small product survey in which we purchased a range of brand-styles at retail and sent them to commercial laboratories for dimensional and physical tests, including banded and non-banded area porosities. Other analyses obtained used chemical, chromatographic, and spectrophotometric (terahertz) techniques to identify main banding agents and additives used with them. One finding from this study, was at least at the time the samples were collected some companies used just one FSC paper for all products while others used multiple FSC papers.

STPOST 37

FDA's proposed NNN product standard: quantitative risk assessment

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The U.S. Food and Drug Administration (FDA) has published a proposed standard for N-nitrosonornicotine (NNN) content in finished smokeless tobacco products. The proposed NNN limit of 1 part per million (ppm) dry weight 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 U.S. 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 greater than or equal to 5 ppm dry weight would result. This range is consistent with, or lower than, historical NNN levels in the smokeless tobacco 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 greater than five-fold lower concentration of NNN will further reduce cancer risks in smokeless tobacco users or otherwise be protective of public health.

STPOST 38

FDA's proposed NNN product standard: toxicological evidence review

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The U.S. Food and Drug Administration (FDA) has published a proposed standard for N-nitrosonornicotine (NNN) in smokeless tobacco products. An independent review of the toxicological evidence was conducted, and 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 smokeless tobacco 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 smokeless tobacco or smokeless tobacco 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 smokeless tobacco products would benefit, or otherwise be protective of, public health.

STPOST 39

Shelf-life of tobacco products: moisture transport modeling

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Shelf-life is a key consideration in most consumer packaged goods including tobacco. Since shelf-life studies typically require testing until the end (or beyond) of the expected shelf-life of the product, they may significantly extend the product development lifecycle. Identifying and solving potential shelf-life issues in the early product development stages is essential for timely commercialization.

One factor that has the potential to impact tobacco product quality and consistency is the moisture content of the tobacco product. To maintain the moisture content within an acceptable range it is common to use packaging to protect the product from moisture gain or loss. To reduce the need for iterative testing, we have developed a mathematical model to predict moisture transport between packaged tobacco products and the environment. The unique feature of this model is to use the product's water activity isotherm to determine the water activity within the packaging, as a function of the product moisture content. This is a significant improvement over typical models that incorrectly assume that water activity within the package is a constant.

The model was validated by comparing the results with experimental stability studies at various conditions (temperature and humidity). Several applications of the model will be demonstrated: identification of packaging materials with the right barrier properties to ensure consistent product quality over the target shelf-life and determination of package water vapor transport rates in assembled packaging for when it cannot be easily determined from the packaging material properties.

STPOST 40

A comparison of two different extraction methods for aromatic amines in mainstream smoke using gas chromatography-tandem mass spectrometry (GC/QQQ)

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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 solid phase extraction (SPE) 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 and offers superior limits of detection compared to the quadrupole mass spectrometer based standard method.

We will present instrument/method precision, accuracy and limit of detection (LOD) and limit of quantitation (LOQ) of both methods.

STPOST 41

Optimization of an analytical method for the determination of PAHs in tobacco and tobacco smoke by GC-MS

MARTIN A.

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Polycyclic aromatic hydrocarbons (PAHs) are an extensive group of compounds formed as a result of incomplete combustion of carbonaceous materials and are known components in mainstream cigarette smoke. Many PAHs have been identified by IARC as probably or possibly carcinogenic to humans and their quantitation is of much interest to regulating bodies. Currently, 16 PAHs are included on the FDA list of harmful and potentially harmful constituents (HPHC).

The purpose of this study was to develop and validate a single method for 15 of the HPHC listed PAHs using gas chromatography mass spectrometry (GC-MS). Several aspects of the instrumental method were critical to ensuring a robust method was developed.

Many of the PAHs are structural isomers and discrimination by GC-MS can prove extremely difficult; retention time becomes critical to the correct identification of each PAH. The choice in column and temperature gradient is driven by the separation of critical pairs of PAHs, which would otherwise co-elute. The temperature of the GC inlet can have a dramatic impact on the outcome of analytical results and must be optimized for the best possible responses for all 15 HPHCs simultaneously. Surprisingly, the optimum inlet temperature for this method was determined to be 250 °C, significantly lower than other investigators and instrument specialists have recommended. Improvement in peak response and peak shape were observed using a combination of reduced inlet temperature and shorter run time (<30 minutes). The most surprising effect was upon the dibenzopyrenes, where the responses were observed to increase at lower inlet temperatures and the calibration curves, initially quadratic, became linear.

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STPOST 42

Identification of candidate biomarkers of tobacco related diseases through gene/disease associations

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Biomarkers can be useful tools in measuring the biological effects of tobacco product use. Although there is a small group of biomarkers that are typically used to assess the biological effect of tobacco use, there are few publications summarizing recent developments in this area. The purpose of this project was to investigate recent (past 5 years) publications to identify potential biomarkers that may be useful in assessment of the biological effect of tobacco product use. Candidate biomarkers were identified by investigating gene/protein associations in the published literature for three indications and a term: COPD, CVD, Lung Cancer (LC) and Tobacco Smoke (TS). Our approach used query terms, association words and database terms using the PolySearch web server and pattern recognition system-based relevancy ranking to identify gene-disease/term associations in the published literature. The search was limited to the ~50 highest ranked gene/proteins for each indication and term. The identified gene/proteins for each indication were then compared with the TS associated genes/proteins. This strategy identified 18 COPD + TS targets, 6 CVD + TS targets, and 10 LC + TS targets. Although no genes/proteins were found in all four conditions, 5 genes/proteins were common across COPD, CVD and TS, and 6 common genes/proteins across COPD, LC and TS. We identified enriched pathways using the Database for Annotation, Visualization, and Integrated Discovery (DAVID 6.8) represented by each set of biomarker candidates. Most enriched pathways were associated with immune and inflammatory response with the top-scoring enriched pathways for all three disease conditions were Jak- STAT signaling and cytokine-cytokine receptor interaction. This approach presents a promising tool for identification of emerging biomarkers to assess biological effects of tobacco product use.

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STPOST 43

Biomarkers of exposure specific to e-vapor products based on stable-isotope labelled ingredients – methods

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E-vapor products (EVPs) are becoming an accepted alternative for nicotine delivery amongst smokers. The popularity of EVPs has been increasing and more systematic research is required in order to assess the impact of e-cigarette use-related exposure to vapor ingredients including potential degradation products. The e-liquid ingredients, propylene glycol (PG), glycerol (G) and to some extent nicotine (Nic) are ubiquitous. Therefore, EVP use related uptake is difficult to distinguish from unspecific environmental uptake. To overcome this problem, stable-isotope tracers are used with mass spectrometry measurements for understanding kinetics, uptake and distribution of various compounds in living organisms. In the current study the e-liquid was partially replaced (10 %) with stable isotope labelled 13C3-PG, 13C3-G and Nic-d_7. By measuring known biomarkers in urine and blood, this approach allows the quantitative assessment of the absorption, metabolism and further fate of PG, G and Nic as well as compounds formed from the precursors in the vapor (or endogenously from the labelled precursors) such as formaldehyde (FA), acetaldehyde (AA), acrolein (ACR) and tobacco-specific nitrosamines (TSNAs) in EVP users. This poster presentation highlights the analytical methods used for the main ingredients and their metabolites by means of GC-MS and LCMS/MS.

STPOST 44

Validation and comparison of the neutral red uptake assay in BALB/c 3T3 and CHO-WBL cells

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The neutral red uptake (NRU) assay is used to evaluate the cytotoxicity of a variety of chemicals, cigarette smoke fractions, e-liquids, medical devices, etc. *in vitro*. Charles River Laboratories-Skokie previously validated the NRU assay in BALB/c 3T3 cells, according to OECD Guidance Document 129, and we report here its validation in CHO-WBL cells according to Health Canada Official Method T-502. CHO-WBL and BALB/c 3T3 cells were exposed to test or control articles, in a 96-well plate format, for 24 hours with newborn calf serum or 48 hours without serum, respectively. After removing the treatment media, fresh media with neutral red (NR) is added for an additional 3-hour incubation. Since only viable cells incorporate and bind the NR, cytotoxicity is detected by a concentration-dependent decrease in NR staining, which is quantitated by the optical density at 540 nm (OD540). Analysis of our current historical control databases, compiled from at least ten independent trials performed under GLP conditions, reveals BALB/c cells are more sensitive to our standard positive control, sodium lauryl sulfate, under these conditions. Both cell lines were seeded at 1 × 104 cells/well, and both undergo ~2 population doublings during the 24- or 48-hour exposure times. However, our standard concentration ranges were 6.8 to 100 µg/mL for Balb/c and 13.6 to 200 µg/mL for CHO-WBL, which produced IC50 values of 19.99 ± 3.35 µg/mL and 89.15 ± 3.38 µg/mL, respectively. Thus, under these conditions, the more sensitive Balb/c cells –serum may be more appropriate to evaluate e-liquids and condensates, which generally have proved to be relatively innocuous, while the more resistant CHO-WBL cells +serum may be more appropriate to evaluate combusted smoke condensates, which are relatively more cytotoxic. Additional experiments to evaluate the effects of serum and treatment time are in progress.

STPOST 45

Stability of the certified 1R6F reference cigarette

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University of Kentucky Center of Tobacco Reference Products (CTRP) has provided reference cigarettes for almost 50 years. These reference cigarettes are widely used in tobacco research including analytical method development and modified risk tobacco product development. In 2014, CTRP obtained a service agreement with the U.S. 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 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 were transferred out stepwise from these storage conditions until they reached room temperature. Cigarettes stored in -20 °C were transferred to 4 °C for 24 hr then moved to room temperature for at least 2 hr. The sampled cigarettes were conditioned for 48 hr at 22 °C and 60 % relative humidity prior to smoking and filler analysis for selected constituents. Filler analysis included oven volatiles, individual alkaloids and TSNAs. Smoke analysis included the measurement of individual alkaloids, TSNAs, CO and TPM under ISO smoking regime. There were no significant changes for the oven volatiles, nicotine and NNN in the filler from 4 °C or -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 the smoke of cigarettes under -20 °C, 4 °C and room temperature conditions. Our experimental data demonstrated 1R6F cigarettes were relatively stable after one year storage at 4 °C or -20 °C for selected constituents. This study will continue for the duration of the 1R6F cigarette supply.

STPOST 46

Validation and comparison of the *in vitro* micronucleus assay in TK6 and CHO-WBL cells

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The *in vitro* micronucleus (MN) assay is used to evaluate the potential clastogenicity and aneugenicity of a variety of chemicals and agents *in vitro*. Charles River Laboratories-Skokie previously validated the *in vitro* MN assay in TK6 cells, according to OECD Test Guideline 487, and we previously performed this assay in CHO-WBL cells in an abbreviated non-GLP screening format. We report here validation of this assay in CHO-WBL cells under TG 487- and GLP-compliant conditions. Both cell types are exposed to test or control articles for four hours with and without metabolic activation (±S9), and for 24 or 27 hours -S9. All CHO-WBL cultures are harvested 24 hours after start of treatment, as are the TK6 cells treated for 27 hours -S9. However, we have found an extended harvest time (44 hours after the start of treatment) provides greater sensitivity for TK6 cells using the short treatment ±S9. Comparisons of our current historical control databases, compiled from at least 10 independent trials, reveals that TK6 cells have lower background MN frequencies for the various treatments than CHO-WBL cells (averaging 0.30 to 0.36 and 0.73 to 0.83 % MN, respectively). Additionally, CHO-WBL cells exhibited lower cytotoxicity to our standard positive controls: mitomycin C (4-hr –S9), cyclophosphamide (4-hr +S9) and vinblastine (24- or 27-hr –S9). While the absolute and fold-increases in MN observed for these positive controls were generally larger in CHO-WBL cells, and they occurred at higher concentrations, positive responses were observed at lower concentrations in TK6 cells, indicating increased sensitivity to clastogens and aneugens. Thus, under these conditions, the more sensitive TK6 cells may be more appropriate to evaluate e-liquids and condensates, which generally have proved to be relatively innocuous, while the more resistant CHO-WBL cells may be more appropriate to compare combusted smoke condensates, which are relatively more cytotoxic.

STPOST 47

Toxicity assessment of e-cigarette vapours on human vascular endothelial cells

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The present study was conducted to provide toxicological data on e-cigarette vapours of different vendors and to compare e-cigarette vapour toxicity to the toxicity of conventional cigarette smoke. Using an adapted version of a previously constructed cigarette smoke sampling device, we collected the hydrophilic fraction of e-cigarette vapour and exposed to human umbilical vein endothelial cells (HUVEC). After incubation of cells with various concentrations and for various time points, we analysed cell death induction, proliferation rates, the occurrence of intra-cellular reactive oxygen species and cell morphology.

Conventional cigarette smoke extract showed the most severe impact on endothelial cells. However, some e-cigarette vapour extracts showed surprisingly high cytotoxicity, inhibition of cell proliferation, and alterations in cell morphology, which were comparable to conventional strong high-nicotine cigarettes. The vapours generated from different liquids using the same e-cigarette showed massive differences, pointing to some flavours as an important source of toxicity. We detected a high variability in the acute cytotoxicity of e-cigarette vapours depending on the liquid and the e-cigarettes used. Nicotine as well as the formation of acute intracellular reactive oxygen species do not seem to be the central elements in e-cigarette vapour toxicity.

STPOST 48

The *in vitro* biological assessment of a novel hybrid tobacco product and comparison with a cigarette smoke

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Cigarette smoking is a risk factor for many diseases including cardiovascular disease, lung disease, and cancer. Recently there has been an increase in the development and consumer acceptance of novel nicotine and tobacco products including tobacco-heating products (THPs) and vapour products such as e-cigarettes.

Using a number of *in vitro* test methods, recently outlined as part of a framework to substantiate the risk reduction potential of novel tobacco and nicotine products, we have assessed the toxicological and biological effects of a novel hybrid tobacco product, iFuse, designed to reduce toxicant exposures. Responses were compared to a commercially available THP (THS) and a 3R4F reference cigarette.

Exposure matrices assessed included total particulate matter, whole aerosol, and aqueous aerosol extracts obtained after machine-puffing using the Health Canada Intense smoking regime. The hybrid tobacco product had little or no activity across all the *in vitro* assays assessing endpoints including mutagenicity (Ames), genotoxicity (γH2AX), cytotoxicity (neutral red uptake), tumour promotion (Bhas cell transformation), oxidative stress (ROS formation, intracellular glutathione content and antioxidant response element activation) and endothelial cell migration (wound healing) when compared to a 3R4F reference product. The THS product also demonstrated significantly reduced responses. These *in vitro* assays have enabled the biological assessment of a novel tobacco hybrid product and results suggest the product demonstrates reduced health risks. Further pre-clinical and clinical assessments are required to understand further the risk reduction of these novel products at individual and population levels.

STPOST 49

In vitro assessment of a novel prototype e-cigarette

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E-cigarettes have rapidly increased in popularity over the last decade and there is a growing consensus that e-cigarettes hold great potential for reducing the risk associated with cigarette smoking.

In this study, the responses of a novel prototype e-cigarette and scientific reference cigarette (3R4F) in cytotoxicity, oxidative stress and endothelial cell migration endpoints were assessed using the neutral red uptake (NRU), glutathione ratio and wound healing assays, respectively. Exposure matrices were whole aerosol and aqueous aerosol extracts (AqE) generated using Health Canada Intense (HCI) regime for cigarettes or CRM 81 regime for e-cigarettes. Nicotine was measured in all exposure matrices.

3R4F whole aerosol induced a concentration dependent increase in cytotoxicity, whereas the prototype e-cigarette resulted in responses comparable to the air control. 3R4F AqE reduced the glutathione ratio at doses >6.25 % indicative of oxidative stress and induced cytotoxicity at doses >12.5 %. Exposure to the prototype e-cigarette AqE, even at 100 % did not affect glutathione ratio or cell viability, aligned to control responses. 3R4F AqE doses >10 % inhibited endothelial cell migration with complete inhibition at doses >25 % AqE. Prototype e-cigarette AqE, even at the 100 % dose, did not inhibit cell migration, equivalent to the control responses.

In all assays, prototype e-cigarette exposure resulted in reduced responses when compared to 3R4F. These data add to the growing weight of evidence that e-cigarettes offer substantially reduced toxicant exposure when compared to conventional cigarettes. Further pre-clinical and clinical assessments are required to fully understand the risk reduction potential of e-cigarettes at individual and population levels.

STPOST 50

Vascular endothelial oxidative stress leading to hypertension: development of an AOP using *in vitro* assays

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Cigarette smoking is associated with diseases including cardiovascular disease. Key events from exposure to cigarette smoke to the development of disease related endpoints can be mapped out in the form of an adverse outcome pathway (AOP). An AOP is a framework that documents a chain of biological effects induced by exposure (cigarette smoke) and describes molecular and biological responses at the cellular, tissue, organ, whole body and population level. AOPs offer promise for researchers, regulators and risk assessors, forming toxicological and biological knowledge frameworks to aid risk assessment based on mechanistic reasoning and supporting biomarker discovery and validation.

We have mapped out an AOP focussing on key events associated with the development of hypertension initiated by cigarette smoke-induced vascular endothelial oxidative stress. Oxidative stress contributes to endothelial dysfunction and can lead to impairment of endothelium-dependent vasodilation and hypertension having an inhibitory effect on endothelial nitric oxide (NO) production, critical for the maintenance of healthy vascular tone.

Using bovine aortic endothelial cells (BAECs) and aqueous extracts (AqE) from a 3R4F reference cigarette, we were able to model and further assess these endpoints *in vitro*. Exposure of BAECs to AqE resulted in significant changes to key events including a decrease in NO production with an increase in superoxide, and generation and accumulation of 4-HNE protein adducts. AqE exposure led to depletion of tetrahydrobiopterin (BH4) and total biopterin levels. Importantly, exposure of BAECs to AqE indicated a central role of the ubiquitin proteasome system (UPS) in AqE-induced eNOS dysfunction.

In conclusion, our results provide strong *in vitro* evidence to support an AOP of vascular endothelial oxidative stress leading to hypertension. These endpoints combined with clinical data can serve as potential biomarkers of cigarette smoke-induced vascular endothelial dysfunction and help to provide comparative data supporting the assessment of novel tobacco and nicotine products.

STPOST 51

RNA-seq-based toxicogenomics shows limited impact of e-cigarette vapour on airway cells compared with cigarette smoke when matching for nicotine delivery

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There is increasing evidence from *in vitro* testing that e-cigarettes cause minimal damage to cell systems: however some studies reported cytotoxicity and inflammatory responses. In this study we compared the transcriptional response of MucilAir™ exposed for one hour to e-cigarette (Vype ePen) vapor and smoke from 3R4F reference cigarettes. The average nicotine delivered to the cells from 3R4F smoke was matched to the e-cigarette. One additional e-cigarette dose was also tested for higher nicotine delivery. RNA was extracted for RNA-seq at 24 hrs and 48 hrs post exposure. 873 and 205 RNA features were differentially expressed for 3R4F at 24 hrs and 48 hrs post exposure (pFDR < 0.01, fold change > 2 threshold), respectively. Differentially expressed RNA from e-cigarettes (49 RNA features and 113 RNA features at higher dose) could only be identified using a looser threshold (pFDR<0.05, no fold change filter), and pooling the two timepoints to increase statistical power. Gene set enrichment analysis revealed a clear response from lung cancer and fibrosis associated genes after 3R4F smoke exposure. Using the less robust thresholds, glucagon metabolism pathway and processes relating to the extracellular matrix were identified for e-cigarette exposures, albeit with a low degree of confidence. Based on equivalent or higher nicotine delivery, an acute exposure to Vype ePen vapour has very limited impact on gene expression compared to 3R4F smoke exposure.

STPOST 53

Non-targeted screening of extractables and leachables in e-cigarettes using a Single Platform UPLC-APGC-QTOF-MS

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Characterization of extractables and leachables is essential for ensuring the safety, quality and efficacy of inhalation tobacco products such as e-cigarettes. The initial step for characterizing extractables from e-cigarettes involves targeted screening, i.e., testing the extracts for known impurities. This is a well-established process that can be performed using analytical techniques such as GC-MS and LC-MS/MS. However the final extracts of e-liquids, refill cartridges and e-cigarette aerosol may have impurities present from the starting materials and other packaging and device components that need to be further evaluated.

In this study, the various components of an e-cigarette (end caps, mouth piece, gauze, heating element and flavor formulation) were extracted separately and subjected to a non-targeted high resolution screening using both ultra-performance liquid chromatography (UPLC) and atmospheric pressure gas chromatography (APGC) analysis on a single QTOF-MS platform. MS and MS/MS data was acquired using alternating low and high collision energy states (MSE) across the full analytical mass range. The data from sample extracts was compared to reagent blank to determine the differences and potential extractables.

Both LC and GC acquisition and data processing were handled on a single instrument platform. The first step in ensuring that the compounds identified in extractables profile do not pose any toxicological risks to the consumer is to identify and quantify the extractables. Common plasticizer (dibutyl phthalate), stabilizing agent (4-methyl benzophenone) and few polymer additives were identified in the e-cigarette component extracts by matching accurate mass precursor and fragment ions, retention time and isotopic patterns against a known library of extractables and leachables. This application demonstrates how non-targeted screening using LC and GC workflows can be adopted on a single high resolution mass spectrometry (HRMS) and software platform for extractable and leachable testing in e-cigarettes.

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STPOST 54

Ion mobility mass spectrometry: a new tool for characterization of complex tobacco products

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Comprehensive chemical profiling and identification of compounds in tobacco and tobacco smoke matrices is a challenging task due to the sample complexity and the wide array of analyses required. LC-MS and GC-MS based methods are typically applied as they offer higher selectivity, sensitivity and peak capacity for complex separations. Recent advancements in ion mobility separation provide an additional dimension of gas phase separation orthogonal to chromatographic separation resulting in high quality mass spectral data. Here we present the use of UPLC separations with IM-MS and novel informatic tools for characterization and confident compound identification in 3R4F tobacco and smoke extracts.

Ion mobility separations differentiate compounds based on size, shape, and charge. Ion mobility data was acquired using Vion IMS QTof. UPLC coupled to ion mobility mass spectrometry (IM-MS) provides accurate mass precursor and fragment ion information, retention time, isotope ratios and averaged collision cross-section (CCS) parameters that can be used to build or search mass spectral database. The CCS value provides additional dimension of separation for confident compound identification and is independent of front end chromatography (LC or GC) and matrix effects. The potential of ion mobility for the separation of isomers and chromatographically co-eluting compounds in tobacco and smoke matrices will be presented.

STPOST 55

Rapid authentication of tobacco flavors using DART-MS

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Characterization of flavors in tobacco products is critical for consumer's perception of aroma, taste, quality and branding. Maintaining consistent flavor in tobacco products requires quality control testing of raw materials and finished products to ensure product quality.

Analysis of flavor compounds is challenging and time consuming as it requires several lab based measurements. Based on the source of the flavor (e.g. natural versus synthetic), the flavor formulations can contain a few hundred compounds with varying solubility, volatility, concentrations and stability. A combination of gas chromatography (GC) and liquid chromatography (LC) based methods integrated with mass spectrometry (MS) detection are typically applied for identification and quantification of tobacco flavors. These procedures require isolation and concentration of flavor compounds from the tobacco matrix prior to instrumental analysis.

This application demonstrates the utility of DART-MS technology (Direct Analysis in Real Time) for rapid, accurate and cost effective flavor characterization, to monitor product quality and reduce production costs. DART-QDa was applied to characterize volatile and semi-volatile flavors in tobacco, whiskey and chewing gums with different characteristic flavors. The flexibility of sample introduction using an ambient ionization technique like DART combined with the sensitivity of QDa detector reduces analysis and decision making time compared to conventional methods and makes this system suitable for quality control applications in the tobacco, food, beverage and consumer goods industries.

STPOST 56

Benefits of mass detection for routine LC analysis of tobacco products

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There are several tobacco industry methods that rely on HPLC separation and optical detection (UV, Fluorescence and ELSD) for identification of target compounds such as carbonyls, nicotine, related alkaloids, sugars and artificial sweeteners. In this application we demonstrate the benefits of QDa mass detector for method development, transfer and routine analysis of tobacco products in combination with UPLC and optical detection workflow.

Peak traceability can be a challenge during method development and routine testing using optical detectors. E-liquid formulations were analyzed under different mobile phase pH conditions and using different UPLC columns to show the benefits of MS detection during method development. In a second example, we show the benefits of using UPLC to drastically reduce the analysis time for determination of carbonyl compounds but also demonstrate how the addition of a mass detector helps to confirm trace analytes and avoid interference from co-eluting compounds.

Finally the benefits of using dual detection (UV and MS) for simultaneous analysis of nicotine and related impurities demonstrate how to measure the major and minor components of an e-liquid formulation in a single analysis. These experiments demonstrate that the use of mass detection greatly reduces the method development and transfer time, identifies interferences from co-eluting compounds, reduces troubleshooting time for out of specification results, which will in turn improve overall lab productivity and analytical data quality.

Kitzbühel - Austria 2017
CORESTA SSPT