2019 CORESTA JOINT STUDY GROUPS CONFERENCE

SMOKE SCIENCE and PRODUCT TECHNOLOGY

ABSTRACTS

ORAL PRESENTATIONS

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

ST 01

United Nations sustainable development goals and impacts of the tobacco and alternative product sectors

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In 2015, the United Nations published 17 sustainable development goals (UN SDGs) and their corresponding targets to be achieved by 2030. The third goal "Good health and wellbeing" specifically refers to tobacco activities with the target to "*strengthen the implementation of the WHO/FCTC in all countries*".

The tobacco and alternative product sectors are likely to impact most of the UN SDGs even if some goals are more particularly relevant than others from a social perspective. In 2018, CORESTA decided to support a project with the objectives to i) elaborate a scientific framework for assessing environmental and social impacts of these sectors, ii) identify scientific tools for performance assessment, and iii) measure the gaps between required and available tools.

During the period December 2018 – February 2019, a survey was conducted among and beyond the CORESTA members to better understand the positive and negative impacts of the tobacco and alternative product sectors with regards to the UN SDGs, to collate examples of actions undertaken for enhancing the positive impacts and mitigating or suppressing the negative ones, and finally, to identify the tools and KPIs used to measure and monitor the consequences of these actions. A workshop was organised subsequently in May 2019 to consolidate the contributions to the survey, to identify the needs and to set priorities for a possible work programme.

The conclusions of this project will be presented along with sets of tools, assessment frameworks, recommendations and possible implications for future activities of CORESTA and/or other organisations.

ST 02

Comparative assessment of selected compounds yields in heated tobacco products

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A diverse range of heated tobacco products (HTPs) has been introduced in a growing number of markets by different manufacturers. They generally differentiate from cigarettes by the absence of combustion and a significant reduction in the levels of harmful and potentially harmful constituents (HPHCs) emitted. The purpose of this study was to propose a standardized method to generate HTP aerosols and to report the obtained HPHC yields in order to perform a comparative assessment of the different products. The yields of selected compounds, such as nicotine, CO, nitrogen oxides, tobacco-specific nitrosamines (TSNAs), and volatile organic compounds were measured in commercially available products, covering different types of HTPs (electrically heated products, hybrid products, tobacco vaporisers), using standardized conditions. The yields of TSNAs and nicotine were the most variable, while the yields of other HPHCs, linked to tobacco degradation at high temperatures (e.g. CO, benzene, or benzo[a]pyrene), were very low for all products in comparison with yields found in cigarette mainstream smoke. The robustness of the products toward slight changes in the aerosol generating regime was also examined on the basis of HPHC yields in aerosols. The use of a reporting per volume basis allowed comparison of results obtained with different puffing regimes. It was found that not all HTPs show the same behavior toward changes in the puffing regime.

ST 03

The effect of socio-economic inequalities: modelling of population health impact of introducing reduced-risk tobacco products into Germany

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Education, income and occupation are the most commonly used descriptors of social inequalities in health. The socio-economic status (SES) of individuals (or groups of individuals) has proven to be important when describing and understanding smoking prevalence in a population. Indicators such as low education level and low income have been associated with higher smoking prevalence, higher exposure to smoking environments, and lower success in quitting smoking. Prevalence of tobacco consumption is high in Germany (28.3 %); it is markedly uneven across the population and strongly influenced by SES. A Population Health Impact Model (PHIM) was used to estimate the impact of introducing a reduced-risk product* (RRP) in Germany and its effect on socio-economic inequalities based on education and income. Input data on smoking prevalence, quit times, and mortality were obtained from widely used German data (publicly available sources and recently published research). Various simulations were carried out to understand the effect of an RRP introduction on major smoking-related diseases (lung cancer, ischemic heart disease, chronic obstructive pulmonary disease, and stroke) in the German population. The simulations were performed for different age, gender, and SES sub-groups over a 20-year period. Study results can be indicative of the outcome of introducing and switching to an RRP within a population characterized by groups of individuals from different SES and in helping to discern the impact of an RRP on the stratified socio-economic context of a country.

* "Reduced-risk products" or "RRPs" is the term we use 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. We have a range of RRPs in various stages of development, scientific assessment, and commercialisation.

ST 04

Paper components for heated tobacco products (HTPs)

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While conventional cigarettes, which burn tobacco, and the consumables of heated tobacco products (HTPs) both use paper components, the requirements on the papers used for heated tobacco products differ substantially from those used on conventional cigarettes.

This study shall provide an overview over paper components used on HTPs, discuss their properties and explain how papermaking technology can be used to fulfil the requirements for HTPs.

Firstly, the paper components of current HTPs are analysed for their properties. These properties include common properties such as basis weight, thickness and air permeability but also thermal properties, such as thermal conductivity, mechanical properties, such as stiffness and other properties that are not usually considered for papers used on conventional cigarettes. Furthermore, anti-staining and special means to prevent the lighting of the consumable like a conventional cigarette will be discussed.

Secondly, some of the components of a consumable of a HTP can be made from paper and contribute to the functionality of the product, for example, by cooling or by filtering the aerosol. Options where paper can complement or replace other materials are discussed.

In a third part it will be explained how papermaking technology can be used to fulfil the requirements on paper components for HTPs. This includes the type and treatment of fiber and filler materials and other technologies such as coating or lamination.

In summary, papers for HTPs generally have higher basis weight, lower permeability and focus more on physical and mechanical properties as they are not burnt together with the tobacco. Compared to papers for conventional cigarettes, there is more freedom to design suitable papers. However, some difficult challenges remain, particularly regarding thermal stability.

ST 05

Determination of ten kinds of polycyclic aromatic hydrocarbon in the aerosol of heat-not-burn products by SPME-GC-MS

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Polycyclic aromatic hydrocarbons are low in mainstream tobacco smoke, but they are among the most hazardous substances and have been shown to be carcinogenic in a number of studies. One polycyclic aromatic hydrocarbon is benzo[a]pyrene, which has been classified as a Group 1A carcinogen by the International Agency for Research on Cancer (IARC). However, the determination of polycyclic aromatic hydrocarbon in the aerosol of heat-not-burn products is still limited to the single determination of benzo[a]pyrene. There are many kinds of polycyclic aromatic hydrocarbons and they all in+volve different degrees of risk of disease and carcinogenicity. Therefore, the selection of the main hazardous aromatic hydrocarbon compounds and the establishment of a comprehensive determination of polycyclic aromatic hydrocarbons in the aerosol of heat-not-burn products can provide a reference for their safety assessment. Polycyclic aromatic hydrocarbons are present in aerosols, but they are extremely low in content and the composition of the aerosol itself is very complex, which greatly interferes with the determination. Therefore, it is necessary to increase the concentration of the aerosol in a single set test as much as possible, and to separate it further after collection and then analyse it. In this study, a pack (20 sticks) of heat-not-burn products of a certain brand sold on the market was selected as a group, and the aerosol was captured by a smoking machine, and the polycyclic aromatic hydrocarbon substances were separated by solid phase microextraction (SPME). The relative contents of ten polycyclic aromatic hydrocarbons were determined by GC-MS with internal standard method. The polycyclic aromatic hydrocarbons are dominated by compounds with low rings. About 80 % are bicyclic and tricyclic aromatic hydrocarbons, and about 20 % are tetracyclic and above polycyclic aromatic hydrocarbons. Therefore, this method can be used for the comprehensive determination of polycyclic aromatic hydrocarbons in the aerosol of heat-not-burn products, and has important reference significance for the safety assessment of heat-not-burn products.

ST 06

Heated tobacco product aerosol generation and aerosol analysis by gas chromatography flame ionisation and thermal conductivity detection

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Heated tobacco products (HTP) aerosol composition is different from the mainstream smoke of a burning cigarette. There are currently no standard methods defined. Identifying potential sources of variation is important to adjust the analytical procedures accordingly.

The objectives of our work were to:

- develop a method to generate HTP aerosol and analyse nicotine, glycerol, propylene glycol (PG) and water by gas chromatography with flame ionisation and thermal conductivity detection.
- assess how the HTP stick conditioning and puff interval duration affect aerosol composition.

Health Canada Intense regime without vent blocking was used to generate HTP aerosol on a linear Borgwaldt RM4 machine. Commercial IQOS Heets and glo™ Kent Neosticks were smoked with a standard puff interval (30 s) and a shorter puff interval (15 s), delivering respectively 12 and 6 puffs.

The puff interval duration did not modify significantly the aerosol composition of both HTPs.

In order to study the influence of sample conditioning before vaping, IQOS Heets and glo™ Kent Neosticks were conditioned 48 hours and 15 days according to ISO3402 standard and compared to sticks stored in sealed packs. For each conditioning, four replicates were vaped with 15 second puff intervals. Long storage time lead to a reduction of PG in tobacco and aerosol. Otherwise, there were only some limited differences, the major observation being more stable results when smoking sticks are out of the sealed pack.

In order to assess the repeatability and reproducibility of the vaping method, five operators vaped IQOS Heets sticks stored in sealed packs four different days with four replicates per day. The relative error was below 15 % for TPM, nicotine, humectants and 23 % for water.

ST 07

Quantitative determination and puff-by-puff analysis on the release pattern of nicotine and menthol in heat-not-burn tobacco material and emissions

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There is a lack of description of nicotine strength in current available heat-not-burn (HnB) tobacco products. Three HnB products were studied regarding the occurrence of nicotine in their tobacco and menthol flavoured cigarettes, and menthol in their menthol cigarettes, with respect to their tobacco material, emissions and puffed filters.

Nicotine and menthol in tobacco material, and menthol in filters of menthol cigarettes were determined by GC-MS. Emissions generation was done with a linear smoking/puffing machine, and the nicotine and menthol trapped on the Cambridge Filter Pad were determined. The puffed tobacco materials and filters were collected and analysed for the leftovers of nicotine and menthol. Puff-by-puff analysis of nicotine and menthol release was also done based on our previous established method.

The nicotine content in the three products were different ranging from 1.9 to 4.6 mg/cig, and 2.1 to 3.2 mg/cig of menthol was determined in their menthol flavoured cigarettes. Average release of nicotine into emissions ranged from 0.25 to 0.53 mg/cig, and 0.69 to 1.6 mg/cig for menthol. The efficiency of nicotine delivery from tobacco material to emission of the three products ranged from 11.5 % to 16.5 %. Different menthol contents were determined in unpuffed filters of all three products. Puff-by-puff analysis showed a release of 0.02~0.09 mg/puff of nicotine and 0.08~0.28 mg/puff of menthol in emissions of these three products. The pattern of nicotine/menthol release showed different consistency throughout the whole puffing session.

In conclusion, HnB products contain various nicotine/menthol contents, and their levels in emissions are different. It might be safer to label nicotine strength on product packaging similarly to conventional cigarettes. Our puff-by-puff analysis was able to monitor the dynamic release of substances of interest, which better mimic real puffing behavior and thus could lead to a better understanding of user exposure to substances. Analysis on the puff basis helps further improve consistency of product performance.

ST 08

Effects of smoking parameters on aerosol delivery of next generation of Heated Tobacco Products (HTPs)

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In recent years there have been many changes in the cigarette market. Cigarette users are accepting novel products along with conventional cigarettes. These novel products are sometimes referred to as next generation products (NGPs), and include items such as e-cigarettes and heated tobacco products (HTPs). These products release aerosol in different ways than that of normal cigarettes. As a result of these market changes a great deal of research has been launched on the puff topography of these novel products. This study investigates the effects of puff parameters on the aerosol delivery of NGPs via a smoking machine. In particular, two types of products were examined, HTP A and B, by altering the puff parameter regime in terms of puff profile, puff volume, and puff frequency, in accordance with features displayed by NGPs, while maintaining a constant puff duration of two or three seconds throughout. In addition, the HTP B was examined under two other conditions: ISO20778 and ISO20768. The results indicate that aerosol delivery for both products is influenced by puff volume, and that it is highly dependent on puff frequency. For the HTP A, aerosol delivery increased in line with increases in puff frequency, while the effect of increases in puff volume was more limited. For the HTP B, aerosol delivery was moderately influenced by changes to the puff profile, with humectant delivery being affected, but not nicotine delivery. Aerosol delivery for the HTP B was also less influenced by differences in the tested puff durations. This study indicates the need to establish clear smoking conditions for NGPs, and provides a basis for the future design and development of test products to facilitate these needs.

ST 09

The ABOUT™ Toolbox: toward the development of consumer-reported outcome measures that matter

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Tobacco harm reduction promotes the substitution of reduced-risk products (RRPs) for cigarettes by adult smokers who would otherwise continue to smoke, while preventing RRP initiation among non-users. To operationalize this public health strategy, there is a need to understand the motivations, perceptions, and behavioral patterns of using RRPs. A prerequisite for this is to have accurate estimates of these underlying perceptions and behaviors, which require adequate measurement tools to be put in place. This work presents the rationale for the development of the ABOUT™ (Assessment of Behavioral OUtcomes related to Tobacco and nicotine products) Toolbox, a portfolio of self-report instruments developed to support population perception and behavior assessment of RRPs. The ABOUT™ Toolbox has been developed using best measurement practices, including generation of a conceptual model, evaluation of content validity, use of an appropriate psychometric model, cross-cultural measurement equivalence, and appropriate access of the validated instruments (original and translations). Moreover, the Toolbox fits into an underpinning behavioral conceptual model, designed to understand switching or transition behaviors, which encompasses several levels of assessment (i.e. individual, product, and environment). The ABOUT™ Toolbox will initially include five self-report instruments focusing on individual level (perceived risk, perceived dependence, product experience, use history, health and functioning). In this work, we focus on the rationale and guiding principles supporting the ABOUT™ Toolbox initiative, while specific instruments will be presented in detail in separate presentations. Making the ABOUT™ Toolbox accessible to the tobacco research and public health community is paramount to addressing tobacco harm reduction challenges. Wider use of the Toolbox could help to rapidly build an evidence base that would allow comparisons of study findings across a wide spectrum of RRPs. In turn, this would potentially enable public health and regulatory communities to make informed decisions for future regulation of RRPs.

ST 10

Development of the ABOUT™–Health & Functioning: results from the preparatory phase

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Switching cigarette smokers to significantly less harmful tobacco or nicotine products, or reduced-risk products (RRPs), has been recognized as a valuable and promising approach to reduce smoking-related population harm. Measuring self-reported experience of the change in health and functioning is crucial to evaluating RRPs' impact on public health. To date, no established smoking-related quality of life measures have been shown to be sensitive enough to detect changes in health and functioning in otherwise healthy smokers who switch to RRPs. The goal of the ABOUT™–Health & Functioning, part of the Philip Morris Products ABOUT™ Toolbox initiative, is to develop a sensitive self-report measure that will assess changes in health and functioning among healthy smokers who switch to RRPs. The project is designed to be carried out over three years and will follow the U.S. Food and Drug Administration's Guidance on Patient-Reported Outcome Measures. The preparatory phase of the measurement instrument development included a scoping literature review, secondary analyses of existing qualitative studies, and convening of an expert panel. The literature review indicated that publications overwhelmingly focused on cigarette use and associated health effects (e.g. respiratory or oral health), with less than 10 % focusing on e-cigarettes and alternative nicotine delivery products. Results from analysis of existing qualitative research identified key drivers for continuing smoking: perceived benefit of tobacco or nicotine product use, dependence, and withdrawal symptoms or fear of withdrawal symptoms. Lastly, the expert panel supported the preliminary conceptual model incorporating the concepts of utility of use, biomarkers, signs and symptoms, functioning, general health perceptions, and health-related quality of life. These activities led to the development of a draft conceptual model for the measurement instrument. In the next phase, the conceptual model will be refined through concept elicitation studies, a global Delphi panel, and the reconvening of the project`s expert panel.

ST 11

Development and validation of the ABOUT™–Dependence measurement instrument to assess perceived dependence on tobacco and nicotine products

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The development of the ABOUT™–Dependence measurement instrument was initiated to enable assessment of perceived dependence associated with the use of different tobacco and nicotine products (TNPs) and in users of multiple TNPs. We initially developed a conceptual framework to define relevant concepts of interests based on expert opinions, literature review, and concept elicitation interviews with TNP users (n = 40). This led to the development of a 19-item draft instrument, which was tested in a cross-sectional survey of TNP users (n = 2,434) to establish psychometric reliability and validity. For the interviews and survey, the sample was recruited in order to have an equal number of single TNP users and poly-users across different products (i.e. cigarettes, cigars/cigarillos, e-cigarettes, smokeless tobacco, waterpipe, pipe, and nicotine replacement therapy). The conceptual framework included symptoms to best represent "lack of control" as the core concept of dependence, recognizing that success in cessation depends mainly on an individual's motivation and willingness. Qualitative data also suggested that TNP users tend to characterize dependence on their product(s) in terms of the intensity and frequency of need/desire to use TNPs and difficulty to limit TNP use. Psychometric evaluation of the 19-item draft version of the instrument supported a final 12-item instrument consisting of three perceived dependence domains (i.e. behavioral impact [five items], signs and symptoms [five items], and extent/timing of use [two items]) and an overall total composite score. Validity of the final instrument was further supported by correlations with existing dependence measures (e.g. Fagerström Test for Nicotine Dependence) and known groups testing (e.g. demographics and product use characteristics). Crosswalk tables were generated to aid the comparability of scores between the new and existing dependence measurement instruments on a common metric. The new instrument advances our ability to measure and, therefore, better understand perceived dependence across different TNPs and user types.

ST 12

Review of current methodologies in evaluating abuse liability of nicotine and tobacco products in the U.S. regulatory environment

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Traditional definitions of abuse and misuse do not apply to a consumer product such as tobacco that consumers essentially use 'as intended'. In contrast to pharmaceutical products, a guidance for abuse liability testing of nicotine and tobacco products has not been issued in a U.S. regulatory context. As a result, no definitive industry guidance exists for abuse liability testing of such products to aid in obtaining a marketing order in the U.S. from the Food and Drug Administration. This presentation is intended to summarize current consensus on the best abuse liability assessment methodologies.

Some of the key issues that will be discussed include the importance of including a variety of abuse liability assessments including chemistry, toxicology, preclinical pharmacology, animal behavioral and dependence pharmacology, pharmacokinetics/pharmacodynamics, human abuse potential laboratory studies, clinical trial data relative to abuse dependence potential, integrated summaries of safety and efficacy, and any foreign experience with the drug. "Gold standard" human abuse potential laboratory studies will be examined in detail. In addition, subjective effects questionnaires measures that evaluate the perceived rewarding effects of the tobacco product, product acceptability, central nervous system (CNS) and peripheral effects of tobacco products will be discussed. All topics will be integrated to assess perceived regulatory requirements and challenges.

ST 13

Importance of e-liquid preparation and stability characterization for a combinatorial safety assessment approach of flavors in e-liquids

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Numerous flavorings are generally recognized as safe for use in food, but there is limited information available to evaluate their potential toxicity by inhalation. Consequently, gaining information on individual flavor safety levels is of significant relevance to define appropriate use of flavors in potentially reduced-risk products (PRRPs), such as electronic cigarettes.

Safety assessment requires pre-clinical studies; considering the large number of potential flavorings, appropriate selection of representative candidates to subject to such studies is a key first step. In addition, it is critical to characterize the test items, including the verification of batch consistency, throughout the study. In this study, we propose a unique combinatorial flavor group-based approach to acquire information on the safety of flavors ingested via inhalation. Using this approach, we clustered 246 flavoring substances into 38 groups of structurally related substances (based on groups defined in European Commission regulation No 1565/2000). From each group, we selected the flavor group representative (FGR) with the predicted worst toxicological profile, aiming to use the toxicological information acquired on the FGRs to predict the toxicity of structurally related flavoring substances within the same group. To reduce complexity of test item preparation, we split the 38 FGRs into six concentrated “pre-blends” based on structural moiety, solubility, and chemical reactivity, and assessed their stability. These pre-blend solutions were stable for up to 28 days. Finally, we mixed those pre-blends to obtain two e-liquid solutions containing all the FGRs. The final formulation containing nicotine was stable for up to three days and the formulation without nicotine was stable for up to ten days. Our research demonstrated the advantages of mixing selected representatives into concentrated and stable pre-blends, thus avoiding laborious daily solution preparation and minimizing efforts and costs associated to batch characterization.

ST 14

Study on capillary-evaporation effect in porous medium of electronic cigarettes

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In order to study the capillary-evaporation effect in the porous medium of electronic cigarettes, a theoretical model was established to simulate the heat and mass transfer process in the heating zone of e-cigarettes. Based on the similarity principle, a scaled-model test bench for e-cigarettes was designed and built. After comparing the numerical simulation results with experimental data, the saturation level and the transport rate of e-liquids in the heating zone were studied by applying the theoretical model.

The results showed that the numerical simulation results were basically in accordance with the experimental data. The theoretical model was proved to be reliable to some extent. The average vaporisation rate, average aerosol mass concentration and effective vaporisation heat efficiency increased linearly with the incremental increase of the heating power. Applying the same heating power and puffing regime, a higher propylene glycol content in the e-liquid resulted in a greater vaporisation rate and aerosol mass concentration. The heat efficiency (at same heating power) of propylene glycol was higher than that of vegetable glycerin. Most likely an increased porosity of the wick also increases the saturation level of e-liquid in the heating zone, which results in a higher capacity of the wick to absorb the e-liquid and therefore the probability of dry heating is reduced. The saturation level of the e-liquid in the heating zone is decreased symmetrically from both edges of the porous medium to its center. The transport rate of e-liquid at the beginning of the heating zone was higher than the rate in the center, therefore dry heating was most likely to occur at the center of the heating zone. The e-liquid saturation level in the heating zone decreased with the increase of the heating power. A higher heating power will therefore increase the probability of dry heating.

ST 15

Crystal structure of nicotine gentisate and its permeability in pig buccal mucosa

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Nicotine compound salt has been widely concerned recently with the continuous expansion of the e-cigarette market. Synthetic nicotine organic acid salt or nicotine complex, instead of monomeric nicotine, is used in the formulation of the next generation tobacco products. Bound nicotine has shown its clear impact on not only the taste but also on the dissolution and absorption of nicotine.

In order to develop a totally different nicotine introduction form for next generation tobacco products, the compound salt of nicotine with gentisic acid was synthesized in this study. The liquid products were crystal cultured in the dark at room temperature. The transparent brown needle crystals were obtained after several weeks. The structure of nicotine gentisate crystal was characterized with the single crystal X-ray diffraction technique. The results indicated that the relative molecular weight was 316.35. The crystal density was 1.342 mg/m^3. The crystal belonged to the monoclinic crystal system and the spatial group was $P2_1$. Transdermal diffusion experiments of nicotine and nicotine gentisate, in which pig buccal mucosa was used as a cross-membrane carrier, were carried out at different pH levels *in vitro*. The results suggested that compared to nicotine, nicotine gentisate had a significant effect on the sustained release of nicotine. In addition, the slowing effect was more significant in low pH medium. This study provides a reference for the research and development of a new form of nicotine and its potential application value to the next generation tobacco products.

ST 16

Selected harmful or potentially harmful constituent yields in the aerosol of commercial closed systems electronic cigarettes

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Over the past few years, electronic cigarettes have rapidly evolved and have been introduced in multiple markets across the world. They are thought to be a potentially less harmful alternative to cigarettes, as they heat a liquid to generate the aerosol instead of combusting tobacco. Electronic cigarettes have different designs, with closed systems (such as cartridges), open systems, and mods. The aim of this study was to examine the aerosol emissions of various cartridge e-cigarettes from different markets. Our efforts were mainly focused on carbonyls, tobacco-specific nitrosamines, metals, benzene, benzo[a]pyrene, and 1,3-butadiene. The study was conducted in three waves from 2015 to 2018, with 18 device and liquid combinations. For each product, three replicates of the devices were used to test product variability and the tests were conducted until total liquid depletion to test the products' consistency. To put the results into perspective, the aerosol constituents of e-cigarettes were compared with smoke from the 3R4F reference cigarette. Many of the harmful or potentially harmful constituents tested were not quantifiable or were below the levels of air blanks. For the examined cartridge systems, the carbonyl emissions were detectable, with formaldehyde being the most variable. Compared with other e-cigarette system designs, formaldehyde in cartridges showed a considerable reduction compared with the emissions from 3R4F mainstream smoke. Several of the products analysed were also tested under diverse conditions (different puffing regimes and analytical puffing machine orientation). The obtained results indicated an overall consistency of products across the various conditions, as the emissions did not change greatly.

ST 17

Comparison of *in vitro* toxicity of heated tobacco products and combustible cigarettes

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In recent years, the paradigm of the tobacco industry has been changing with the development of heated tobacco products (HTPs) that have a similar taste to cigarettes but significantly reduce the amount of smoke components. The purpose of this study was to compare the *in vitro* toxicity of HTPs with that of combustible cigarettes.

We used two kinds of HTPs which have different design characteristics such as heating temperature and method, and a 3R4F reference cigarette. The mainstream smoke/aerosol were generated under the Health Canada (HC) smoking regime and both total particulate matter (TPM) and gas vapour phase (GVP) were collected for toxicity assessment. Bacterial reverse mutation assay (Ames test) and the NRU cytotoxicity assay, which are widely used in the tobacco industry, were conducted to evaluate their *in vitro* toxicity.

In the Ames test, TPMs obtained from 3R4F was mutagenic in TA98, 100, 1537 in the presence of S9 mix whereas TPMs obtained from HTPs were not mutagenic under any of the conditions tested, although the highest obtainable concentration in the vehicle, 5 mg/plate was treated. In the NRU cytotoxicity test, TPM and GVP from HTPs showed more than 90 % reduction in cytotoxicity as compared to those from 3R4F and the order of cytotoxic potential was: 3R4F >> HTP-A > HTP-B.

However, in the case of HTPs, the EC_{50} values could not be determined since HTPs did not show enough cytotoxicity to calculate them. Therefore further studies are needed to develop reliable *in vitro* assays for HTPs.

As a result, our data confirm that the toxicity of the heated tobacco product was significantly lower than that of conventional cigarettes and reduced the risk potential.

ST 18

Human precision-cut lung slices as a screening tool: increased throughput, cryopreservation, and detection of DNA adducts for next generation tobacco product evaluation

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Human-relevant, *in vitro*/*ex vivo* assays are considered an ethical and economically viable manner by which to screen the many chemicals requiring hazard assessment, such as next generation tobacco product (NGTP) ingredients. Human precision-cut lung slices (PCLS) are often considered the most physiologically relevant pulmonary test system, but low throughput and difficulties in cryopreservation have hampered PCLS use. Our objective was to increase PCLS production, optimize cryopreservation, and demonstrate 3R4F-induced DNA adduct formation using PCLS.

A tissue slicer modification allowed simultaneous slicing of three tissue cores. Increased production was quantified using agarose and tissue cores over 15 minutes. We tested five PCLS cryopreservation formulations against PCLS maintained in culture. Viability in each of the groups was assessed with the WST-8 viability assay, prior to fixation and histological evaluation. For mass-spectrometer analysis of DNA adduct formation, PCLS were exposed to ten 3R4F cigarettes (10 puffs each) using the Health Canada Intense regime or exposed to air.

The slicer modification resulted in 2.8-fold and 2.4-fold more slices from agarose cores, and lung cores, respectively. Cryopreservation efforts indicated freezing after slicing yields better average viability (48-73 % of fresh, non-frozen control) than culturing overnight and freezing (13-54 % of control) when assessing health over 4 days, post-thaw. Cryopreservation buffers containing University of Wisconsin preservation solution preserved viability the best (54 %-90 % of non-frozen control). Histological findings concurred with WST-8 viability results (> 75 % of normal healthy lung tissue features), post-thaw. DNA-adduct evaluation indicated every 3R4F exposed (N = 6) but no air-exposed, (N = 6) PCLS yielded acrolein DNA adducts.

The increased PCLS production indicates larger screening studies can be initiated from one lung and cryopreservation results suggest slices can be banked. Further, utilizing human PCLS for DNA adduct analysis demonstrates a relevant screening endpoint to evaluate NGTP safety.

ST 19

In vitro biological impact evaluation of whole cigarette smoke by using pathway-based approach

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Harm-reduced cigarettes have been greatly developed to minimize the negative impact of smoking on health. However, the practical effects of such products are still under debate. Along with the application of high-throughput sequencing technologies (such as RNA-seq) and advanced smoking equipment, accurate algorithms are needed to assess the biological effects of inhaled substances on human health. A Functional Class Scoring-based approach has been developed in this study.

The main work is shown as follows: (1) Traditional Over-Representation Analysis method only considers the number of differential expressed genes and completely ignores the magnitude of the actual expression changes. To solve the problem, the method presented here sorts all genes and calculates the score of the given pathway based on scoring and penalizing algorithms; (2) Through 500 random simulations, the previously calculated score is normalized and a significance test is performed to check the confidence of the pathway; (3) Due to the different numbers of genes in different pathways, the contribution of pathways to the overall evaluation score is different, therefore the method weighs the normalized score by the number of genes; (4) Integrated with the information of REACTOME database, the impact on the pathway is translated into the impact on the main functional categories. Then an Effect Score is finally given to provide an intuitive judgment. By comparing e-cigarettes (Magma), a widely-used cigarette Brand A from U.S. (8 mg tar) and a widely-used cigarette Brand B from China (8 mg tar), our data suggests that the e-cigarette has less impact on human bronchial epithelial cells, while Brand A obtained the highest Effect Score.

The Effect Score could not only be used by cigarette industries or governmental bodies to discuss the risks and regulation of new tobacco products, but also could facilitate smokers in the selection of tobacco and related tobacco products.

ST 20

On the scientific value and practical utility of the comparative tests of nicotine-free aerosols *in vitro* and *in vivo*

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The presence of nicotine in emissions from conventional and electronic cigarettes is an important physiological and associative factor combining these two classes of products. Besides a comparable nicotine delivery, according to other characteristics, these types of products show little resemblance. Nevertheless, lawmakers tend to impose a common regulation for both product categories. To unfold the fundamental differences in biological effects between these product types, it is sufficient to figure out the importance of the role of nicotine in their emissions. Depending on the research model, the contribution of the nicotine component to various biological effects derived from the whole cigarette smoke ranges from 0 to 20 %. For e-cigarettes, this contribution should be significantly higher, but this issue is still poorly studied and direct evidence is still missing. The nicotine fraction in the smoke amounts to 1:5000-1:7000. Aerosols of e-cigarettes are not that rich and diverse in composition, yielding to smoke in the number of components hundreds of times, thus, it seems that the biological weight of nicotine in these aerosols is much higher than in the smoke of a regular cigarette. In this work, we investigated the changes in a number of biochemical and physiological indicators (including the activity of circulating leukocytes, cardiac parameters and respiratory function) upon exposure to nicotine-free aerosol derived from an e-cigarette and nicotine-free smoke of a combustible cigarette. As a result, it has been found that aerosol emissions formed exclusively from propylene glycol (a filler of any e-cigarette), as well as the nicotine-free smoke, in general, exhibit a noticeable potential activity towards physiological functions of the body and its immune system. Another important conclusion drawn from our study is that the biological effects assessed for the same objects *in vitro* and *ex vivo* often exhibit the opposite direction compared to that observed *in vivo*.

ST 21

A model based on cigarette density segmentation method for predicting burning cone drop rate of slim cigarettes

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Burning cone drop is a potential safety problem and quality defect for slim cigarettes. In order to conveniently monitor the burning cone drop rate, a model based on cigarette density segmentation method was established and used for predicting the burning cone drop rate of cigarettes. This method is nondestructive and can predict the burning cone drop rate of cigarettes according to tobacco rod density and its variation rate.

The established model is based on a straight line $\rho=A \times \rho'+B$ in the Cartesian coordinate system with abscissa, the variation rate of tobacco rod density ρ' and ordinate, tobacco rod density ρ. The straight line splits the coordinate system into two regions. By measuring the density of a slim cigarette, the position of point (ρ', ρ) is determined, and the burning cone drop tendency can be predicted accordingly.

The accuracy of the model was validated. The results suggested that the predicted value and the measured value were basically near the 1:1 line. The coefficient of determination (R^2) and the index of agreement (D index) between the predicted and measured values were 0.98 and 0.99 respectively, and the normalized root mean square error (nRMSE) was 11.8 %. The coefficient of variation of the predicted burning cone drop rate was 6.5 % for 10 consecutive replicates.The cigarette density segmentation model is suitable for predicting burning cone drop rate of slim cigarette samples with varied cut tobacco density or cut tobacco distribution and provides a valuable reference for the production and quality inspection of slim cigarettes.

ST 22

Study of combustion patterns for ignition propensity test using several kinds of substrates and cigarettes

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The ignition propensity of cigarettes can be measured in accordance with ISO 12863:2010. It recommends the use of Whatman No. 2 filter paper as a substrate. Actually most laboratories use this filter paper and there may be a need to replace Whatman No. 2 filter paper by other materials. The Routine Analytical Chemistry (RAC) Sub-Group of CORESTA evaluated the ignition propensity of three test cigarettes on Whatman No. 2 filter paper and three alternative substrates. The project only focused on the ignition propensity and there was no analysis of other factors that could have affected the experiments.

The objective of this study was to find the key factors that affect the ignition propensity by using a thermal camera. We evaluated the basic physical properties of two cigarettes (CORESTA Ignition Propensity Approved Monitor No. 2, NIST® SRM® 1082 Cigarette Ignition Strength Standard) and three substrate papers. In addition, we analysed the combustion patterns of these cigarette/substrate combinations with a thermal camera. All the experiments were carried out in accordance with ISO 12863:2010.

We found a significant difference in the combustion patterns. Especially, the minimum temperature for continued smoldering was identified as the key factor for self-extinguishment and it was highest for Whatman No. 2 filter paper.

In this study it was found that combustion patterns of substrates are closely related to the ignition propensity and they can be used to develop alternative substrates for ISO 12863:2010 test.

ST 23

Functional filter plug wrap paper for control of the thermal energy of the aerosol from heated tobacco products (HTPs)

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Next generation products, which include all kinds of e-vapour items as well as heated tobacco products (HTPs or “heat sticks”), have successfully entered the tobacco market in addition to conventionally combustible cigarettes. From the basic geometrical concept, HTPs resemble traditional filter cigarettes with various formats, and therefore, heat sticks require filter wrapping materials. Filter wrapping materials are thin and flexible sheets made of paper or other web substrates in order to enable specific quality and technical properties. In general, filter wrapping materials can be separated into tipping paper and filter plug wrap paper. The purpose of the present study is to focus on filter plug wrap paper and to describe individual technical functionalities of the paper. The application of a physically active substance on the plug wrap paper will hereby be demonstrated to lower the temperature of the aerosol generated by HTPs. As some heat stick versions are geometrically distinctively shorter than combustibles, the cooling rate of the aerosol is comparably low yielding high remaining temperature of the inhaled evaporation and distillation result. Selective surface treatment of the plug wrap paper through coating generates enhanced thermal absorptivity, and its efficiency will be quantitatively investigated under pilot plant test conditions as well as evaluated with heat absorption calculations and phase transition aspects. Moreover, mechanical embossing of the plug wrap paper is carried out to increase the effective area of the coated surface thus providing a stronger contribution to the temperature reduction.

ST 24

Vapour temperature in polylactic acid (PLA) free heat-not-burn products

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For the purpose of tobacco harm reduction, new generations of heated tobacco (heat-not-burn - HnB) products (HTPs) were introduced in the market (Savareear et al., 2018). Most existing HnB products are using polylactic acid (PLA) films or fibers to reduce vapour temperature. In this study we investigate different filter designs to lower vapour temperature without using PLA. Although PLA is made from natural renewable sources, its degradation rate is very slow in ambient temperatures showing no significant advantages against conventional plastics (Bagheri et al., 2017).

Various combinations of acetate filters, outer shape filters, acetate and paper tubes were combined to reach full flavour delivery and low vapour temperature. For the tobacco column we used commercially available tobacco segments of HnB sticks. Regular HnB sticks were used as standards with which we compared our design filters. Vapour temperature was measured puff-by-puff and vapour condensate was collected on Cambridge filters. Vapour phase condensate was collected, extracted and analysed on Agilent 5977 Intuvo GCMS.

Some HnB combinations showed temperatures comparable to commercially available products containing crimped PLA films. We believe that HnB cigarettes containing paper and acetate tow parts have a good potential to achieve low vapour temperatures and tor become an environmentally friendly product with balanced taste properties.

ST 26

Digital evaluation of tobacco style and quality by using a support vector machine algorithm with thermal analysis spectra

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In the processes of tobacco blend design and product maintenance of cigarettes, the evaluation of tobacco style and quality is very important. At present, the evaluation method mainly relies on artificial sensory analysis, which is subjective and relatively unstable. In order to realize the digital evaluation of tobacco style and quality, the pyrolysis and combustion characteristics of tobacco are taken as an efficient index, which is closely related to cigarette burning behavior and can be expressed by thermal analysis spectra. In this study, the thermal analysis spectra of 88 single-grade tobacco leaves were obtained by thermal analysis technology, and Support Vector Machine (SVM) algorithm was employed to classify 74 single-grade tobacco leaves with different qualities and styles into eight categories. The accuracy of the SVM algorithm for the training set was up to 98.6 %. Afterwards, the SVM algorithm was applied to predict the quality and styles of the other 14 single-grade tobacco leaves, and the accuracy was 92.9 % for the testing set. The results showed that this method could not only greatly improve the accuracy of digital evaluation of tobacco quality and style but could also verify the effectiveness and practicality of thermal analysis spectra for the digital evaluation of tobacco.

ST 27

Determination of petroleum ether extracts in flue-cured tobacco leaves by NIR combined with random frog wavelength selection method

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In order to improve the prediction accuracy and model stability for quantitative calibration in near infrared spectroscopy, the random frog wavelength selection method combined with partial least squares (PLS) was employed to establish the prediction model of near infrared spectra for petroleum ether extracts in flue-cured tobacco leaves from various geographical regions. A combination of the standard normal variate and the first derivative was selected as the spectral pre-treatment method. The samples were subdivided into a calibration set (285 samples) and a validation set (95 samples) according to 3:1 by the Kennard-Stone (KS) algorithm. The random frog method was used for spectral variable selection. The partial least squares regression (PLSR) calibration models were established to predict the content of petroleum ether extracts, and compared with the results of full spectra. The results showed that better prediction was obtained by the random frog method compared to full spectra PLS modelling, moving window PLSR (MW-PLSR) and Monte-Carlo uninformative variable elimination PLSR (MC-UVEPLSR). The determination coefficient of calibration set (R^2), root mean square error of cross validation (RMSECV), determination coefficient of validation set (r^2) and root mean square error of prediction (RMSEP) were used to evaluate the quality of the model. R^2, RMSECV, r^2 and RMSEP of the full spectrum PLS model were 0.9902, 0.0896 %, 0.8573, and 0.3415 %, respectively. While R^2, RMSECV, r^2 and RMSEP of the random frog PLS model were 0.9934, 0.0830 %, 0.9700 and 0.1547 %, respectively. The result showed that using a variable selection method such as the random frog method could effectively select the characteristic wavelengths of NIR spectrum to improve the model applicability and robustness. The proposed method could effectively simplify the model and was suitable for fast and reliable determination of petroleum ether extracts in flue-cured tobacco leaves.

ST 28

Simultaneous determination of twenty amino acids in tobacco leaves by multiple heart-cut two-dimensional liquid chromatography

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Amino acids are important aroma precursors in tobacco. They can react with reducing sugar in the processes of tobacco curing, aging or smoking, which is called the Maillard reaction. Various heterocyclic aroma compounds are generated in this reaction, which affects the quality of tobacco. Besides, amino acids will decompose and generate various compounds at high temperatures, including flavour components, irritant components and harmful components. Therefore, the analysis of amino acids in tobacco could provide theoretical guidance for tobacco curing, cigarette blend, etc. A variety of chromatographic or chromatographic/mass spectrometric methods have been used to determine the amino acids in tobacco. However, due to the high complexity of tobacco substrates, there is no generally accepted quantitative detection method at present. In this article, a multiple heart-cut two-dimensional liquid chromatography method was developed for simultaneously determining 20 amino acids in tobacco leaves. After extracting with hydrochloric acid solution and deriving with 6-aminoquinolyl-N-hydroxysuccinimidylcarbamate (AQC), tobacco samples were separated with the cyano column in the first dimension, then trapped and separated by C_{18} column in the second dimension. Finally the derivatives of these amino acids were detected by fluorescence detector. This method greatly reduced the matrix interference, and the chromatogram of tobacco samples was close to that of the standard amino acid samples, and the quantitative reliability of the method was promoted. The results showed that: the correlation coefficients (r^2) was higher than 0.993 for all calibration curves, the standard recoveries of the spiked samples ranged from 72.55 % to 104.91 %, the detection limits were from 0.06 to 12.25 µg/g, the quantitation limits ranged from 0.18 to 34.07 µg/g, the intra- and inter-day relative standard deviations (RSDs) were 0.05 %-7.78 % and 0.20 %-9.11 %, respectively. This method features high resolution and high sensitivity, and is suitable for the simultaneous determination of amino acids in complex matrix samples.

ST 29

Thermochemical and thermophysical mapping of burning superslim and kingsize cigarettes

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After presenting and publishing the proof of principle of the *in-situ* thermochemical and thermophysical mapping inside a burning Super Slim (SS) cigarette, we now extended the study scope to other cigarette formats and smoking regimes[1].

A Chinese Virginia-style Super Slim and the respective King Size (KS) cigarette were investigated under ISO as well as Health Canada Intense (HCI) smoking regime.

A micrometre sampling bench was used to accurately position the *in-situ* sampling probes for insertion into the cigarettes. An array of 0.254-mm thermocouples (for gas-phase temperature determination) and multiple 0.35-mm diameter quartz tubes connected to transducers (for pressure determination) were inserted into the SS cigarette. For chemical analysis, a single heated 0.5-mm chemical sampling microprobe was also inserted and coupled to a single-photon soft ionisation (SPI) mass spectrometer through a heated transfer line. The different measurement techniques were synchronised by mapping two probes at one point in time (e.g. temperature/pressure or temperature/chemistry).

The mapping approach provides complex as well as dynamic variating data that allows the comprehensive description of the main thermophysical and thermochemical phenomena. Due to the different geometric parameters of SS and KS cigarettes, differences between the SS and KS cigarettes in the chemical fingerprint patterns during a puff were observed. In addition, the higher intensity of the puff under HCI in comparison to the ISO regime alters the thermophysical and thermochemical profile inside the burning cigarette.

The spectrum of the generated information ranges from simple process understanding, such as the spatially resolved thermal desorption of nicotine or the thermal degradation of nicotine, to more complex mechanistic physical-chemical understanding inside a burning cigarette.

[1] Huapeng Cui, Sven Ehlert, Fuwei Xie, Jan Heide, Nan Deng, Bin Li, Chuan Liu, Kevin McAdam, Andreas Walte, Ralf Zimmermann; Integration of time and spatially resolved *in-situ* temperature and pressure measurements with soft ionisation mass spectrometry inside a burning superslim cigarette, *Journal of Analytical and Applied Pyrolysis*, Volume 135, 2018, Pages 310-318.

ST 30

Assessment of within-package and lot-to-lot variability associated with quartz collection pads in the determination of metals in aerosol

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Determining aerosol metals routinely requires an assessment of differences between test sample aerosol results and collection blanks. When using pad collection principles, the background levels associated with metals from the quartz pad can make the interpretation of test sample results difficult. The objectives of this study were to (1) quantify the within-package, among-package and among-lot variability for levels of 20 different metals from untreated quartz pads and (2) utilize that variability data to assess potential limitations in the determination of differences between metals measured in the particulate trapped on quartz pads from aerosols and collection (air) blanks.

Quartz pads were tested by removing the first five pads (closest to the packing material) from each package, prior to use. Each pad was extracted with 35 mL of 10 % (v/v) ultrahigh purity nitric acid solution, and analyzed directly for 20 metals using ICP-MS. Statistical analysis was performed on the analytical results to assess the within-package, among-package and among-lot variability for each metal.

Of the 20 metals tested, the largest proportion of total variance was attributed to the lot (65-70 %) for two metals (Zn, Pb), and the package (55-80 %) for nine metals (including Al, Cr, As, Mo). For the remaining nine metals (of which only Fe was quantifiable), the majority of the variance could not be explained by lot or package of the quartz pad. The pooled residual variance obtained (in units of ng/collection), after accounting for among-lot and among-package differences, was highest for Al and Mo (110, 58) and lowest for Sr and Pb (2.0, 1.1) for metals that were quantifiable on the quartz pads.

Both lot and package within lot are significant factors in the levels of quantifiable metals found on shipments of quartz pads. Thus, the pooled residual variances from the statistical analysis of the untreated quartz pads can be used to estimate the levels of “background” metals that exist on pads within any given package. This, in combination with instrument sensitivity (limit of quantification), help define the limitations of the overall test method.

ST 31

Impact of device variability on the determination of aldehyde compounds in e-cigarette emissions

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The determination of aldehyde compounds (formaldehyde, acetaldehyde and acrolein) in e-cigarette emissions has been widely reported in scientific literature. Recent reports have focused on the contribution of flavor compounds in e-liquids and on the production of aldehydes in the resulting aerosol. Contradictory results have been reported on the role that flavors play in the production of aldehydes in e-cigarette emissions. One possible reason for the lack of consensus, is the inherent variability in aldehyde production found in e-cigarettes. In this study, we collected replicate data from a tank-based e-cigarette device using both flavored and matching unflavored liquids. Using ten unique devices per test condition, we found that typical, across device, variability ranged from 75 % to 200 % for all measured compounds. With this approach it was not possible to discern the impact of flavor compounds on the production of aldehydes. In an attempt to reduce variability, this study was repeated, except that the same devices were cleaned and reused for all samples. This allowed for a direct comparison of the impact of flavor compounds on the production of aldehydes. With this approach, we were able to determine that all flavored e-liquids that were tested yielded a slight increase in acetaldehyde emissions, two of the six flavored e-liquids tested yielded a slight increase in formaldehyde emissions, and that none of the e-liquids tested yielded an increase in acrolein emissions. Our data suggests that the production of aldehydes in e-cigarettes aerosol is extremely device-dependent and care must be taken to ensure that adequate replicates are collected to determine the impact of device variability on analytical measurements of e-cigarette emissions.

ST 34

E-cigarette data standards and open platform architecture for risk and compliance management, supply chain optimization, and real-time benchmarking

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In the next few years, the digital maturity of the e-cigarette sector will evolve offline electronic devices to products that connect via a communication device such as a smart phone or router, and ultimately to Internet-of-Things (IoT)-enabled products, which can communicate via the internet. These advancements will be driven by market trends, new technologies and consumer safety regulations. Almost all e-cigarettes available today are offline electronic devices, but the share of connected and IoT-enabled products will grow at an accelerated pace due to the measurable benefits that the data generated by e-cigarettes can deliver combined with the decreasing cost of wireless communication. To accelerate the digital transformation in the e-cigarette sector, manufacturers need standards for measurement, data and communication, as well as a neutral and open platform for building best practices and producing real-time benchmarks.

The objective of this research is to help e-cigarette manufacturers to accelerate their transformation from offline devices to digital devices by providing best practices for designing digital e-cigarettes that can produce greater consumer and business value. The result is a catalogue of consumer pain points and issues that e-cigarette companies report in their value chains, definitions of the data required to resolve each B2B and B2C scenario, and a recommended architecture for a neutral and open platform for consolidating real-time consumer behaviour and product performance data. Some specific recommendations are presented for using the data to improve consumer satisfaction and safety, streamline compliance, and produce real-time benchmarks. The study includes an analysis of the data that is generated by the few connected e-cigarettes that are available on the market today and an outline of lessons learned, which manufacturers of the next wave of connected and IoT-enabled vaping products can use to decrease their development costs and risk, shrink their time to market, and increase consumer adoption.

ST 35

Chemometric and analytical tools directed to improve the selection of raw materials for long-filler cigar manufacture

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Defining chemical specifications of raw materials, and having adequate analytical methodologies with known tolerances at a reasonable cost is a need for tobacco companies. They allow a better management of batches of raw materials produced. Three experiments were conducted simultaneously from 2012 to 2017: 1) Assessing the fitness for purposes of analytical procedures for total alkaloids as nicotine and total nitrogen under intermediate precision conditions and three mass fraction levels without using certified reference materials; 2) Developing calibration models for analysing the same parameters and total ash through near infra-red spectroscopy (NIRS); 3) Predicting the sensory strength of cigar mainstream smoke through instrumental measurements. Two batches of raw materials from consecutive tobacco seasons were sampled from the point of sale and utilised for preparing 322 dust samples and 1890 cigars. A positive indication was obtained of the fitness for purpose of the reference and NIRS analytical procedures. The relative systematic effect at the intermediate mass fraction level for total alkaloids as nicotine (2.87 %) was smaller than in previous collaborative studies (3.80 % and 4.10 %). The same figure obtained for total nitrogen was 6.39 % and there were no data from collaborative studies. The relative standard error of prediction of the NIRS models for the same parameters and the total ash (3.90 %; 2.79 %, and 2.32 %) were smaller than that reported for traditional analytical procedures under reproducibility or intermediate precision conditions, respectively (4.10 %; 3.63 %; and 2.47 %). The smoke analytes quotient nicotine/tar and the relative nicotine transfer, together with the ones mentioned in the products can predict the sensory strength of the cigar mainstream smoke. In spite of the limitations of cigar emissions testing, this work sheds light on the possible use of chemical information for selecting raw materials for cigar manufacture.

ST 36

HPHC market map study for U.S. machine-made cigars – Part 1: Physical properties, filler and smoke HPHC variability

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Market map studies have been used in the cigarette industry for many years to aid in the characterization of the marketplace. These studies provide comparative values and predictive models for aiding in the assessment of other products. However, the characterization of the physical properties and smoke and filler harmful and potentially harmful constituents (HPHCs) of cigars has been much more limited than with cigarettes.

We examined the physical properties and the filler and smoke HPHCs of 24 machine-made cigars from the U.S. marketplace. The goal was to establish HPHC ranges for filler and smoke yields and to develop predictive relationships to estimate smoke yields of cigars not included in this study.

Products were smoked using the CORESTA, ISO, and Intense smoking regimes for the constituents on the FDA abbreviated HPHC list for cigarettes. The cigars were also tested for each of the filler constituents on the FDA abbreviated HPHC list for cigarettes. Cigars show much greater variability in weight and resistance to draw than cigarettes and that variation is reflected in much greater variability in smoke yields than is seen with cigarettes.

The relative variability of smoke HPHCs and the product yield orderings are similar with all three smoking regimes. Filler HPHCs are less variable than smoke HPHCs. The smoke HPHCs are correlated to the overall yields of the products as measured by TPM, tar, or carbon monoxide yields. Many of the smoke yield correlations are further improved by taking the tobacco filler HPHCs into account.

This work is discussed in a two-part presentation. Part 1 focuses on the description of the products and the inherent variability of cigars. Part 2 focuses on predictive models.

ST 37

HPHC market map study for U.S. machine-made cigars – Part 2: Predictive models

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ST 38

Optimized method for determination of selective phenolic compounds in cigarette and cigar smoke by UHPLC-FLD

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Phenolic compounds, including phenol, catechol, and o-, m-, and p-cresol are included in the "Established List of the Chemicals and Chemical Compounds Identified by the FDA as Harmful and Potentially Harmful Constituents [HPHCs] in Tobacco Products and Tobacco Smoke". CORESTA has developed and published a consensus standardized method for the determination of phenolic compounds in cigarette smoke, the CORESTA Recommended Method No. 78 (CRM 78): "Determination of selected phenolic compounds in mainstream cigarette smoke by HPLC-FLD". CRM 78 has a run time of 34 minutes. We have developed a high throughput method that is based on CRM 78, which has a run time of ten minutes and uses Ultra-High Pressure Liquid Chromatography (UHPLC) and fluorescence detector (FLD) with a sub-2 µm pentafluoro-phenylpropyl phase analytical column. Data generated with the improved method were consistent with data generated using CRM 78. All requirements for method validation were met including linearity, accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), robustness, and standard and sample extract stability. For example, the repeatability for each analyte was less than 10 %, and the linearity was demonstrated with a coefficient of determination of > 0.995 for the calibration ranges of 0.05 µg/mL - 20 µg/mL for hydroquinone, catechol, and phenol, and 0.01 µg/mL - 4 µg/mL for resorcinol, p-cresol, m-cresol, and o-cresol. This optimized method provides a significant reduction in instrumental run time and larger dynamic range as compared to CRM 78. Furthermore, the method has been demonstrated to be fit-for-purpose for the analysis of cigar smoke where the levels of phenolic compounds are higher than in cigarette smoke. Data from commercial cigars and cigarettes will be presented.

ST 39

The mechanism of evaporation in conventional wick-and-coil e-cigarettes compared to an alternative volumetric heater approach

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Conventional electronic cigarettes facilitate the so called wick-and-coil-systems for liquid evaporation. This technology shows essential drawbacks inherent to the system. Due to the non-uniform temperature distribution throughout the heated volume there is an erratic risk of liquid getting overheated locally. Overheating causes the emission of carbonyls and metals and can neither be overcome by automated production of the nowadays typically handmade cartridges nor by sophisticated control algorithms of the power supply, which will be shown and discussed within the presentation in detail.

The aim of this study is to compare the characteristics of a newly developed volumetric heater to conventional wick-and-coil-evaporators and show its potential.

Within the study the differences between both evaporation methods will be described and underlined by comparative testing. For the volumetric heater system, total particulate matter (TPM) consistency, droplet size distribution and carbonyl assessment will be presented. It can be seen that the amount of hazardous substances can be reduced significantly compared to conventional wick-and-coil systems operated at comparable vapour emission. The benefits of the volumetric heater approach for use in electronic cigarettes as well as a door opener to precise inhalative dosing applications will be discussed. It may also serve as a reference method for testing e-liquids under precisely controlled conditions.

ST 40

A new approach for the determination of glycols and sugars in tobaccos and e-liquids

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The objective of this research has been to develop a better method for the determination of glycols such as glycerol (VG) and propylene glycol (PG) and sugars in tobacco and tobacco products as well as for e-vapor products. While gas chromatography (GC) has been used for the determination of VG and PG in tobacco with methanol (MeOH) or MeOH/water extracting solutions, complete extraction of the VG can be difficult, especially for cased, redried Burley tobacco. Aqueous extraction of the tobacco is preferred for both glycols and sugars, but that generally meant liquid chromatography (LC also known as HPLC). While several LC approaches have been developed, they generally involve use of columns packed with ion-exchange resins (require operation at > 60°) or amine-functionalized silica, the latter reportedly being prone to formation of Schiff bases with reducing sugars. However, both problems can be solved using a Type-C silica packing that has primary amide groups bonded to it, such as a Cogent Amide column (250 mm × 4.6 mm ID, 4 µm 100) using a mobile phase of 80 - 90 % acetonitrile with the balance being water at a flow rate of 1 ml/min. This system can be used with a refractive index (RI) detector (Waters 410) or a UV dectector at 195 nm (Waters 486). The column can be used at ambient temperatures, but when the RI detector is used, it and the column are kept at 35 °C. Results will be presented for determination of VG, PG, and sugars in tobacco products, determination of VG, PG and other components in e-liquids, and the determination of VG and PG that transfer from e-liquid aerosols to artificial saliva in a glassmouth. LC-UV characterization of e-liquids will also be shown with aqueous normal phase (ANP) and reverse phase chromatography using the amide column.

ST 41

Human chemical signature – investigation on the influence of human presence and selected activities on concentrations of airborne constituents

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Available indoor air quality (IAQ) studies on tobacco- and nicotine-containing potentially reduced-risk products (PRRPs), such as electrically heated tobacco products and e-cigarettes, demonstrate substantial reduction of environmental emissions compared with cigarette smoking. The IAQ studies on these products apply common IAQ markers (carbonyls, volatile organic compounds [VOC], particulate matter) together with specific tracers, such as nicotine. All indoor environments naturally contain certain levels of the general IAQ markers, while they are usually free of the specific tracers. These present a particular challenge for the assessment of the environmental aerosols generated by potential RRPs. Thus, special attention should be taken to assess the qualitative and quantitative impact of additional pollution sources (e.g. the study participants). In addition, for studies in real-life environments, the impact of different daily life and recreational activities must be considered. To better assess the influence of these parameters on the concentrations of selected air constituents, an exploratory study was performed under simulated residential conditions in an environmentally controlled exposure room. The human subjects either remained for a certain time in the exposure room, or participated in predefined scripted activities (drinking wine, participating in sporting activities, using cosmetics, and cooking). Each activity was assessed separately using our analytical platform and exposure room under controlled environmental conditions. The results showed that prolonged human presence and activities indoors led to an increase in the levels of selected VOCs, cyclic volatile methyl siloxanes and formaldehyde. These constituents were found in higher amounts above the background level when the study participants remained for some time in the exposure room or participated in certain activities. The learnings were applied to optimize the experimental set-up of further studies aiming to assess the IAQ impact of using RRPs in real-life settings.

ST 42

Study on aerosol deposition in the respiratory tract of consumers using cigarette, electronic cigarette or heat-not-burn product

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The deposition of aerosols inhaled into the human respiratory tract is key to evaluate exposure and risk during product use.

A method based on a differential mobility spectrometer (DMS) was established for determining the physical properties of smoke aerosols generated from a combustible cigarette, an electronic cigarette and a heat-not-burn product. The concentration, the count median diameter (CMD) and the geometric standard deviation (GSD) of aerosols generated from all three types of product were determined. On the basis of aerosol physical properties, a Multiple Path Particle Dosimetry (MPPD) model was used to predict the deposition of aerosols in different parts (buccal cavity, tracheal bronchi, alveoli) of human respiratory tract and lungs.

The results showed that: (1) There was no significant difference in the concentration of smoke aerosols between the three different products. The CMD of aerosols from the electronic cigarette (CMD, 17.8-22.4 nm) was lower than for the heat-not-burn product (CMD, 51.8-53.9 nm) and the cigarette (CMD, 174-191 nm); (2) For the aerosols from all three products, the deposition rate was the lowest in the buccal cavity, moderate in the tracheal bronchi, and the highest in the alveoli. The total deposition rate of aerosol was found to be the highest for electronic cigarettes (45.9 %) > heat-not-burn cigarettes (37.2 %) > cigarettes (28.3 %). The highest aerosol deposition rate for all three products was in alveoli, which accounted for more than 60 % of the total deposition; (3) Inside the lungs, the highest deposition rate of aerosol was found in the right lower lung.

ST 43

The value of integrated testing

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In today's competitive market, development times need to be minimised so that products can be launched in response to changing consumer needs. However, as we know, e-vapour products often take considerable timescales to develop as there are both electrical and fluidic system interactions to consider. By observing these interactions during a puff, insights can be made as to how the device is performing, and this data can be used to make better design decisions and cut development time.

When a device is under test different sets of information can be gathered such as:

- Shot weight – the total mass of vapour delivered during a puff. Using automated systems, this can be measured on a puff by puff basis and this greater resolution allows evaluation of liquid feed through life. This is measured to 0.0001 g to allow resolution per puff.
- Vapour trace – this looks at the density of vapour during a puff. Usually done optically, and recorded at 25Hz during a puff, significant information about the atomisation process can be gathered including wick performance.
- Electronics output – By measuring the output of the electronics, usually by piggybacking a second printed circuit board (PCB) to the device, information about power delivery and heater temperature can be ascertained.
- Carbonyl production – detecting the concentration of thermal decomposition products in the aerosol can provide a marker of flavour degradation and use exposure to harmful compounds.

All these methods give valuable information, but when these measurements are combined, additional insights can be made to device performance. The vaporisation efficiency, extracted from a combination of shot weight and electrical power delivered to the heater, can reveal not only deterioration of the liquid feed, but also heating that results in no additional vapour production. The vapour trace can be compared to the measured mass loss to infer when liquid is exiting the device rather than vapour. Comparing the vapour trace to the device's data output can reveal the heater temperature at which vaporisation starts to occur, and how and why the rate of vapour generation changes within a puff. We will illustrate these insights by taking the consumables from two commercially available products (*my*blu™ pods and V2 cartomizers) and simultaneously measuring the puff-by-puff shot weight, the vapour trace and the electronics output from a custom drive system.

ST 44

Lung biomarkers of harm/effect for tobacco regulatory research: opportunities and challenges – a literature review

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Biomarkers of potential harm (BoPH) are indicators of biological perturbations in response to smoking, which may contribute to smoking-related disease. In this review, a project of the CORESTA Biomarkers Sub-Group, we critically assessed the available lung biomarkers of biological effect / BoPH in human lung disease for potential use in evaluation of the effects of tobacco and nicotine products. A Scopus literature search was conducted on lung disease biomarkers that had been used in a clinical setting in humans over the last ten years. Inclusion criteria included human studies only, data on the association of cigarette smoking, smoking cessation with the biomarker and data on smoking vs. non-smoking, preferably with data on changes with smoking cessation. This process identified 1171 papers which were then further screened using commercially available software (Sciome Active Screener). We identified 68 publications that met our preset criteria. This identified five potential sources of biomarkers: Imaging (~9 papers), Blood (~36 papers), Lung Sampling, (~14 papers), Lung Function (~14 papers), and Miscellaneous (~1 paper). This critical review identified several physiological and biochemical measures that are potentially relevant for evaluating the impact of tobacco products on lung health.

ST 45

Meta-analysis of two biomarkers of exposure of tobacco products

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In the context of tobacco products, broadly two types of biomarkers exist, biomarkers of exposure (BoE) and biomarkers of potential harm (BoPH). BoE measure exposure to tobacco constituents, e.g. carbon monoxide (CO) and nicotine equivalents (NEQs). The purpose of the study (a project of the CORESTA Biomarkers Sub-Group) was to establish population level estimates for biomarkers of cigarette smoke exposure to serve as a baseline against change in exposure. A meta-analysis of data published 2008-2018 was conducted to estimate population levels for blood carboxyhemoglobin (COHb) and urinary NEQs.

A protocol for literature assessment was developed, followed by an evidence-based table to identify and select studies. The data template identified elements in four major categories: Labels, Design, Results and Demographics.

A total of 28 scientific articles met the pre-set criteria for COHb; 18 articles published by tobacco companies and ten by academia. By comparison only nine articles met the criteria for NEQs, and these were from tobacco companies. The database was organized by categories, filtered, and data weighted according to the size of the groups.

Much of the data for COHb was derived from smokers (19282) followed by never smokers (NS, 1949) and former smokers (FS, 278). Not surprisingly, smokers had the highest %COHb (5.22 %) compared to FS (1.75 %) and NS (1.05 %). Taking into account the spread of the data, only two groups were significantly different for %COHb: smokers vs NS and FS.

For NEQ the majority of data was from smokers (1506) followed by NS (129) and FS (129). Smokers had the highest %NEQ (14.14 %) compared to FS (0.68 %) and NS (0.06 %). Taking into account the spread of the data, only two groups were significantly different for %NEQ: smokers vs NS and FS.

In summary, baseline exposure for smokers is significantly different from NS and from FS, but FS are not significantly different from NS.

ST 46

Analysis of aroma components in oral fluids of cigarette smokers

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The sensory quality of cigarettes depends on the effects of chemical components in the mainstream smoke on the consumer's sensory organs. To provide a reference for cigarette quality assessment, a method for analysing aroma components in the oral fluids of cigarette smokers was established based on solid phase extraction and heart-cutting two-dimensional gas chromatography-mass spectrometry. After smoking, smokers used a small amount of purified water to rinse their oral cavities, and their oral fluids was collected as experimental samples. The sample pretreatment conditions of solid phase extraction (SPE) were optimized. The established method featured good repeatability (the relative standard deviations ranged from 1.51 % to 6.98 %) and was applied to analyse the aroma components in oral fluids collected from smokers of different types of cigarettes (including Chinese Virginia type, modified Virginia type and blended type). The results showed that the aroma components in the oral fluids of smokers of different types of cigarettes were significantly different, and the major differential components were phenols, caramelization products, Maillard reaction products, etc.; which might be related to the differences of cut tobacco structure between cigarettes of different types. The types and contents of aroma components differed significantly between mainstream cigarette smoke and the oral fluids of cigarette smokers. The distribution ratio of each compound was different in mainstream cigarette smoke and oral fluids, namely the contributions of different compounds to the oral senses of consumers were different. In general, compounds like pyridines, pyrrolidines, pyrazines, cyclopentenones, furfurals and phenols were more likely to be retained in oral fluids. In conclusion, the aroma components in the oral fluids of smokers could reflect their oral sensations more intuitively and might provide a reference for the improvement of cigarette quality.

ST 47

A high-throughput and sensitive bioanalytical method for quantification of nicotine and cotinine in rat/mouse plasma using reversed-phase liquid chromatography–tandem mass spectrometry and its application in inhalation study sample analysis

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Nicotine and cotinine are used as primary biomarkers to estimate the exposure of reduced-risk products, such as e-liquids/heated tobacco products, in comparison with cigarettes. The objective of this study was to develop and validate a method per current regulatory bioanalytical guidelines to ensure accuracy, precision, ruggedness, repeatability, reproducibility, and stability of the analytes in the matrix to quantify the nicotine (NIC) and cotinine (COT) in plasma inhalation study samples.

The method of determination of biomarkers for NIC and COT in rat and mouse plasma using reversed-phase liquid chromatography–tandem mass spectrometry (LC-MS/MS) has been developed using a protein precipitation technique. The detection was carried out with selective mass transition ion-pair of m/z 163.2→132.0 for NIC, 153.1→134.1 for Nornicotine d_4, 177.1→80.0 for COT, and 180.2→80.3 for Cotinine d_3.

Spiked (4 %) calibration curve and quality control plasma samples (20 µl) containing EDTA as anticoagulant were aliquoted into an Eppendorf tube containing deuterium-labelled internal standard. The mixture was vortexed and precipitated using precipitation solvent. The precipitant was then centrifuged at 15,294 g at 10 °C for 5 minutes, and 10 µL of the supernatant was directly injected into the LC-MS/MS. All validation parameters were assessed on an Agilent 1200 series HPLC System coupled with a Gerstel MPS2 autosampler and ABSciex QTRAP 4000 LC-MS/MS using an *Xselect HSS T3* column with a total run time of 3.0 minutes.

The method was validated for a linear range of 0.627-12.693 µmol/L for NIC and 0.583 to 11.804 µmol/L for COT, with a correlation coefficient = 0.99. The intra-run and inter-run precision and accuracy were within 10 %, with overall recoveries > 90 %.

In conclusion, the validated method is simple, fast, sensitive, and requires only 20 µL rat/mouse plasma volume for the quantification of nicotine and cotinine.

ST 48

Analysis of four volatile nitroso-amines, four alkaloids, 18 polycyclic aromatic hydrocarbons in oral tobacco products based on simultaneous extraction technique and GC-MS/MS detection

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Many kinds of harmful or potentially harmful components (HPHCs) exist in oral tobacco products. Usually, each kind of chemical needs a specific analytical method, which will cause high cost and low throughput. Therefore, the objective of this study was to develop a high-throughput simultaneous extraction scheme with two phase solution (5 % NaOH water solution: CH_2Cl_2) for 18 polycyclic aromatic hydrocarbons (PAHs), four volatile nitroso-amines (VANs), nicotine and three minor alkaloids. With reference to the 18 PAHs and four VANs of ng/g level, the solid phase extraction step with a silica column was designed for further purification. After optimization, the three classes of targets were determined by two GC-MS/MS equipped with an EI and CI source respectively. The developed analytical method was systematically validated. The limits of quantification for the 18 PAHs, four VANs, three minor alkaloids and nicotine were 0.2~1.2 ng/g, 0.2~0.4 ng/g, 0.6~1.0 µg/g, 10.2 µg/g, respectively. The inter-day precisions and the intra-day precisions for all of the targets were all controlled less than 8.5 %, 11.2 %. Using CRP1, CRP2, one chewing gum, and one dissolvable product as substrates to prepare calibration lines, the ratios of the slope of the calibration line by matrix preparation to the slope of the corresponding calibration lines by solvent preparation were in the range of 89 %~111 % for all the targets. With the low, medium, high spiked levels, the measured recovery rates for all the targets were all between 83 % and 110 %. Therefore, the developed method was proven to be of good repeatability, high sensitivity and accuracy. The developed method was then used to assay 18 oral tobacco products.

ST 49

Automation of analytical procedures for mycotoxins, acrylamide, aldehydes and NDMA using Tecan Fluent 1080 automation workstation system

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Mycotoxins, acrylamide, aldehydes and N-nitrosodimethylamine (NDMA) are constituents included on the FDA "Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List" and considered as large volume analyses at many tobacco industry laboratories. However, the analytical procedures for these analytes are time-consuming and difficult, involving analytical steps, such as derivatization (aldehydes) or solid-phase extraction (SPE) clean-up (acrylamide, mycotoxins). To increase the throughput and reduce the labour-intensive manual steps in an analytical procedure, parts or the whole analytical procedure can be automated.

Here, we present the automation of analytical procedures for mycotoxins, acrylamide, aldehydes and NDMA using Tecan Fluent 1080 (Tecan Group Ltd.) automation work-station system. The system is custom-built to meet our needs. It has an orbital shaker with 96 positions, a positive pressure unit intended for use with 96-well filter-/SPE-plates, a centrifuge, an evaporator, a 96-well plate shaker and a hotel for 96-well plates. The system can, in addition to liquid handling and extraction, also perform liquid-liquid extraction, SPE clean-up/filtration, centrifugation and evaporation in 96-well format.

The methods are fully automated and the extracts, ready for instrumental analysis, are obtained in a 96-well plate format. Sample preparation of 96 samples, from weighed analytical sample to extracts ready for instrumental analysis takes 2-3 hours, depending on analytical method. The sample throughput for all the methods has increased, from 50-60 samples/day to 288 samples/day. The methods have been validated for snus, moist snuff, tobacco flour and nicotine pouches.

Altogether, the Tecan Fluent 1080 automation workstation system provides sample preparation with great accuracy and precision and high sample throughput while labour-intensive manual sample preparation steps are eliminated. Moreover, introduction of such a system also improves the physical working environment in the laboratory.

ST 50

In vitro dissolution testing of nicotine release from smokeless tobacco products

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Developing dissolution testing methods to measure the nicotine release profiles from smokeless tobacco products is valuable for product assessment and for product-to-product comparisons. Furthermore, it can allow for understanding which physical or chemical parameters have an impact on nicotine release from smokeless tobacco products.

In this work, we developed a robust dissolution method to study the *in vitro* release of nicotine from smokeless tobacco products using the U.S. Pharmacopeia flow-through cell dissolution apparatus 4 (USP-4). We further developed and validated a sensitive UPLC-PDA method for the accurate quantitation of the released nicotine into artificial saliva, which is our selected dissolution medium. We have successfully shown the applicability of this validated method by investigating the release profiles of nicotine from various commercial and CORESTA reference smokeless tobacco products [CRP 1.1 (Swedish style snus pouch), CRP 2.1 (American style loose moist snuff), CRP 4 (loose-leaf chewing tobacco) and CRP 4.1 (chopped loose-leaf chewing tobacco)].

Nicotine release profiles were analyzed by calculating the difference factor (f1) and similarity factor (f2) by adopting methodology referenced in Guidance for Industry from FDA's Center for Drug Evaluation and Research (CDER) and also by fitting the release profile curves to first order kinetic models. Nicotine release was found to be dependent on the form and cut of the smokeless tobacco products, with a slower release observed for Swedish snus and loose leaf, compared to chopped and loose smokeless tobacco. This dissolution methodology can be extended to measure and compare release of other constituents from smokeless tobacco and novel oral tobacco products, and has the potential for method standardization.

ST 51

Examining the impact of very low nicotine content (VLNC) on cigarette use patterns – a review of current evidence and suggestions for further research

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A number of scientists and regulatory bodies have called for a reduction in the nicotine content of cigarettes to a minimally addictive level in the expectation that there will be a subsequent increase in the number of smokers who quit and a reduction in the number of non-smokers (especially youth) who commence cigarette smoking. A New Work Item Proposal was initiated within the CORESTA Product Use Behaviour (PUB) Sub-Group to review publicly available study designs and methods which have been used to evaluate very low nicotine content (VLNC) cigarettes in adult users of tobacco products. The review focussed on clinical, behaviour and perception studies, categorized by whether they aimed to examine or reported (i) smoking cessation or switching to other non-combusted tobacco after switching to VLNC cigarettes (ii) changes in product exposure (e.g. cigarettes per day (CPD), biomarkers) after switching to VLNC cigarettes (iii) changes in product use (e.g. topography) after switching to VLNC cigarettes and (iv) changes in perceptions (e.g. risk, sensory) after switching to VLNC cigarettes. Each study was referenced against accepted study design criteria and assessed as to what extent their result addressed identified VLNC research gaps (e.g. as described by Hatsukami et al. 2013, Nicotine Reduction: Strategic Research Plan). Finally, the review identifies the VLNC research gaps which remain and suggests appropriate human study designs measures and methods which could help resolve these outstanding issues.

ST 52

Application of differential ion mobility spectrometry-tandem mass spectrometry for improved assay selectivity and sensitivity in the quantitation of tobacco biomarkers NNN, NNAL and 2-/3-HPMA in human urine

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Differential ion mobility spectrometry (DMS) has recently emerged as an orthogonal gas-phase ion separation technique which, when interfaced between liquid chromatography (LC) and tandem mass spectrometry (MS/MS), promises improvements in both assay selectivity and sensitivity by differentiating analyte from interference based upon physical cross-section. In the current research, we have established the potential for DMS technology to address existing LC-MS/MS assay limitations associated with the quantitation of the urinary biomarkers 2-/3-hydroxypropylmercapturic acid (HPMA), total N-nitrosonornicotine (NNN) and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).

Leveraging a SCIEX 6500+ TripleQuad with SelexION DMS in an LC-DMS-MS/MS workflow, total NNN method detection limits could be lowered ten-fold from those previously reported in the literature, to 0.20 pg/mL. This represents a relevant improvement in sensitivity since 25-30 % of smoker urine contains baseline NNN concentrations < 2.00 pg/mL. Achieving a 0.20 pg/mL detection limit required only a fivefold concentration of extract, accomplished without concomitant matrix effect or interference. Further, the additional selectivity offered by the LC-DMS-MS/MS approach suggested that previous reports of augmented NNN response in urine left at room temperature were due to a chromatographically unresolved isobaric interference, which proved separable from NNN when leveraging ion mobility.

For total NNAL, a five-fold improvement in signal-to-noise ratio (SNR) using LC-DMS-MS/MS allowed extracts to be reconstituted without concentration, resulting in an absence of ionization suppression and increased assay robustness for a 5.0 pg/mL detection limit (SNR 40:1). In the determination of 2-/3-HPMA, the sensitivity for 2-HPMA by LC-DMS-MS/MS was increased two-fold compared to LC-MS/MS, whilst an interference at the retention time of 3-HPMA could be eliminated by ion mobility, reducing complex and prolonged sample preparation and chromatographic separation.

Each of the three LC-DMS-MS/MS methods was successfully validated according to the criteria established by the U.S. FDA in the 2018 Bioanalytical Method Validation Guidance document for small molecule quantitation.

ST 53

NNN – a suitable biomarker of exposure from smoke and new nicotine delivery products?

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N-Nitrosonornicotine (NNN) is believed to play a critical role in the development of cancer in tobacco users and was therefore classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen. Hence, urinary NNN is commonly determined within the toxicological risk assessment of new nicotine delivery products (e.g. heat-not-burn, smokeless tobacco). Recent studies have shown that NNN can be formed by nitrosation of nornicotine in saliva. Our own investigations have also shown occasionally high levels of NNN in smokers, which could not be explained by their exposure. Thus, we systematically investigated the artefactual formation of NNN in urine. We performed incubation experiments with non-smoker urine samples by adding the precursors for nitrosation – nornicotine and nitrite/nitrate. We found that pH was most critical for artefactual NNN formation while varying the added amounts of precursors had a rather low impact. High concentrations of NNN (> 50 pg/mL) were formed within minutes after addition of the precursors at acidic conditions (pH = 2-5) with increasing formation over time. Even at neutral pH (pH = 6-7), NNN formation was observed albeit at much lower concentrations (2-4 pg/mL). Our results indicate that artefactual NNN formation can occur due to the presence of nornicotine in urine even though no NNN exposure from the product was expected (e.g. from e-cigarettes). Hence, we developed an LC-MS/MS method for NNN in human plasma and saliva in order to circumvent NNN analysis in urine. The validated method is characterized by very high sensitivity (LOQ: 0.5 pg/mL), broad linear range (0.5-1000 pg/mL) and high throughput. The new method is thus well suited for large biomonitoring and PK studies, and will be used in future investigations to explore the suitability of NNN as a biomarker of exposure in plasma as an alternative matrix to urine.

ST 54

Clinical biomarkers of compliance for use in potentially reduced-risk product switching studies

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Preclinical assessments and clinical studies have shown significant reductions in toxicants in tobacco heating product (THP) aerosols compared to smoke from conventional cigarettes. Further studies to assess if these toxicant reductions translate to reductions in smoking-related health risks are being carried out. As these designs tend to be ambulatory in nature with the subjects visiting the clinic at specific timepoints, the potential for non-compliance, where the subject smokes conventional cigarettes and not the assigned THP, is high.

In our current study examining health effect indicators when a smoker switches to using a THP in the U.K. (ISRCTN81075760) we employed a biomarker of compliance, the haemoglobin adduct of acrylonitrile cyano-ethyl valine (CEVal). This paper presents data from the cessation arm, where subjects have ceased all tobacco and nicotine use except for being allowed nicotine replacement therapy (NRT), and the never smoker arm over a period of 90 days.

This study was approved by a local research ethics committee and run in accordance with ICH-GCP. Subjects were of either gender (aged 23-55 years) and comprised of regular smokers who intended to quit and were provided with assistance with quitting (NRT/varenicline/counselling) or were participants who have never smoked. At the 90-day stage of this study, subjects had attended a total of four non-residential clinic visits plus a screening visit. Blood samples were taken at baseline, and days 30, 60 and 90 for the cessation arm, whereas never smokers' samples were taken at baseline and day 90. The haemoglobin adduct CEVal was measured in all blood samples and showed reductions in levels in the cessation arm between baseline and day 90. Whereas there was no change in CEVal levels in the never smokers.

This data shows that CEVal has the potential to be a long-term biomarker of compliance in potentially reduced-risk product (PRRP) switching studies.

ST 55

An open-label, parallel-group, controlled study to evaluate changes in biomarkers of cigarette smoke exposure and biomarkers of potential harm in adult smokers who completely switch to using e-vapor products for 24 weeks

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The purpose of this study was to characterize biomarkers of exposure (BoE) to select harmful or potentially harmful components (HPHCs) and biomarkers of potential harm (BoPH) in adult smokers (AS) who switched to exclusive use of e-vapor products (EVPs) for 24 weeks. Generally compliant subjects from an initial 12-week study were invited to be followed for an additional 12 weeks. The subjects enrolled (N = 150, ~50 in each group; 49 % male; 30-65 years of age; daily smokers who were not planning to quit, and smoked > 10 cigarettes per day (CPD), for > 10 years) had been randomly assigned to continue to smoke (CC) or switch to one of the EVPs in the initial 12-week study and continued using the assigned products in the 12-week follow-up. Select BoEs, BoPHs and FEV1/FVC were measured at Baseline, 12 and 24 weeks. Statistically significant differences in absolute change from Baseline (p99 %) were identified in the EVP groups. In conclusion, we demonstrate that significant reduction or complete elimination of many of the HPHCs in EVP aerosol results in significant reductions in BoE and favorable changes in BoPH after switching to EVPs for 24 weeks. The observed biomarker changes, other than nicotine, approached those reported for smoking cessation studies over a comparable time period suggesting that switching to exclusive use of the EVPs tested in this study may be less harmful than continuing smoking.

ST 56

Effects of acidic components in cigarette smoke on lipid metabolism in salivary glands of rats

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Some components in tobacco, such as acidic components, stimulate the salivary secretion in smokers. However, their effect on lipid metabolism in salivary gland tissues remains unclear. In this study, the effects of acidic components in cigarette smoke on the salivary secretion of rats were investigated. Firstly, immunohistochemical analysis was employed to confirm the rise of AQP5 (Quaporin) expression after acidic component stimulation. Secondly, based on this, lipid metabolomics in the submandibular gland of rats before and after acidic component stimulation were studied by ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) combined with multivariate data analysis. 49 representative differential metabolites were screened out by VIP and p values of lipid components, among which 19 lipids, including ceramide, diphosphatidyl glycerol, lysophosphatidyl choline, lysophosphatidyl ethanol amide, were up-regulated; while 30 lipids, including diacylglycerol, triglyceride, were down-regulated. Meanwhile, increasing the dosage of acidic components did not significantly influence the expression of lipid compounds. The above results reveal that acidic component stimulation enhances the expression of AQP5 in the submandibular gland of rats and thus increases its salivary secretion; furthermore, it affects lipid metabolism in the submandibular gland with no obvious metabolic abnormality. This study provides a technical support for evaluating the effects of tobacco components on the lipid metabolism in salivary glands.

WORKSHOP
21st Century Toxicology for Next Generation Tobacco and Nicotine Products

STW 01

The application of *in vitro* Toxicity Testing in 21 Century (TT21C) for next generation products

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The aim is to demonstrate the feasibility of TT21C *in vitro* methods as part of an overall assessment framework for next generation products (NGP).

TT21C methodologies can be used to assess harm reduction potential of NGPs using human-derived cellular systems and biological responses. The potential harm reduction potential of three different NGP products were compared to the reference cigarette (3R4F) in a series of *in vitro* assays. Either whole smoke/aerosol or phosphate buffered saline (PBS) trapped smoke/aerosol were used. The products investigated were the Kentucky reference cigarette (3R4F, 1.8 puffs/ml), a tobacco heated product (THP), a hybrid product (HYB) and a *my*blu™ e-cigarette (Tobacco Flavour 1.6 % Nicotine). The 3R4F and THP were smoked using the HCI Intense smoking regime. HYB and *my*blu™ were vaped according to CORESTA Recommended Method No. 81 (CRM 81). Four puffs/ml of PBS was used for all NGPs.

Smoke/aerosols were captured in PBS to enable the use of *in vitro* systems where direct exposure to smoke/aerosol was not possible. Chemical characterisation was conducted on whole smoke/aerosol (WHO TobReg 9) and on aerosol/smoke PBS solutions to measure nicotine and 8 carbonyls.

Regulatory accepted *in vitro* assays (NRU, Ames, IVMNT) were employed for testing whole smoke/aerosol from the different platforms. All assays indicated reduced cytotoxic and genotoxic activity of the THP compared to 3R4F. There were limited to no effects observed in each assay for HYB and *my*blu™. Test samples were also analysed in several TT21C assays to provide a wider mechanistic understanding, including cardiovascular disease (scratch wound), COPD, tumour promotion (Bhas cellular transformation assay) and cellular health (high content screening); 3D reconstituted bronchial epithelia with repeated exposure; multicellular/organ profiling (DiscoverX) and developmental toxicity. The results of all assays indicated limited to no toxicity for *my*blu™ aerosol or extracts. Based on the tests conducted the overall ranking in terms of the most active product was 3R4F > THP > HYB ≥ *my*blu™.

WORKSHOP
21st Century Toxicology for Next Generation Tobacco and Nicotine Products

STW 02

Contemporary high-content screening approaches to assess the biological impact of single compounds and complex mixtures *in vitro*

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High-content screening (HCS) is an automated image-based screening technology that allows analysing macroscopically the biological impact of single compounds or complex mixtures on cells growing in multi-well culture dishes. The technology involves fluorescence microscopy that is paired with high-throughput image acquisition and a series of algorithms and software tools for image processing and analysis. Taking advantage of fluorescent labeling using different dyes, multiplexing to simultaneously measure various cellular parameters can be achieved. To run a comprehensive analysis of a cytotoxic impact, HCS, through a battery of different assays, usually analyses structural or quantitative changes in cellular compartments or individual biomolecules such as nuclear size, DNA structure, mitochondrial mass, mitochondrial membrane potential, cytochrome C release and cellular membrane permeability. In addition, changes in subcellular localization of biomolecules upon cytotoxic impacts can be monitored. Resulting from the development of confocal versions of HCS readers, assays previously established for two-dimensional (2D) cellular models may also being applied for HCS analysis of reconstituted human three-dimensional (3D) organotypic cell models. These models more closely mimic the physiology of the corresponding tissue counterpart in humans and therefore provide enhanced translatability of results. HCS may be combined with omics technologies to expand the assessment to the systems level and allow to investigate more readily the underlying mechanisms of toxicity.

This presentation intends to provide a general overview on HCS and how it can be used in *in vitro* respiratory toxicology of next generation tobacco and nicotine products, illustrated by examples of testing aerosol fractions and individual constituents measured in 2D cell culture, as well as applications of HCS to 3D organotypic cell models.

WORKSHOP
21st Century Toxicology for Next Generation Tobacco and Nicotine Products

STW 03

Organotypic *in vitro* models for assessment of biological impact

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Historically, the assessment of biological effects on humans was predominately based on animal testing, examining whole organism responses to toxicants and chemicals. However, it has been demonstrated that some toxicities observed in animals are less likely to be relevant in human toxicity. An expanding area of research aims at improving toxicological assessment by using human-relevant *in vitro* systems for accurate and cost-effective prediction of biological impact on humans.

Among the *in vitro* models, interest in the use of three-dimensional (3D) organotypic cell culture systems has grown significantly in recent years. Most of the published data is derived from experiments performed using two-dimensional (2D) conditions. However, these conventional 2D cultures, grown under simplified and unrealistic conditions, do not fully reflect the characteristics of genuine tissues. 3D cell culture environments enables for greater cell-to-cell contact, allowing cells to differentiate into more complex structures. Therefore, 3D cell culture systems have the potential to better mimic morphological, physiological and functional responses of complex tissues than cells cultured as a submerged monolayer. Generally, it is also recognized that 3D cell cultures have greater stability and a longer lifecycle.

The intention of this review is to provide a general overview of 3D organotypic cell culture systems and examples of application with a focus on respiratory and oral tissues for assessing the biological impact of next generation tobacco and nicotine products.

WORKSHOP
21st Century Toxicology for Next Generation Tobacco and Nicotine Products

STW 04

Multi-organ-on-a-chip platforms to assess the biological impact of toxicants as well as PBPK properties *in vitro*

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The merging of three-dimensional (3D) organotypic *in vitro* models with multi-organ-on-a-chip (MOC) technology has taken *in vitro* assessment of chemicals to an unprecedented level. By connecting multiple 3D organotypic models, MOCs enable the study of organ cross-talk, thereby placing the evaluation of compound safety and efficacy much closer to human exposure conditions. In particular MOCs, utilizing human airway epithelial air-liquid interface (ALI) culture models, could advance the toxicological assessment of aerosols *in vitro*. In contrast to models kept in static culture conditions, the microfluidics connecting the organ models in MOCs mimic the dynamic conditions in the blood circulation. Therefore physiologically-based pharmacokinetic (PBPK) modelling, based on MOC data, can better simulate exposure-responses *in vivo*. Furthermore, the presence of metabolically active 3D organotypic liver spheroids in lung-liver MOCs can provide valuable information about biotransformation of aerosol constituents and their potential harm.

This presentation intends to provide a general overview of MOC platforms and their applicability to next generation tobacco and nicotine product assessment. Additionally, a MOC recently developed by us will be highlighted as an example for a lung-liver chip model where the metabolizing capacity of liver spheroids present in the chip can modulate the biological impact of a toxicant.

WORKSHOP
Population Modelling

STW 05

Introduction to statistical population modeling of tobacco products and their impact on health

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This presentation will introduce the basic concepts of population modeling in a non-technical, conceptual manner. The utility of modeling will be described, emphasizing the importance of such modeling in the context of FDA's population-impact standards for Pre-Market Tobacco Applications and Modified Risk Tobacco Product Applications, which require considering and balancing or netting-out impacts on users and non-users of existing tobacco products, in a pre-market setting. Several examples of modeling will be briefly presented to illustrate their common focus on transitions among states of tobacco product use (including non-use). Other common elements, such as the extensive use of sensitivity testing and tipping-point analyses will be discussed. Following the description of elements that are common to all modeling approaches, the presentation will then turn to how models may differ with regard to such matters as their assumptions, sources of inputs, nature of their outputs, and their underlying mechanics. In addition to introducing modeling in general, these concepts lay the foundation for the presentations that will follow, which will present specific models and applications.

WORKSHOP
Population Modelling

STW 06

Statistical modeling in support of a population health perspective

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Modeling is best suited for estimating trends and likelihoods. Among the lessons learned with the recent application of statistical, or population modeling in support of a tobacco product regulatory application – in this case, a modified-risk tobacco product application for Camel Snus – was that it is ultimately the direction and magnitude of modeling projections that substantiate a population health perspective. This presentation identifies and discusses a number of modeling features that serve to provide confidence in modeling projections, including (1) the use of a validated model, (2) the inclusion of a wide range of unintended harmful changes in tobacco use that may occur with the product's use, (3) the reliance on empirically derived model inputs, and (4) extensive sensitivity testing of model inputs. The last of these features – that is, extensive sensitivity testing of inputs used for the modeling – provides an opportunity to demonstrate consistency in direction of the estimated population health effects that are of sufficient magnitude to provide confidence in the modeling projections. The presentation concludes with an example of how to convey modeling projections in a manner that best addresses regulatory application requirements.

WORKSHOP
Population Modelling

STW 07

Refining the modeling assumptions to understand the population health impact after introducing a reduced-risk product into a market

BAKER G.

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The Population Health Impact Model (PHIM) was developed prior to marketing our reduced risk product (RRP)*, to help assess and understand the potential impact of marketing such products on population health within specific countries.

The PHIM uses publicly available data on smoking prevalence and smoking-related disease-specific mortality, together with estimates of the exposure of the RRP relative to a cigarette. The original modeling used a set of assumptions to define possible scenarios that could happen after the introduction of an RRP into a market, in order to assess the possible prevalence of cigarette and RRP use patterns (including combine product use). It allowed us to compare various scenarios with and without the introduction of the RRP during the same time period (1990-2010) to predict the change in smoking attributable deaths (SAD) and life years saved from the major smoking-related diseases (lung cancer, ischemic heart disease, stroke, and COPD).

In Japan, our heated tobacco product has been marketed nationally since 2016 and there are multiple products that have entered the market since. Initially we modeled the RRP uptake base-case scenario where ten years after marketing the RRP, 55% of the smokers had switched to the RRP (heated tobacco products such as IQOS). The results demonstrate that within 20 years Japan could see 0.96 million life years saved (75,820 SAD reduction). Now that the product is in the market, we are able to use actual population-level data to replace some of the estimates of product uptake, and redefine scenarios based on what is known.

It is important that we not only assess the impact of tobacco harm reduction pre-market, but that we are actively collecting and using data from the real world to better understand the actual population impacts after actually introducing RRP into a market as a replacement to cigarettes.

* ”RRP” is the term PMI uses 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.

WORKSHOP
Population Modelling

STW 08

Modeling the impact of a tobacco product with a modified risk claim on population health: the role of sensitivity analyses

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Computational models can be used to assess the population health impact of a modified risk claim on a tobacco product, while taking into account tobacco users and non-tobacco users. We present here a case study of a smokeless tobacco (ST) product with a modified risk claim. Using best modeling practices, we developed and validated a Cohort model using the U.S. population. There are two key components of the model: (1) risk of exclusive ST use compared to cigarette smoking; (2) changes in product use patterns due to the introduction of the claim. The primary outcome is the number of premature deaths prevented, calculated by comparing a Base Case (cigarettes and ST) and a Modified Case (cigarettes and ST with a claim). An epidemiological study was conducted to determine the mortality risk of ST use relative to cigarettes (Component 1). Published nationally representative data were used to estimate transition probabilities in the Base Case (Component 2). In the Modified Case, the Base Case transition probabilities were adjusted using ST product-specific behavioral data from a claims comprehension and intentions study. The model predicted a benefit of ~93,000 premature deaths prevented over the 60 years following authorization of the claim. Since population models are not intended to predict outcomes with numerical precision, it is important to conduct extensive risk versus benefit sensitivity analyses. We examined numerous "what if" scenarios offering insights into the dynamics of the model projection. For example, concurrently varying key transitions such as ST initiation and switching (cigarette smokers switching completely to ST use) indicated that the initiation rate (risk) would have to move to relatively high levels to outweigh the projected benefit from switching, holding other modified rates constant. These types of sensitivity analyses can play an important role in population modeling when examining the robustness of the projected outcome.

SUSTAINABILITY FORUM

In 2015, the United Nations published 17 sustainable development goals and their corresponding targets to be achieved by 2030. Like other sectors, the tobacco and alternative product sectors are likely to impact most of the goals. Consequently, it is important to understand and measure these impacts for organisations aiming to enhance their positive impacts, and reduce the others. In 2018, CORESTA decided to support a project with the objective to identify available scientific tools for performance assessment from which a framework could be elaborated. 16 sectorial gaps were identified and each translated into proposals for guideline and method developments. In 2019, the CORESTA Scientific Commission decided to arrange a panel discussion during the SSPT Conference in Hamburg to exchange on topics associated with these gaps.

The first topic discussed concerns the risk assessment of tobacco and derived products and their positioning against a risk continuum. Dr Christopher Russell, psychologist and Deputy Director of the Centre for Substance Use Research (CSUR), Glasgow, Scotland, presents one of his most recent studies on perception and behavioural research on electronic cigarette users. Dr Russel's presentation is followed by an industry perspective delivered by Joe Thompson, Director of Group Science and Regulatory Affairs at Imperial Brands.

The second topic discussed focusses on the carbon footprint and other GHG emissions along the tobacco and derived product supply chain. Following a general presentation by Stéphane Colard, Secretary General of CORESTA, on the context and global issues, Diane Raverdy-Lambert, SWM Chief Scientist & Director Regulatory Affairs, provides specific and detailed insights on approaches, initiatives and perspectives from a paper manufacturer's point of view.

2019 CORESTA JOINT STUDY GROUPS CONFERENCE

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

Methods to evaluate the heat resistance of heat-not-burn cigarette paper

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Heat-not-burn cigarettes are becoming more and more popular as a new kind of tobacco product. Compared to conventional cigarettes, the characteristic of heat-not-burn cigarettes is that to release the flavour the tobacco is heated not burnt. The tobacco burning temperature of a conventional cigarette is up to 900 °C, consequently there are harmful and potentially harmful constituents (HPHCs) released from the tobacco and other materials in the cigarettes due to burning, pyrolyzation, and thermosynthesis reactions. However, the heating temperature of heat-not-burn cigarettes is below 500 °C and as a result there are less HPHCs and the tobacco extract is released from the heating zone rapidly. This allows the customers to have the same sensations as when smoking conventional cigarettes, but with less harmful materials.

There is much research on aerosol constituents and toxicology of heat-not-burn cigarettes, but little reference related to heat-not-burn cigarette paper. This study researched evaluation methods related to heat resistance of heat-not-burn cigarette paper. It was shown that observing the colour of the heated cigarette paper can give us a direct result. The oxygen index can give us data more directly, but this method was not designed for cigarette paper and there can be large errors. Thermogravimetric analysis was more accurate than other methods. It was shown that the heat resistance of heat-not-burn cigarette paper by these methods could be evaluated synthetically.

STPOST 02

Electro-thermal simulation analysis of heaters in tobacco heating devices

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Heat-not-burn tobacco products have gradually become an important development direction and a key research topic of tobacco products due to their remarkable advantages in reducing the release of harmful components. As a heat source for cigarette heating, the heater is one of the most important parts of tobacco heating devices. In order to improve the design efficiency of tobacco heating devices, an electro-thermal simulation model for the prediction of temperature distribution on heaters of tobacco heating devices was established. The temperature distribution on two kinds of heaters at different voltages was determined by simulation software. The simulation results were verified by the temperature distribution measured with an infrared temperature meter. The results showed that: (1) the simulated temperature distribution on the heater well agreed with the experimental result, and there was a good correlation in the longitudinal and transverse temperature distribution trends between them; (2) the higher temperature region on the heater was near its pointed end, and the temperature difference between the front and back surfaces of the heater was small; (3) as a result of the positive feedback mechanism of temperature to the resistivity of conductive loops, the maximum temperature difference on the whole heater was higher than 250 °C. Electro-thermal simulation is a reliable method for predicting the temperature distribution on heaters of tobacco heating devices, and thus provides a reference for the design optimization of the heaters.

STPOST 03

Measurement of temperature regulation performance of the JUUL nicotine salt pod system

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Combustible cigarettes operate at temperatures that can exceed 1000 °C, consequently emitting over 4000 degradation products and HPHCs (harmful and potentially harmful constituents). By contrast, the JUUL Nicotine Salt Pod System (NSPS) is a pre-filled (closed), non-modifiable, vapor-based electronic nicotine delivery system (ENDS) featuring automated temperature regulation. The system is designed to minimize degradation products from aerosolization, including HPHCs.

The purpose of this study was to investigate NSPS temperature control performance across a range of use and operating conditions through complementary measurement approaches.

Actual atomizer wick-and-coil temperatures were measured simultaneously by infrared (IR) thermography, electronic temperature control mechanisms in the device, and by a sensor buried inside the fluid-conducting wick. Pods with two different wick materials were studied.

Mean atomizer temperatures were consistently below 300 °C independently of vapor flow rate and duration. IR thermography images showed the crucial maximum surface temperatures both during vaporization and in dry wick conditions. Real-time coil electronic impedance, used by internal device software to infer average coil temperature, corroborated IR results.

NSPS temperature control mechanisms were tested under multiple use and design conditions showing mean and peak operating temperatures below 300 °C. These findings corroborated results from computational simulation studies reported separately.

STPOST 05

Computational simulation of aerosol generation and temperature regulation performance of nicotine salt pod system

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The JUUL Nicotine Salt Pod System (NSPS) is a pre-filled (closed), non-modifiable, vapor-based electronic nicotine delivery system (ENDS) featuring automated temperature regulation. The system is designed to minimize the generation of degradation products, including harmful and potentially harmful constituents (HPHCs).

The purpose of this study was to investigate the performance of the NSPS using multiphysics simulation. Performance criteria, such as energy efficiency and vapor generation, were simulated as a function of heater temperature, wick material, wick saturation, puff volume, and flow rate.

Simulations were performed using a computational fluid dynamics (CFD) EXN/Aero. EXN/Aero solved mass, momentum, and energy conservation equations for the fluid, solid and porous media that comprise the NSPS. Simulations also included the electro-static fields and current flow in the heating element and liquid vaporization processes. The computational domain was discretized using finite control volume approaches with 2^{nd} order accurate temporal and upwind-biased spatial approximations. EXN/Aero used multigrid acceleration and GPU computer architectures for fast solution on large computational meshes.

Simulation results provided both overall performance measures and detailed fluid flow and temperature distributions below 300 °C. Detailed temperature fields of heater, wick, and air/vapour mixture, as well as vapour mass fraction profiles and energy consumption were simulated for a range of operating conditions.

CFD-based multiphysics models are reported which can, for the first time, extend conductive and convective heat transfer mechanisms into quantitative descriptions of vapor generation and related thermodynamic parameters, simulating product performance under a range of operating conditions.

STPOST 06

Correlation between heating temperature of the JUUL e-cigarette and carbonyl formation

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Electronic cigarettes (EC) have the potential to pose reduced risk to the consumer compared to combustion products, due to the absence of toxic byproducts of combustion. These products function by heating and vaporizing e-liquid at lower temperatures. One concern related to heating temperature in EC is the elevated production and subsequent exposure to toxic carbonyl compounds (formaldehyde, acetaldehyde, acrolein, etc.), particularly in devices that do not directly control coil temperature. By contrast, the JUUL device is a closed system with temperature regulation designed to maintain temperature consistency and minimize the generation of degradation products.

The purpose of this study is to correlate heating coil temperature to the concentration of carbonyl compounds present in the aerosol delivered by a JUUL device.

The methods used were as follows:

1. Prototype JUUL devices of different coil temperature settings are created by alteration of the device firmware.
2. Total particulate mass (TPM) as a function of coil set temperature are determined under fixed puffing conditions.
3. E-liquid aerosol composition, total nicotine, propylene glycol, and glycerol collected during TPM measurements are quantified by gas chromatography.
4. Aerosol samples are collected in impingers with DNPH solution and analyzed for carbonyls via high performance liquid chromatography-mass spectroscopy.
5. Atomizer temperatures are measured simultaneously by infrared (IR) thermography and real-time electrical resistance of the heating coil wire.

TPM, carbonyl analysis, and IR thermography were executed using heating coil temperature set points of 237 °C to 417 °C centigrade. TPM and carbonyls increase with the coil temperature. IR thermography measurements corroborate with heating coil electrical resistance measurements.

These findings suggest that the carbonyl yields from JUUL under nominal operating coil temperatures are significantly below levels in combustion cigarettes and at the low end of published data on e-cigarettes. Higher temperatures can produce more carbonyls, suggesting the importance of temperature control mechanisms within EC hardware.

STPOST 07

HPHC analysis of seven flavors of a temperature-regulated nicotine salt-based pod system

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The JUUL Nicotine Salt Pod System (NSPS) has no user modifiable settings and is temperature regulated to minimize the generation of combustion related byproducts.

Aerosol generated from NSPS pods using a cotton wicking material was evaluated for harmful and potentially harmful constituents (HPHCs).

Seven flavors (tobacco, mint/menthol, and fruit flavors; 9 mg/mL nicotine) were analyzed for HPHCs listed in the U.S. FDA premarket tobacco application (PMTA) draft guidance document for ENDS. Testing was conducted by an accredited ISO 17025 laboratory using validated methods. Machine topography was 70 mL volume, 3 second puff duration, and 30 second inter-puff interval. Each analytical result was generated using a unique pod with 10 replicates collected for each assay. A panel of 22 analytes from six categories of HPHCs was assessed: tobacco specific nitrosamines (TSNAs), polyaromatic amines (PAAs), polyaromatic hydrocarbons (PAHs), carbonyls, volatile organic compounds (VOCs), and metals. Comparator reference combustible cigarettes (3R4F) were also evaluated.

HPHCs were reduced by 99 % in NSPS aerosol vs. mainstream smoke of the 3R4F comparator cigarette. The majority (95 %) of NSPS aerosol analytes were below the level of quantification. Notably, VOCs (acrylonitrile, benzene, 1,3-butadiene, isoprene, and toluene) and select carbonyls (diacetyl, acetyl propionyl, and crotonaldehyde) were uniformly below the level of detection in the aerosol generated by all seven flavors.

Consistent with previous testing, NSPS pods using a cotton wicking material demonstrated significant reductions in HPHCs on a puff-for-puff basis compared to reference combustible cigarettes.

STPOST 08

Puff-by-puff analysis of carbon monoxide yield for THP systems

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Tobacco heating products (THPs) generate carbon monoxide (CO) although not combusting tobacco. The levels of CO formed are much lower than for traditional products and formation process is less well understood.

A novel detection system for CO is explored that gives simple to understand information on CO deliveries on a puff by puff basis. The suitability of such a detection system can only be demonstrated through practical experimentation and correlation with more familiar detection systems.

A detection system based on an electrochemical cell is constructed and each puff CO level is logged for two different THPs (@two different devices). The applied puffing regime is based upon Health Canada Intense (HCI) used for conventional cigarettes. The validity of the data is derived through correlation with data from a more traditional nondispersive infrared detector (NDIR) system that determines CO concentrations in an aggregated manner. However, the NDIR system cannot resolve CO in aerosol puff by puff, whilst the novel detection system can.

The system devised is shown to have a repeatability of 10 ppm and correlates well with the NDIR system when THP gas mixtures are deployed. Under the given test regime, the two THPs (@two different devices) are shown to deliver CO concentrations between 300 ppm and 700 ppm per puff. One product (THP1) shows a near uniform CO delivery throughout the puffing session, whilst the second system (THP2) shows an increasing pattern of delivery of CO, nearly doubling per puff from first to last puff.

The CO detection system proposed shows considerable merit as both a screening tool in new product development and as an analytical tool for characterising the CO delivery behaviour for this category of heat-not-burn (HNB) devices.

STPOST 09

Considerations when determining yields from THP systems

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Tobacco heating products (THPs) present particular challenges when performing "traditional" tar, nicotine and carbon monoxide (TNC) analysis and these must be considered when developing future standards for test and analysis.

A challenging aspect is the complete capture of all aerosol formed. It is proposed that significant aerosol may be lost as dead volume increases which gives rise to concerns regarding exposure or capture studies. This paper shows the relationship between dead volume as well as capture efficiency and measures that can be taken to maximize total aerosol capture.

Through manipulation of the length and topography of the path to the capture pad, the effect on system capture efficiency is demonstrated via simple mass balance experiments for a variety of THP devices and smoking topography.

The relationship between capture efficiency and dead volume is shown not to be a simple one with the majority of aerosol (40 %) being lost from a capture system within the first 10 mm of the butt end of the THP. Reducing the dead volume path length to 4 mm brings the majority of the aerosol onto the capture pad.

Overcoming the condensation of aerosol before capture is one of practical engineering as the study shows. The implications for exposure (*in vivo* or *in vitro*) are less clear and should give some pause for thought in experimental design.

STPOST 10

A study on production and delivery of volatile aroma substances from heat-not-burn products' tobacco material to emissions

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Volatile substances in emissions from heat-not-burn (HnB) products contribute to sensory quality. These substances including aldehydes, ketones, alcohols, and esters. To better understand the production and delivery of volatile substances, volatile compounds in HnB products' tobacco materials, emissions and puffed filter tips were identified and determined by a semi-quantitative method.

Volatile substances in three different HnB cigarette brands (tobacco and menthol flavour) were extracted with an extracting solution containing a known amount of naphthalene that served as an internal standard in the subsequent quantification by GC-MS. Emissions were generated by linear puffing/smoking machine, and the trapped volatile substances on the filter pad were extracted and determined. The puffed cigarettes and filter tips were collected and leftover volatile substances were determined. Puff-by-puff analysis was carried out with previous established methods to investigate the dynamic release of volatile substances.

30~46 different volatile compounds were detected in three cigarette brands, and the semi-quantitative determination showed a content range of 1.6 to 9.2 mg/Cig. Fewer compound types (14~30) were detected in emissions and a much smaller amount was determined (0.1~1.4 mg/Cig). The heated tobacco materials and puffed filter tips were found to contain more volatile compounds than that of unheated cigarettes and emissions, and the heated tobacco materials contained the most significant number of volatile compound types (33~52). The majority of volatile compounds produced upon heating were trapped by the filter system when puffing. Puff-by-puff analysis showed that some cigarettes had better consistency with respect to volatile substance release than others.

More volatile compounds were produced during heating, however the current filter systems trap a significant number and amount. An optimization of filter systems could be done to elevate the quality of aerosol from HnB products in terms of sensory experiences. Volatile compounds showed different delivery efficiency from material to emission upon heating depending on compound types.

STPOST 11

Simultaneous determination analysis of glycerol, propylene glycol, nicotine, menthol and water by discharge ionisation detector on gas chromatography

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CORESTA developed a recommended method (CRM 84) for determination of Glycerin (G), Propylene Glycol (PG), nicotine and water in the aerosol of e-cigarettes by gas chromatography (GC) with flame ionisation detector (FID) and thermal conductivity detector (TCD). In addition, several analytical methods for determination of G, PG, nicotine, menthol and water in the aerosol of both e-cigarettes and tobacco vapour products were reported at CORESTA. In the methods, these five components were determined by two detectors such as FID for G, PG, nicotine, menthol and TCD for water, respectively. Furthermore, there were several reports about simultaneous determination analysis of those components by TCD. However, the sensitivity of TCD is less than one hundredth compared with that of FID. Thus, it was difficult to determine those five components in common aerosol samples by TCD. Therefore, a new type of detector, the sensitivity of which is comparable with that of FID, is required to determine these five components simultaneously. Discharge ionisation detector (DID) was found to be an optimal detector for analysing those components.

The final aim is to develop an analytical method for simultaneously determining G, PG, nicotine, menthol and water in the aerosol of both e-cigarettes and tobacco vapour products by DID. In this study, the applicability of DID was investigated by using a standard mixture of these five components. As a result, it was confirmed that peaks of these five components in the standard mixture were separated within ten minutes. In addition, it was found that the limit of detection and the limit of quantification were sufficient to determine those five components in this study.

STPOST 12

Determination of nicotine in gas vapour phase in phosphate buffered saline by UHPLC-MS/MS

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A liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method was developed to quantify nicotine in gas vapour phase (GVP) collected in calcium and magnesium free phosphate buffered saline (CMF-PBS). In this method the aerosol was trapped in impingers containing ice cold CMF-PBS. An aliquot of the solution was then diluted with a solution containing pyridine as internal standard. The method has been shown to be accurate, precise and linear with a high coefficient of determination ($R^2 > 0.9993$).

The Limits of Detection (LOD) as well as of Quantification (LOQ) are 0.08 µg/mL. The initial objective was to achieve a LOQ of 0.05 µg/mL, however this was not feasible, as the variability of the nicotine levels in the blanks (with internal standard) was too high. The working range of the method was from 0.08 up to 50 µg/mL. The inter-run precision (n = 15) is ≤ 10 %; the inter run accuracy (n = 15) for is < ±10 %. The intra run-precision (n = 5) is ≤ 20 %; the intra run accuracy (n = 5) is < ±20 %.

Sensitivity of the method was assessed by dilution experiments; i.e. samples were ten-fold diluted. Five replicates from two samples were analysed for sensitivity. The determined average concentrations of the diluted samples were within the acceptance criteria of < ±15 % of the theoretical concentration.

A stability assessment was performed for both, the refrigerated (2-8 °C) and the frozen (20 °C) samples. QC samples (n = 5) were analysed at time point '0' (study begin) and at the end of the study (after 18 days). All analysed samples, i.e. under each storage condition as well as at each point in time, passed the stability criteria of < ±15 % for accuracy and the %RSD.

This method was developed in order to support the characterisation of GVP samples for *in-vitro* testing. To ensure proper air-liquid interface exposure during sampling, additional solvent traps were used during sample generation.

STPOST 13

Analysis of propylene oxide and glycidol in tobacco smoke and e-cigarette vapour

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We recently performed a clinical study with e-cigarette users (vapers) and smokers. The stable-isotope labeled e-cigarette constituents propylene glycol, glycerol, and nicotine in the test e-cigarette were used in order to investigate the e-cigarette specific uptake of these compounds as well as their degradation products, which may occur during vaping. As a positive control, a smoking group was included, which consumed spiked cigarettes with the same stable-isotope labeled constituents in a similar way to the vaping group. We observed elevated levels of the mercapturic acids (MA) of the epoxides ethylene oxide (HEMA), propylene oxide (2-HPMA), and glycidol (DHPMA) in urine of smokers. In contrast, only the MA corresponding to glycidol was found in the vapers at lower concentrations compared to smokers. The metabolites for ethylene oxide and propylene oxide were not detectable in vapers. In conclusion, a specific uptake of glycidol was observed after vaping the test product. In order to be able to correlate these biomarker data with smoke and aerosol concentrations, we developed a GC-MS method for the quantification of epoxides after liquid trapping. This poster presentation summarizes the results of the epoxide analyses in smoke and vapour, linking the smoke/aerosol data to the obtained metabolite levels in urine.

STPOST 14

Determination of untargeted compounds in e-liquids using high-performance liquid chromatography-time of flight-mass spectrometry (LC-ToF-MS)

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Techniques like headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GCMS) can identify a wide range of volatile and semi-volatile compounds in e-liquids associated with flavor active compounds of different classes. The objective of this study was to determine what additional, or characteristic, compounds of the e-liquid can be identified using LC-ToF-MS.

The ToF-MS was operated with electrospray and atmospheric pressure chemical ionization (ESI; APCI), both in positive and negative modes. Operating parameters included an acquisition mass range of 70-750 m/z, typical of most small molecules. Samples were prepared using 100 mg of e-liquid diluted with 25 mL of 5 mM ammonium acetate and isotopically labelled compounds acting as internal standards. This ratio provided a balance between sensitivity for detection of trace-level analytes, and the risk of detector or column saturation from compounds in greater abundance. Five replicates of four e-liquids were evaluated.

Preliminary evaluation of positive mode ESI shows approximately 200 features, or distinct compounds, between masses 70-300 da. Many additional features, including hundreds of features above 300 m/z, typical of phthalates or other common plastic contaminates, were detected. These are suspected to have originated with sample preparation materials. Negative mode ESI shows > 400 distinct features between 70-400 m/z. Many of these are isomeric compounds corresponding to chemical formulas typical of modified sugars such as sugar alcohols and acids, as well as, C6-C18 fatty acids. Positive mode APCI shows approximately 60 distinct features between 70-500 m/z while negative mode shows approximately 150, over the same range using a lower corona voltage condition. Few features above 500 m/z were detected.

LC-ToF-MS can identify different compounds present in e-liquids compared to HS-SPME-GCMS, based on the mode of operation of the ToF-MS. The combination of both techniques is useful to determine characteristic profiles of the e-liquids. This can be a valuable tool to identify changes in the characteristics of an e-liquid or product.

STPOST 15

Real-time light scattering instrument for measurement of e-vapor size and concentration with no dilution

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At the 2018 Tobacco Science and Research Conference (TSRC), Holve D. et al presented results of a new light scattering instrument (Orion-A) for application to e-cigarettes without need for dilution. Orion-A provides automatic puff flow measurements based on measured pressure drop, display of complete size distribution, and size uncertainty estimation (typically 3-4 %) based on three independent scattering ratio measurements. Results have shown typical mass mean aerodynamic particle sizes in the range of 300 nm for liquid e-cigarettes (LECs), and 250 nm for heated and combustion cigarettes. Puff mass measurements are generally uniform in mass delivery for LECs over a series of puffs, while combustion and heated cigarette concentrations generally increase with puff sequence. A recent study (Sosnowski T.R. et al, 2018) highlights the importance of EC aerosol size distribution for respiratory regional deposition.

Current work focuses on extension of the Orion size range to include supermicron particle sizes, and effects of variable dilution and transport times. Orion-A uses angular MIE scattering DC signals to measure EC and combustion cigarette submicron aerosols. There is further interest in measuring the quantity (if any) of supermicron particles which would have an important impact on vapor transport. In addition to Orion-A, another measurement technique is under development, known as Variance Ratio (Orion-V), to measure larger particles in the size range of 1-100 microns, using the same optical instrument system as Orion-A. This method measures the RMS and DC scattering signals, which can be interpreted to give size and concentration. Results for nominal 5 micron nebulizer aerosols and larger sprays confirm the variance method measurement capability. Initial measurements of ECs show negligible contributions of supermicron vapors and that transport over variable distances have minimal impact on size and concentrations.

STPOST 18

Comparison of a flame ionization detector (GC/FID) to a nitrogen-phosphorus detector (GC/NPD) for gas chromatographic determination of nicotine in conventional and ultra-low nicotine tobacco blends

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The established CORESTA method to determine nicotine in tobacco uses a gas chromatograph coupled with a flame ionization detector (GC/FID) (CRM 62) that uses mass spectrometry (GC/MS) (CRM 87). These methods were developed to evaluate conventional tobacco. Currently, there are no standardized methods available to quantitate the nicotine level in both conventional and in ultra-low nicotine tobacco. Other selective instruments such as a gas chromatograph coupled with a nitrogen-phosphorus detector (GC/NPD), high performance liquid chromatography coupled with an ultraviolet detector (HPLC/UV) and a gas chromatography–mass spectrometry (GC-MS) have been reported for conventional tobacco but have not been widely reported for the evaluation of ultra-low nicotine tobacco.

In the study we will compare the nicotine results between a GC/FID and GC/NPD system using an ultra-low nicotine tobacco blend (NIST SRM 3222), CRP1.1, conventional mid-level tobacco leaf, and a conventional tobacco blend (1R6F).

We will present instrument precision, method precision, accuracy and LOD & LOQ of both methods. We will also present the advantages and disadvantages of the two detectors.

STPOST 19

On-line puff resolved analysis of cigarette smoke for product profiling using soft-photoionisation mass spectrometry

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Photo ionisation-time of flight mass spectrometry (PI-TOFMS) has been established for the on-line analysis of complex gas mixtures. Therefore, it is a suitable tool for puff resolved chemical fingerprint screening of cigarette smoke. This capability can be used for quality control, to determine product variability during manufacturing, or even to identify counterfeit products.

In this study a deuterium lamp is used as the light source for single photon ionisation (SPI). The respective wavelengths/ionisation energy, i.e. the energy required to ionise the organic target compounds, is roughly in a range of 7 to 11 eV (177 nm to 112 nm). As most matrix gases such as oxygen, nitrogen, carbon dioxide and especially water vapour have higher ionisation energies of 12 eV and more, they will be suppressed efficiently by the applied technology as such.

Various commercially available cigarette brands and types are included in the study to prove the measurement principle. Multiple cigarettes of each brand/type were smoked as replicates applying the ISO 3308 smoking protocol. The organic compound fingerprint spectra of each single puff of the replicates was analysed and statistically evaluated. Samples with the same product properties are clustered accordingly, whilst product variability becomes statistically visible. Defective products as well as counterfeit cigarettes can be identified by the applied technology and statistical model, in case significant differences of the chemical fingerprint at puff level were observed.

By increasing the number of analysed products, the statistical model can be continuously improved, so that the quality of the classification of each new sample with respect to product variability, outliers or even counterfeit cigarettes is enhanced.

STPOST 20

On-line puff resolved analysis of cigarette smoke, e-cigarette vapour and vapour of tobacco heating products

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Photo ionisation-time of flight mass spectrometry (PI-TOFMS) has been established for on-line analysis of complex gas mixtures. Cigarette smoke, e-cigarette vapour and the vapour of tobacco heating products provide good examples for such complex gas mixtures. Many toxicants, such as butadiene, acetaldehyde, naphthalene, phenol or polycyclic aromatic hydrocarbons (PAH), can be detected with single puff-resolution in the smoke or vapour of products.

Vacuum PI-MS can be differentiated into SPI (single-photon-ionisation), ionising a wide range of organic molecules, and REMPI (resonance-enhanced-multi-photon-ionisation), focusing primarily on aromatic structures. Especially the more sophisticated complementary use of SPI and REMPI can provide access to profound information allowing mechanistic understanding of processes.

Soft photoionisation can be applied in various research fields and applications dealing with complex gas mixtures that need to be observed in real time. The high time resolution especially enables the investigation of fast and dynamic processes. Considering the variety of cigarettes and innovative aerosol products, such as e-cigarettes and tobacco heating products (THP), photoionisation enables a puff resolved investigation of released compounds from nicotine to harmful or potentially harmful compounds (HPHCs). In addition to time resolved detection, spatial occurrence of smoke constituents in the cigarette can be investigated. Based upon spatiotemporal data, chemical heat maps can be generated, which allow the understanding of the formation and degradation processes even inside a cigarette.

Other products, e.g. tetrahydrocannabinol (THC) containing smoking products such as joints, have been investigated on a puff-by-puff resolved basis.

In summary, the release of the desired active compounds (e.g. nicotine or Δ9-THC) and undesirable HPHCs is dependent on a broad set of parameters, such as puff regime, environmental conditions and physical product design features. The puff-by-puff resolved release profiles derived by on-line photo-ionisation MS enables a reliable understanding of mechanistic processes and reaction pathways.

STPOST 21

Determination of nitrogen oxides in the vapour phase of cigarette smoke: comparison of two methods

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At present, the ISO TC 126 is tasked with working on a new ISO Standard ISO/NP 23924 "Determination of nitrogen oxides in the vapour phase of cigarette smoke under intense smoking conditions -- Chemiluminescence method".

In the ISO document/NP 23924 it is defined that for the determination of nitrogen oxides ($NO+NO_x$; as NO_x) the gas phase of 170 puffs, 55 ml each (intense regime), is collected in a gas bag. After completion of the smoking run the amount of NO_x is determined in the gas bag by using a chemiluminescence detector.

NO_x is known to be unstable over time, e.g. after sampling, therefore it is important to investigate the stability of the NO_x in the gas bag during and after sampling.

In the tobacco industry a different method to ISO/NP 23924 method for the determination of NO_x is in use: the NO_x concentration in the vapour phase is determined on-line by a chemiluminescence detector, so that for each puff and the entire cigarette a value for NO_x can be calculated. Obviously, this detection principle would exclude the issue of aging the vapour phase with the potential impact on the NO_x concentration.

In our study we compared the 'gas bag' against the 'on-line' method for the determination of NO_x in the vapour phase of cigarette smoke. On a smoking machine type RM 200 A2 equipped with an online NO_x analyser both methods are performed in parallel. While one part of the vapour phase is used for the online measurement, the remaining amount is collected as per ISO/NP 23924 in a prepared collection bag for determination of NO_x after completion of the smoking run. To investigate the influence of time on the aging and therefore the concentration of NO_x in the gas bag, measurement of NO_x in the gas bag was carried out at different points in time after sampling.

STPOST 22

Determination of primary aromatic amines in smokeless tobacco products

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The list of harmful and potentially harmful constituents (HPHCs) published by the FDA in 2012 features six primary aromatic amine (PAA) compounds: 1-aminonaphthalene (1-ANP), 2-aminonaphthalene (2-ANP), 4-aminobiphenyl (4-ABP), o-anisidine (o-AND), 2,6-dimethylaniline (DMA), and o-toluidine (o-TOL). Of these six PAAs, 2-ANP, 4-ABP, and o-TOL have been deemed to be Group 1 carcinogens by the International Association for Research on Cancer (IARC). PAAs in mainstream smoke are thought to be combustion products formed from the tobacco's nitrogen-containing constituents. Smokeless tobacco products (STPs) would, therefore, be expected to be relatively free from PAAs, but some tobacco curing processes may introduce PAA contaminants through exposure to smoke and heat. In this study, we sought to determine if PAAs are present in a range of STPs and if there is any correlation to the tobacco types used in the product. Using a procedure adapted from a draft CORESTA method for the analysis of PAAs in mainstream cigarette smoke by GC-MS, we screened a variety of reference and market products for their PAA content. For many products, the levels were below either the verified quantitation or detection limits for each analyte. However, elevated and quantifiable levels of the Group 1 carcinogens, o-TOL and 2-ANP, were observed in products containing significant proportions of dark-fired tobacco. The CORESTA research product CRP3.1, for instance, is a dry snuff product for which the tobacco blend is 62 % dark-fired tobacco, and was found to contain approximately 4.9 ng/g o-TOL and 0.17 ng/g 2-ANP. Elevated levels of the Group 2B carcinogens DMA and o-AND were also found (0.48 and 0.57 ng/g, respectively). For comparison, a Kentucky 3R4F cigarette smoked under ISO conditions generates an average of 45 ng/cig o-TOL, 5.8 ng/cig 2-ANP, 3.2 ng/cig DMA, and 2.5 ng/cig o-AND. Results from our market product survey will be presented and discussed.

STPOST 23

Determination of three isothiazolinone fungicides content in tobacco paper by ultrasonic extraction coupled with gas chromatography-mass spectrometry

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Isothiazolinone are highly effective fungicides, used in papermaking to inhibit the deterioration of pulp, however direct contact of the human body with isothiazolinone may cause dermatitis, allergies and other reactions. The objective of this study was to develop a method for the simultaneous determination of three isothiazolinone fungicides in tobacco paper by ultrasonic extraction coupled with gas chromatography-mass spectrometry (GC-MS). An experimental plan of two-levels plus center point was designed and the response surface methodology was used for three factors extraction time, extraction solvent amount and extraction power in the extraction process. The optimal factor levels were respectively obtained as an extraction time of 40 min, an extraction solvent amount of 20 mL and an extraction power of 250 W.

Supernatant was obtained by stasis, derivatized by N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), and then selected for ion scanning analysis by GC-MS, and quantified by using an internal standard. The results showed that the three isothiazolinones had good linear calibration curves in the range of concentrations from 0.50 g/mL to 16.0 g/mL, and R^2 was 0.9952 to 0.9973. At three standard concentration levels, the standard recovery was 90.9 % to 96.4 % and the relative standard deviation was 1.5 % to 6.9 %. The method limit of quantification (LOQ) was 0.014 mg/kg to 0.074 mg/kg and under these conditions the signal to noise ratio was 10. This method has the advantages of simple operation, accurate quantification and low detection limit.

STPOST 24

A preliminary comparison of flavoured waterpipe tobacco aerosol with cigarette smoke – Part 1: "Tar", nicotine and carbon monoxide (TNCO) machine derived yields

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It is often asserted that a waterpipe session is equivalent to smoking 100 or more cigarettes. This originates from a 2005 statistic used by the World Health Organization (WHO) comparing the volume of aerosol produced during a waterpipe session to the volume of smoke produced by a single cigarette, without reference to the composition of the respective emissions or different consumer behaviours and consumption patterns.

The aim of the present study was to evaluate this assertion, based on an assessment of "*tar*", nicotine and carbon monoxide yields from a commercially available flavoured waterpipe tobacco product (Al Fakher "*Two Apples with Mint*") and the University of Kentucky 3R4F reference cigarette.

Waterpipe aerosol was collected in accordance with the International Organisation for Standardisation (ISO) draft Technical Specification 22486 "*Water pipe tobacco smoking machine — Definitions and standard conditions*", using a Cerulean SPS-Shisha smoking machine with conventional charcoal as the heating source (Global Laboratory Service Inc., U.S.A.). Waterpipe aerosol TNCO yields were compared with those from the 3R4F under a machine-smoking regime of "*puff*" volume 55 ml, "*puff*" duration 2 s and "*puff*" interval 30 s.

Machine derived waterpipe tobacco aerosol was found to contain significantly reduced concentrations of "*tar*", nicotine and carbon monoxide (84 %, 99 % and 90 % respectively) compared to the smoke generated from a single 3R4F. Furthermore, in contrast to the numerous toxicants present in the "*tar*" from the 3R4F cigarette, waterpipe tobacco residue was found to be primarily composed of humectants (i.e. glycerol, propylene glycol and triacetin) and water.

These data do not support the WHO statistic that conflates the volume of aerosol produced from a flavoured waterpipe session with the yields associated with smoking 100 or more cigarettes.

STPOST 25

A preliminary comparison of flavoured waterpipe tobacco aerosol with cigarette smoke – Part 2: Hoffmann analytes machine derived data

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Smoking-related harm and disease are directly caused by long-term exposure to the toxicants found in combusted tobacco smoke. In contrast to cigarettes, flavoured waterpipe tobacco (also known as *shisha*, and *hookah*) is heated which can significantly impact, inter alia reduce, aerosol toxicant yields.

The aim of the present study was to assess the Hoffman analyte yields from a commercially available flavoured waterpipe tobacco product with those from the University of Kentucky 3R4F reference cigarette.

Forty-two analytes comprising the Hoffmann list were quantified in the aerosol from a commercially available waterpipe tobacco product (Al Fakher "*Two Apples with Mint*"). Waterpipe aerosol was collected in accordance with the International Organisation for Standardisation (ISO) draft Technical Specification 22486, using a Cerulean SPS-Shisha smoking machine with conventional charcoal as the heating source (Global Laboratory Service Inc., U.S.A.). Waterpipe aerosol Hoffmann analyte yields were compared with those from the 3R4F under a machine-smoking regime of "*puff*" volume 55 ml, "*puff*" duration 2 s and "*puff*" interval 30 s.

In contrast to the 3R4F, 29 Hoffman analytes were not quantifiable in the collected waterpipe aerosol, including: NNN, NNK, NAT, NAB (LOQ < 25 ng), Hydroquinone, Catechol, Resorcinol (LOQ < 8 µg) p-Cresol, o-Cresol (LOQ < 2.4 µg), 1-Aminonaphthalene, 2-Aminonaphthalene, 3-Aminobiphenyl, 4-Aminobiphenyl (LOQ < 0.5 ng), Propionaldehyde, Butyraldehyde (LOQ < 2.5 µg), Lead, Cadmium, Chromium, Arsenic (LOQ < 0.025 ng), Mercury, Selenium (LOQ < 0.25 ng), Pyridine, Styrene (LOQ < 10 µg), Quinoline (LOQ < 1 µg), 1,3-Butadiene, Isoprene, Toluene (LOQ < 10 µg), Acrylonitrile (LOQ < 1 µg), and Hydrogen cyanide (LOQ < 14 µg).

Thirteen Hoffmann analytes were detected in waterpipe aerosol at quantifiable levels, including; Benzo[a]pyrene (34.2 ng), NO (24.8 ppm), Phenol (13.77 µg), m-Cresol (7.65 µg), Formaldehyde (117.22 µg), Acetaldehyde (131.41 µg), Acetone (12.82 µg), Acrolein (11.27 µg), Methylethylketone (3.82 µg), Crotonaldehyde (457.38 µg), Nickel (436 ng), Benzene (242.8 µg) and Ammonia (296.65 µg). The concentration of these analytes in waterpipe aerosol was reduced by ≥94 % compared to smoke from a single 3R4F.

These preliminary results suggest that flavoured waterpipe tobacco products offer the potential to substantially reduce exposure to toxicants for consumers who use such products as alternatives to cigarettes.

STPOST 26

Impact of glycerol content on the yield of carbonyl compounds in machine-derived aerosol of flavoured waterpipe tobacco products

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Investigations in the past have demonstrated no link between glycerol levels in commercial cigarettes and the formation of carbonyl compounds. Whilst a number of substantial differences exist between cigarettes and waterpipe tobacco (e.g. combusted versus indirectly heated respectively), the potential impact of glycerol on the formation of carbonyl compounds warrants further investigation for waterpipe tobacco.

The aim of the present study was to assess the impact that varying the quantity of glycerol used in the manufacture of flavoured waterpipe tobacco products has on the formation of eight carbonyl compounds; Acetaldehyde, Acetone, Acrolein, Butyraldehyde, Crotonaldehyde, Formaldehyde, Methylethylketone and Propionaldehyde.

Representative samples of flavoured waterpipe tobacco containing 11 %, 20 %, 24 % and 30 % (w/w) glycerol were manufactured by modulating the quantity of a non-tobacco ingredient. Aerosol was collected in accordance with the International Organisation for Standardisation (ISO) draft Technical Specification 22486 "*Water pipe tobacco smoking machine — Definitions and standard conditions*", using a Borgwaldt Shisha Smoker with conventional charcoal as the heating source (ASL Analytic Service Laboratory GmbH, Germany).

Five carbonyl compounds were not quantifiable in waterpipe aerosol regardless of glycerol content; Acetone (LOQ < 12 µg) Butyraldehyde (LOQ < 35 µg), Crotonaldehyde (LOQ < 12 µg), Methylethylketone (LOQ < 18 µg), and Propionaldehyde (LOQ < 12 µg).

Three carbonyl compounds were quantifiable in waterpipe aerosol; Acetaldehyde (158 µg 11 %, 212 µg 19.3 %, 264 µg 24.3 %, and 191 µg 30.2 %), Acrolein (0 µg 11 %, 58 µg 19.3 %, 69 µg 24.3 %, and 43 µg 30.2 %) and Formaldehyde (167 µg 11 %, 374 µg 19.3 %, 398 µg 24.3 %, and 232 µg 30.2 %). Carbonyl yields were not associated with variations in glycerol content.

The formation of carbonyl compounds from flavoured waterpipe tobacco products is not associated with the quantity of glycerol used in its manufacture.

STPOST 27

Next generation product aerosol bubbled extracts show little to no effect in high content screening endpoints when compared to cigarette smoke bubbled extracts

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Tobacco-based and tobacco-free next generation products (NGPs) are understood to be a less harmful alternative to conventional combustible cigarettes.

The objective of this study was to compare the *in vitro* biological response of cigarette smoke and a selection of NGP aerosols bubbled through Phosphate Buffered Saline (PBS). Normal Human Bronchial Epithelial cells were exposed to smoke/aerosol in PBS from Kentucky reference cigarette (3R4F), a tobacco heating product (THP), a hybrid product (HYB) and a *my*blu™ e-cigarette (Tobacco Flavour 1.6 % Nicotine). *In vitro* response of each extract was determined after 4 and 24 hours exposure using high content screening.

The 3R4F and THP were smoked using the Health Canada Intense method. HYB and *my*blu™ were vaped according to CRM 81. The smoke and aerosols were bubbled through a series of three impingers containing PBS. For every test day, fresh PBS solutions with 1.8 puffs/ml and 4 puffs/ml for the 3R4F and NGP samples respectively were produced. Chemical analysis of the 3R4F PBS solutions detected nicotine with an average of 86 ± 12µg/ml. The three NGP solutions contained nicotine levels from 70 ± 1 µg/ml (HYB), over 150 ± 17 µg/ml (THP) to 175 ± 17 µg/ml (*my*blu™).

The 3R4F smoke/aerosol in PBS caused a significant dose dependent decrease in cell count and significantly altered γ-H2AX, NfKB, p-c-Jun and oxidative stress endpoints (at concentrations > 1 %). The THP solution induced a few similar endpoints to 3R4F, however the concentration of THP used to produce a response was considerably higher (2-4-fold) with lower effect (2-3 fold) than with 3R4F. By contrast, *my*blu™ and HYB extracts did not induce any significant activity in all the parameters tested at the maximum concentration used (10 % for THP, max of 4 % for 3R4F).

This data suggests that the extracts from NGPs elicit little to no *in vitro* biological activity, even at higher exposure concentrations, compared to combustible cigarettes under the conditions tested.

STPOST 28

The *in vitro* biological assessment of a tobacco heating product and comparison with 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 panel 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 biological effects of two commercially available THPs, designed to reduce toxicant exposures. Responses were compared to a 3R4F reference cigarette.

Exposure matrices assessed included total particulate matter (TPM), whole aerosol (WA), and aqueous aerosol extracts (AqE) obtained after machine-puffing using the Health Canada Intense smoking regime. Endpoints assessed included cytotoxicity (neutral red uptake viability assay), oxidative stress (antioxidant response element (ARE) activation in lung epithelial reporter cells) and endothelial cell migration (wound healing). In addition, we used a high content screening (HCS) approach to assess ten different toxicity endpoints in primary human bronchial epithelial cells (HBEC).

THPs had little or no activity across all the *in vitro* assays when compared to the 3R4F reference product. WA from THPs induced cytotoxicity in H292 cells, but only at much higher doses than 3R4F WA. Similarly, the response to TPM treatment in the ARE assay and HCS was significantly lower for THPs than for 3R4F, at both 4 hour and 24 hours. AqE from TPM did not significantly inhibit endothelial wound healing, while 3R4F AqE exhibited a concentration-related response in this assay.

Together, these *in vitro* assays have enabled the biological assessment of THPs, and results suggest the products demonstrate 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 29

Reversibility of *in vitro* biological effects in cigarette smoke-exposed 3D lung tissues following switching to a tobacco heating product

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Tobacco heated products (THPs) potentially offer a safer alternative to combustible cigarettes. Recent *in vitro* studies have shown reduced THP biological effects compared to 3R4F reference cigarette smoke. Existing *in vitro* data, however, has been generated performing acute, single exposures not reflective of consumer use. Furthermore, the reversibility of the biological effects of cigarette smoke following switching to THPs has not been extensively studied *in vitro*.

A feasibility study was conducted to assess the potential of using MucilAir™ tissues in a 4-week repeated exposure study. Tissues were exposed to 3R4F smoke (15 mins × 3 times a week) for two weeks after which the cohort were split into three groups, further 2-week repeated exposure to 3R4F, switched to THP or air. The Borgwaldt RM20S generated whole aerosols at the Health Canada Intense smoking regime. Endpoints assessed included cytotoxicity, tight-junction integrity (TEER) and cytokine expression. The results were compared to a continuous air exposure control at week 4.

During the 4-week repeated exposure, LDH release remained below 10 % for all tested conditions and TEER above 500 Ω/cm^2, indicative of tissue integrity. At two weeks 3R4F repeated exposure, an increase in cytokine expression was observed, however following 4-week 3R4F repeated exposure, a strong differential cytokine expression was demonstrated, with 14 responsive cytokines in the culture media including MMP-9, IL-6, IL-4, IL-1β, VEGF at $p < 0.05$, $FC > 1.5$. However, tissues that were switched to THP aerosols for two weeks following 3R4F repeated exposure, demonstrated lower cytokine expression with only eotaxin-3 and MMP-9 remaining significantly increased ($p < 0.05$, $FC > 1.5$).

We have demonstrated the feasibility of repeated aerosol exposure *in vitro* with MucilAir™ tissues remaining viable over the exposure duration. Switching to THP after two weeks repeated 3R4F exposure, reversed *in vitro* biological effects with inflammatory cytokine expression greatly reduced compared to 4-week 3R4F exposure.

STPOST 30

In vitro RNA-seq-based toxicogenomics assessment shows reduced biological effect of tobacco heating products when compared to cigarette smoke

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The battery of regulatory tests used to evaluate the risk of novel tobacco products such as heated tobacco products (THPs) presents some limitations including a bias towards the apical endpoints tested, and limited information on the mode of action. This is driving a paradigm shift to more holistic systems biology approaches. In this study, we used RNA-sequencing to compare the transcriptomic perturbations following acute exposure of a 3D airway tissue to the aerosols from two commercial THPs and a reference 3R4F cigarette. An acute exposure was performed for one hour at an intense smoking regime using cells from one donor. 2809 RNAs were differentially expressed for the 3R4F treatment and 115 and 2 RNAs for the two THPs (pFDR < 0.05, FC > 1.5), respectively. The data was validated by qPCR with 20 genes selected from the RNA-seq data using cells from three donors. 17 out of 20 genes from the 3R4F smoke exposure agreed with the RNA-seq data whilst fewer positive responses could be confirmed with the THPs. A correlation coefficient of 0.8 was also obtained when comparing the RNA-seq data from two donors exposed to 3R4F smoke using the same conditions. The relationship between the identified differentially expressed RNA features and gene ontologies were mapped showing a strong association with stress response, xenobiotics metabolism, and COPD-related terms for 3R4F. In contrast, fewer ontologies were found enriched for the THPs aerosols. “Response to wounding” was a common COPD-related term over-represented for the two THPs but at a reduced significance. Quantification of a panel of 33 cytokines confirmed a pro-inflammatory effect of cigarette smoke only with nine induced cytokines including IL-6, -8, and -13. In conclusion, THPs have a reduced impact on gene expression in 3D airway tissue compared to 3R4F.

STPOST 31

Comparative *in vitro* toxicological evaluation of novel tobacco heating product

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In vitro studies have been widely used to support toxicological evaluations of chemicals and complex mixtures including cigarette smoke. More recently the same assays have been employed for comparative assessment of tobacco heating products (THP) against cigarette smoke.

In this study, traditional *in vitro* toxicological approaches using total particulate matter (TPM) and advanced aerosol exposure techniques were used to assess Kentucky Reference 3R4F cigarette against a commercially available THP. *In vitro* mutagenicity, cytotoxicity and tumour-promoting activity approaches were employed across TPM and whole aerosol test matrices.

The Ames bacterial mutation assay was employed using TA98, TA100, TA1535, TA1537 and TA102 ±metabolic activation (S9). The mouse lymphoma assay (MLA) was assessed ±metabolic activation with short 3-hr and longer 24-hr -S9 exposures. The Bhas 42 cell transformation assay was used to supplement traditional approaches and incorporate ability to detect tumour promoters. The neutral red uptake (NRU) assay provided an acute measure of cytotoxicity. The *in vitro* micronucleus (IVMN) assay was employed ±S9 with short and long exposures. The Ames assay was also employed with TA98, TA100, TA1535, TA97 and TA102 using a scaled down 35 mm methodology for the assessment of whole aerosols.

Cigarette smoke from TPM test matrices was deemed positive under almost all test conditions in all assays. For NRU, Ames, MLA, Bhas 42 and IVMN assays, responses were observed at 60 µg/mL, 240 µg/plate, 60 µg/mL, 50 µg/mL and 30 µg/mL, respectively. In contrast, THP TPM failed to elicit a response in the assays up to 240 µg/mL. Cigarette smoke was also deemed positive in the Ames assay at doses up to 5 µg/cm^3, THP aerosols were negative at doses exceeding 25 µg/cm^3.

Given a weight of evidence approach, these data demonstrate that THP test matrices are negative at doses equivalent and exceeding those of cigarette smoke where positive responses are observed, suggesting THPs may offer significant reduced risk potential compared to cigarette smoking.

STPOST 32

Enumeration of TBNK cells: A case for a bioanalytical assay to support clinical studies for the development of modified risk tobacco products

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Smoking has been reported to lessen the body's immune response making it more susceptible to infections. Chronic cigarette smoke exposure causes imbalance in immune homeostasis affecting the circulating immune cells. Peripheral blood lymphocytes are heterogeneous in nature. Based on biological function and cell-surface antigen expression, lymphocytes are classified into three major populations: T lymphocytes, B lymphocytes and NK (Natural Killer) lymphocytes (TBNK). Measurement of these subsets can be valuable tool in understanding the impact of modified risk tobacco products (MRTP) in comparison with traditional combustible tobacco products.

Current clinical protocols for enumeration of specific cell populations within whole blood require that the sample be analysed within 24-48 hrs. Staining fresh whole blood is not practical in large clinical studies, and it is not possible to batch or re-run samples. Furthermore, it is very challenging if not impossible to ship samples from clinical study sites to a central analytical laboratory given these time constraints. Isolation and cryopreservation of PBMCs for downstream analysis by cell flow cytometry is an alternative, but is technically demanding, highly variable and labor intensive.

Here we report a flow cytometry-based TBNK assay, which overcomes the limitations of a clinical diagnostics assay mentioned as above. The assay utilizes stabilized whole blood simplifying the sample collection at the clinical sites. We have established stability in whole blood with stabilizer for more than 30 days post-freezing at -80 °C. This innovation allows the analysis of clinical samples in batches by subject minimizing the process related variability within subjects. The innovation allows the preparation and use of quality control samples in each batch for the monitoring of assay performance. During the method validation relative accuracy, precision (inter-operator and inter-instrument), post-staining stability, pre-process stability, post-staining stability and freeze-thaw stability were also tested. The method was found to be an attractive solution and alternative to highly variable clinical diagnostics for the enumeration of TBNK for the purposes of MRTP evaluation in a clinical setting.

STPOST 33

Quantitative risk assessment (QRA) indicates reduced risk potential for carcinogenic and non-carcinogenic effects of the aerosol of the next generation products (NGPs) compared to reference cigarettes

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In order to understand the potential relative health risks of NGPs compared to cigarettes, we compared the relative risks of aerosols from tobacco-containing and tobacco-free NGPs to the smoke generated from a reference cigarette. Quantitative Risk Assessment (QRA) is a scientific, evidence-based analytical process that combines chemical and biological data to quantify the probability and potential impact of defined risks. This work will present a comparison of reduced exposure and corresponding potential risks for the *my*blu™ e-cigarette and heated tobacco (HT) in comparison to the reference cigarette. QRA was used to estimate the potential carcinogenic and non-carcinogenic effects of *my*blu™ e-cigarette and Pulze HT aerosols and these were compared to smoke from a reference cigarette (3R4F).

Selective harmful and potentially harmful constituents (HPHCs) were measured in *my*blu™ e-cigarette aerosol and converted to µg/L. For the HT and reference cigarette, the analytes were measured on a per unit basis (i.e. µg per cigarette). Exposure concentrations (EC) were estimated assuming a lifetime continuous exposure using the equation EC = AC × CPD (SPD) × ED × EF / DIR × AT for the HT stick and cigarette or EC = AC × (PC × PV) × ED × EF / DIR × AT for the e-cig, where EC: exposure concentration, AC: analyte concentration, CPD (SPD): cigarettes/HT sticks per day, ED: exposure duration (64.4 years), EF: exposure frequency (356 days), DIR: daily inhalation rate (20 m^3/day), AT: averaging time (23506 days), PC: puff count (worst-case 400 puffs), and PV: puff volume (0.055 L).

Non-cancer risks were quantified using the hazard quotient (HQ) approach and cancer risks were estimated by calculating the incremental lifetime cancer risk (ILCR), utilizing non-cancer and cancer toxicity values issued by government agencies or published in the peer-review literature.

As may be expected, the modelling of exposure to HPHCs in both e-cigarette and HT aerosols lead to a marked reduction when compared to the smoke from reference cigarettes, indicating the potential for reduced non-cancer and cancer health hazard risks. Exposure to some analytes was found to be below LOD or LOQ for the test method.

STPOST 34

Next generation product aerosols induce lower biological activity than combusted cigarettes: a comparison of *in vitro* cell migration in the scratch wound assay

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Smoking combustible cigarettes is a cause of serious diseases in smokers, including heart disease. There are many commercially available next generation products (NGPs), such as tobacco-free e-vapour products, aiming to provide an alternative to smoking with a significant reduction in harm. The study aimed to compare the cardiovascular-related effect of NGP aerosol to cigarette smoke using the *in vitro* endothelial migration (scratch wound) assay.

Products investigated were the Kentucky reference cigarette (3R4F), a tobacco heating product (THP), a hybrid product (HYB) and a *my*blu™ e-vapour product (Tobacco Flavour 1.6 % Nicotine). The 3R4F and THP were smoked using an intense smoking regime, with HYB and *my*blu™ vaped according to CORESTA Recommended Method No. 81. Freshly generated smoke and aerosols were captured in phosphate buffered saline and added at concentrations up to 10 % of total cell media. Artificial wounds 700-800 µm wide were created in the human umbilical vein endothelial cell monolayer using a WoundMaker™ device. The impact of the test articles on endothelial cell migration activity was determined over 30 hours following exposure.

The scratch wound assay showed significant differences in rate of wound closure between test articles. A highly significantly, dose-dependent increase in wound closure inhibition was observed following 3R4F exposure. Trapped HTP and HYB aerosols showed lower migration activity over control indicating slight inhibition of the wound healing activity. The Dunnett's multiple comparison test confirmed statistically significant effects for the HTP only. Trapped *my*blu™ aerosol did not show any significant inhibition of cell migration over control, even at the highest concentrations, under the conditions of test.

The data demonstrates clear differences between 3R4F smoke and next generation product (NGP) aerosols on endothelial cell migration, with no measurable effect observed for the tobacco-free *my*blu™ e-vapour product. These findings add to the increasing body of scientific evidence supporting the harm reduction potential of NGPs.

STPOST 35

Neutral red uptake (NRU) cytotoxicity analysis of aerosol generated from a temperature-regulated nicotine salt based product utilizing cotton wicking material

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The JUUL Nicotine Salt Pod System (NSPS) has no user modifiable settings and is temperature regulated to minimize the generation of combustion-related byproducts. Aerosol generated from NSPS pods using a cotton wicking material was evaluated for cytotoxicity potential.

Seven flavors (tobacco, mint/menthol, and fruit flavors; 9 mg/mL nicotine) were evaluated. Testing was conducted by an accredited ISO 17025 laboratory using validated methods. Cytotoxic potential was assessed by the neutral red uptake *in vitro* assay (OECD, TG 129). CHO-K1 cells were treated with NSPS aerosol condensate, a positive control, or vehicle control for 24 hours utilizing an ethanol extraction methodology. NSPS aerosol exposure under ethanol extraction was evaluated across a dose range of (0-300 µg/mL). Cell viability following NSPS aerosol exposure was compared to mainstream smoke from a reference cigarette (3R4F) at a dose range of (0-150 µg/mL). Additionally, cells were exposed to the positive control (SLS) at 40 and 80 µg/mL.

No aerosol mediated significant toxicity was observed at any of the tested NSPS flavors or concentrations. EC_{50} for the NSPS aerosol and carrier control aerosol could not be calculated because cell viability was greater than 50 % for all test conditions. Additionally, TPM from the 3R4F reference cigarette demonstrated expected toxicity with an EC_{50} of 59 µg/mL.

In conclusion, under the experimental conditions and based on the criteria for Evaluation of Cytotoxic Response (ISO 10993-5), all NSPS aerosol condensates generated from the 9 mg/mL NSPS test articles were found to be non-cytotoxic.

STPOST 36

Use of *ex vivo* precision-cut lung slices as a screening tool for potential respiratory toxicity of e-liquids

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The Family Smoking Prevention and Tobacco Control Act gave the FDA regulatory authority over next generation tobacco products (NGTP) such as e-vapor products. E-vapor product liquids contain a variety of ingredient combinations that should be assessed for human risk. One human lung-relevant testing platform with reasonable throughput, is human precision-cut lung slices (HuPCLS). HuPCLS are arguably the most complex non-animal model of the lung, retaining native architecture and immune-competent cells over multi-week culture periods. HuPCLS were exposed to three concentrations (0.1 %, 0.5 %, and 1.2 %) of propylene glycol (PG; an e-vapor product constituent) continuously for 16 days. Exposure-effects were evaluated biochemically (WST-8 assay) and histologically (viability assessment of H&E stained slides). Positive control treatments consisted of 10 µM phortress and 13 µM bleomycin. HuPCLS were fed every day with fresh medium ± treatment and harvested at days 4, 8, and 16. Untreated control (UC) HuPCLS viability was confirmed using protein and adenylate kinase assays. Over 16 days in culture, UC lost 30 % viability while WST-8 results indicated no loss over 16 days in culture. Phortress caused severe damage by day 4 and bleomycin by day 8 (histologically & WST-8 viability). Prolonged 1.2 % PG exposure diminished WST-8 viability by ~30 % at day 16 which agreed with histological results. High osmolality is the suspected mechanism of toxicity. There was no effect histologically or via WST-8 viability for prolonged exposure to 0.1 % and 0.5 % PG. In summary, PG, a common e-vapor product ingredient, at 1.2 % had adverse effects in a human pulmonary model in an exaggerated exposure regimen (prolonged exposures with changes in osmolarity). The HuPCLS platform has huge potential to serve as a screening tool for e-liquid (and other materials of concern) by elucidating potentially relevant, long-term events following NGTP ingredient exposure.

STPOST 37

Use of cell media nicotine concentration as a marker to predict cells surface deposited nicotine in transwells after fresh smoke/aerosol exposure

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Exposure of organotypic 3D lung models at the air liquid interface (ALI) to fresh whole smoke/aerosol provides a more human relevant exposure assessment of combustible cigarettes and e-vapour products.

The aim of this study is to present a method for determination of nicotine deposition at the 3D MucilAir™ tissues surface, in transwells of 24 MWP after repeated exposure to 30, 60 or 90 puffs of tobacco cigarette (3R4F, 1:17 dilution) or undiluted *my*blu™ e-vapour aerosol over four weeks. Exact measurement of nicotine deposited at the cell surface is difficult due to absorption of nicotine into the cells. The nicotine serves as a general marker of exposure.

We wanted to determine if cell media nicotine concentration was correlated with cell surface deposition. To measure this, the deposition efficiency onto a glass plate inserted directly into the transwell was determined and was compared to glass discs with cells grown on the surface (BEAS-2B, V79).

During the four weeks of repeated exposure of MucilAir™ tissues at ALI to 3R4F smoke and *my*blu™ aerosol, basal culture media were collected for nicotine quantification. Additionally nicotine deposition on to glass discs representing the cell surface area was measured. Nicotine based toxicological effects observed over the exposure time in comparison to the puff numbers were compared.

Nicotine deposition on glass discs in the transwells correlated well with the deposited nicotine in the cell media, with increasing puff numbers, dilution factors and the surface area of the glass plates. Therefore, the nicotine concentration measured (using LC-MS/MS) in exposed basal medium can be considered as a proxy in relation to nicotine deposition on the cell surface. As expected, due to different physical characteristics of the products, smoke from 3R4F and aerosol from *my*blu™, relationship cell media and nicotine deposition had different correlation coefficients.

STPOST 38

In vitro cytotoxicity and genotoxicity of representative e-liquid flavor mixtures

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Flavor compounds “generally recognized as safe” for oral consumption are commonly used in inhalable e-vapor products for which insufficient safety data exists. The hazard characterization of each flavor and various flavor mixtures is resource and time-demanding. Here we explored a pragmatic approach, where we selected representative flavors and tested for *in vitro* cytotoxicity and genotoxicity. From a group of commonly used individual flavors (> 200) in e-vapor products, 38 flavors were selected using structural grouping and available toxicological data. These flavors were mixed in a carrier (PG/VG/water) to prepare a test mixture (prototype flavor mixture with up to 18 % flavor load), with and without nicotine, and were subjected to a standard CORESTA battery of *in vitro* cytotoxicity (Neutral Red Uptake [NRU]) and genotoxicity (Ames and micronucleus [MN]) assays. Test mixtures (with and without nicotine) were negative in the Ames mutagenicity assay but showed cytotoxicity in all three assays including NRU assay. In the MN genotoxicity assay, the test mixture with nicotine was negative but the test mixture without nicotine provided equivocal results. To further identify the potentially responsible flavor(s) for the cytotoxicity response in the NRU assay of the test mixtures, we divided the 38 flavors into five sub-group mixtures according to their solubility and chemical reactivity, and tested them using NRU cytotoxicity assay. Results suggested that sub-group mixtures containing certain flavors – for example, ethyl maltol, furaneol and isopulegol – were more cytotoxic, consistent to literature findings as *in vitro* cytotoxicant/irritant. The results align with the overall systematic toxicity evaluation approach, deconstructing mixtures into subsets of flavors, ultimately in support of flavor read-across assessment.

STPOST 39

Risk assessment of a novel tobacco vapour product using ToxTracker® assay and high content screening *in vitro*

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A novel tobacco vapour product (NTV) showed its lower genotoxic and cytotoxic potentials in the *in vitro* bacterial reverse mutation assay, micronucleus assay, and neutral red uptake assay compared with a combustible tobacco product. This study aimed to further evaluate and compare the risk assessment of an aerosol from the NTV with that of smoke from the 3R4F reference cigarette. We employed (1)ToxTracker®, in which six mouse embryonic stem reporter cell lines were designed to exhibit fluorescence upon induction of various pathways relevant to (geno)toxicity and cancer, and (2) human bronchial epithelial cells (BEAS-2B) were analysed for DNA double-strand breaks (γ-H2AX immunostaining), oxidative stress (superoxide and glutathione indicator), and apoptosis (activated caspase-3/7 fluorescence). Total particulate matter (TPM) of the 3R4F smoke or aerosol collected mass (ACM) of the NTV aerosol was collected on a Cambridge filter pad. The trapped constituents on the pad were extracted with dimethyl sulfoxide to prepare the test material solution. In ToxTracker®, DNA damage, oxidative stress, p53 activation, and the unfolded protein response were significantly induced in each reporter cell line treated with the TPM of the 3R4F smoke, but no significant induction was observed in any of the reporter cell lines treated with the ACM of the NTV aerosol. In addition, the 3R4F TPM markedly modified γ-H2AX, superoxide, glutathione, and caspase-3/7 levels in BEAS-2B cells, whereas the NTV ACM brought no significant changes. We found that the NTV aerosol had a different risk assessment profile from that of the combustible cigarette smoke under the present study conditions.

STPOST 40

A seven-month systems toxicology inhalation study in C57BL/6 mice demonstrates reduced pulmonary inflammation and emphysema following smoking cessation or switching to e-vapor aerosol exposures

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Cigarette smoking causes lung cancer, emphysema, and other serious diseases. While cessation remains the most effective approach to minimize smoking-related diseases, alternative nicotine delivery products that limit the generation of combustion by-products may offer reduced risk to those who would otherwise continue to smoke. E-vapor products are one set of such promising nicotine-delivery products. The health risks of long-term inhalation exposures are unknown. We designed a chronic inhalation (4 h/day, 5 d/week, 7 months) study in C57BL/6 mice to evaluate the long-term respiratory toxicity of MarkTen® e-vapor aerosols in comparison to the reference 3R4F cigarette smoke (CS). Additional groups were added to explore the impact of CS cessation or switching to e-vapor exposures after three months of CS exposure. There were no significant changes in in-life (body weights, clinical signs) observations in e-vapor groups compared to the sham control. The CS group had lower body weight and showed transient signs of distress post-exposure and reduced respiratory function during exposure. Following seven months of exposure, e-vapor resulted in no or minimal increase in pulmonary inflammation, while the CS induced consistently elevated pulmonary inflammation (infiltration of activated macrophages, T and B cells, neutrophils) and emphysema. Biological changes in the switching group were similar to those in the cessation group. Transcriptomics analysis showed that the CS exposure elicited a large number of differentially expressed genes (DEG) in the lung and nasal epithelium, while drastically reduced gene expression changes were observed in response to e-vapor exposure. Compared with the CS group, the number of DEGs was much smaller in the lungs of the switching or cessation groups, while the reduction in nasal epithelium was less pronounced in the switching or cessation groups. In conclusion, exposures to e-vapor aerosols instead of CS can significantly reduce the respiratory disease risk associated with cigarette smoking.

STPOST 41

Sharing and reusing data: toxicology assessment meta-analysis of various e-liquids and heat-not-burn platforms with INTERVALS datasets

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Extensive scientific studies are conducted to assess the relative risks of various candidate modified risk tobacco products compared with those of smoking cigarettes. As the scientific community conducts such assessments for diverse products and in a variety of laboratory models, knowledge on toxicity is spread across numerous scientific articles. We believe that by fostering the consolidation of data and knowledge gained from studies assessing novel tobacco/nicotine delivery products on a community platform, new hypotheses may be generated, and the weight of evidence may be increased. We have therefore created and are further developing INTERVALS (www.intervals.science), an online platform supporting independent, third-party collaboration by proactively sharing detailed protocols, tools, and data from assessment studies. Data files are accompanied by relevant information to foster reproducible research and encourage data reanalysis.

We will present a meta-analysis of *in vitro* toxicology assessment studies, including aerosol characterization, neutral red uptake assay, and mouse lymphoma assay, for various e-liquid and heat-not-burn platforms compared with the 3R4F reference cigarette. These studies have been conducted by multiple organizations using different methods and models. The content of the separate publications has been curated and included in INTERVALS in an interoperable format so that a meta-analysis of results can be performed.

The direct comparison of the platforms tested in separate studies with different study designs (e.g. different lists of chemicals quantified in the aerosols) makes it difficult to compare every result across individual studies. However, the overall result is consistent in that all of the studies included in this analysis demonstrate the reduction of harmful or potentially harmful chemicals and of toxicity assessed *in vitro* for the tested platforms compared with cigarettes. As the scientific community integrates more studies and datasets into INTERVALS, it will become easier to conduct such meta-analyses and review results obtained across institutions, models, and platforms.

STPOST 42

Characterization of whole mainstream smoke/aerosol delivery within the Vitrocell® Ames 48 high throughput exposure module using different tobacco product types

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The preferred means of assessing combustible or other aerosol-generating tobacco products *in vitro* is by direct exposure of cell cultures to freshly generated whole mainstream smoke/aerosols. This approach eliminates the fractionation of smoke/aerosols that occurs when collecting particulate matter on filter pads and gas vapor phase via liquid traps. Whole smoke/aerosol exposure systems are commercially available and have been utilized to assess combustible and tobacco heated products (THP) as well as electronic nicotine delivery systems (ENDS) *in vitro*. However, a challenge with such systems is ensuring a sufficient number of doses and sample throughput for *in vitro* toxicological studies in a timely manner. Vitrocell® has developed a high throughput whole smoke/aerosol exposure module (Ames 48 module) designed to concurrently deliver up to seven different doses (six wells per dose) of smoke/aerosol and a clean air control to 48 wells of bacterial cell cultures. Characterization of smoke/aerosol delivery within this system was conducted in a series of experiments designed to assess smoke/aerosol delivery and biological responses from a Kentucky Reference 3R4F combustible cigarette or a commercially available THP. Dilution airflows consisting of 0.5 - 10 L/min for 3R4F and 0 (undiluted) - 4 L/min for the THP were evaluated. Smoke/aerosol deposition was quantified on a mass delivered basis using fluorescence measurements (Ex 355/Em 485) of captured particulate matter and chemical analysis (e.g. glycerol, nicotine) of either DMSO (3R4F) or PBS (THP) liquid traps within the module. The mutagenicity (Ames assay) of whole smoke from the 3R4F cigarette was assessed with the Ames 48 module using Salmonella strains TA98 and TA100 (±S9). Results demonstrate a dose-dependent deposition of smoke/aerosol constituents (3R4F and THP) and a characteristic dose-dependent increase in revertant counts (3R4F). Current test results from the Ames 48 module are comparable to historical 3R4F results generated using the standard Vitrocell® exposure modules.

STPOST 43

Dosimetric analysis of aerosol generated by a Vitrocell® VC10® Smoking Robot – investigations on dose dependency and consistency of application

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Quartz Crystal Microbalances were used to assess the suitability of the Vitrocell® VC10® exposure system. The main objective of the study was to deliver smoke aerosol in a consistent and dose dependent manner across a range of module formats.

Dose resolving power, uniformity of deposition within the module, and repeatability across experiments were all assessed by the application of the 3R4F aerosol after dilution with clean air at flow rates of 10, 8, 4 and 1 SLPM (n = 3). Twelve 3R4F were smoked per experiment according to the HCI Smoking regimen (Health Canada Test method T-115). Exposure duration in the system was 66 minutes. Vitrocell® Ames 4, 6/4, 12/4 and 24/4 exposure modules were assessed.

The ability to resolve statistically significant differences between the 8, 4 and 1 SLPM dilution levels was observed across all modules in the test, however none of the module formats were able to detect a significant difference between dilution rates between 10 and 8 SLPM.

Uniformity of deposition within the module was assessed on a per experiment basis and found to have no significant differences and high reproducibility (adjusted $P > 0.98$) in all module formats tested and across the full range of airflows.

Repeatability was assessed per airflow over three experiments and showed no significant difference at airflows of 10, 8 and 4 SLPM, however a significant difference between experiments was found in 9 out of 12 cases at 1 SLPM airflow.

Our assessments show that the Vitrocell® VC10® is capable of delivering cigarette aerosol in a consistent and dose dependent manner. However, it was found that resolution of small differences in dose (particularly at higher dilution rates) is critical and therefore at low dilution rates variability of deposition should be expected on a per experiment basis.

STPOST 44

In vitro testing of an ethanol collection method combining particulate and gas-vapor phase components: *in vitro* micronucleus assay

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Health Canada (HC) guidelines (T-502) for the collection and testing of cigarette smoke are used frequently for *in vitro* testing and although the guidelines allow for the collection and testing of the particulate phase (PP) and the gas-vapor (GVP) phase separately, the method has several limitations. The PP is extracted in DMSO while GVP components are trapped in PBS, which limits the trapping efficiency of volatile or non-water-soluble compounds. Because of this limitation, GVP is only routinely tested for cytotoxicity using the NRU assay, but not for genotoxicity (MN assay) or mutagenicity (Ames assay). We have evaluated the use of ethanol as a trapping solvent for PP and GVP components. ETOH-extracted PP+GVP was compared to PP or PP+GVP collected based on HC methods. CHO-K1 cells were exposed in the absence and presence of S9 metabolic activation for 3 hrs. (21 hrs. recovery) for cytotoxicity and genotoxicity (MN induction) following HC T-503 guidelines. Manual counting of MN was conducted in fixed cells stained with acridine orange. Dose dependent increases in MN were observed in all three types of extracts. Without metabolic activation, HC (PP), HC (PP+GVP) or ETOH (PP+GVP) exposure resulted in a mean fold MN increase in a dose dependent manner from 1.0 to 5.0× for all three extract types. With metabolic activation, HC (PP) and HC (PP+GVP) exposure resulted in a mean fold MN increase in a dose dependent manner from 1.0 to 5.0× while ETOH (PP+GVP) resulted in 1.0 to 3.7× fold MN increase. The ethanol method tested here allows for combined trapping of PP+GVP components yielding a single whole-smoke extract. The ethanol PP+GVP extract tested produces comparable cytotoxicity and %MN results when compared to DMSO extracted PP while allowing the testing of GVP and PP components together. NRU, Ames, and chemistry results are presented separately.

STPOST 45

In vitro testing of an ethanol collection method combining particulate and gas-vapor phase components: Bacterial Reversion Mutation (Ames) Assay

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Health Canada (HC) guidelines (T-502) for the collection and testing of cigarette smoke are used frequently for *in vitro* testing. Although the guidelines allow for the collection and testing of the particulate phase (PP), the gas-vapor (GVP) phase, and a combination of both (PP+GVP), this method has several limitations. The PP is collected in DMSO and can be tested for cytotoxicity (NRU assay), mutagenicity (Ames assay), and clastogenicity (MN assay), while the GVP phase is collected in PBS and only tested using the NRU assay. PBS has limited trapping efficiency of volatile or non-water-soluble compounds and must be used within 60 minutes of collection. These limitations could be overcome with a method that allows collection of the PP and GVP together in a solvent providing enhanced trapping and stability of GVP components. We have tested the use of ethanol to collect PP and GVP components using the Ames assay (HC T-501 guideline). Reference 3R4F cigarettes were used. Five strains of Salmonella bacteria (TA98, TA100, TA102, TA1535 and TA1537) were tested in the absence and presence of S9. Results showed that the ethanol condensate collecting PP+GVP together resulted in increased bacterial lawn cytotoxicity in TA98, TA100, TA1535 and TA1537. DMSO-extracted PP showed toxicity in TA98, TA100, TA1537 while PP+GVP in PBS only showed toxicity in TA1537. With metabolic activation, TPM+GVP in ethanol induced an 18-fold and 11-fold increase in the number of revertants in TA98 and TA1537 respectively. In comparison, DMSO extracted PP induced a 16-fold and 8-fold increase, while TPM+GVP in PBS induced a 14-fold and six-fold increase in the same strains. The ethanol method allows for combined collection of PP+GVP components yielding a single whole-smoke extract significantly increasing the mutagenic and cytotoxic effects observed in the Ames assay. NRU, MN, and chemistry results are presented separately.

STPOST 46

In vitro testing of an ethanol collection method combining particulate and gas-vapor phase components: neutral red assay

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The Neutral Red Uptake (NRU) assay Health Canada (HC) guidelines (T-502) for the collection and testing of cigarette smoke are used frequently for *in vitro* testing. Although the guidelines allow for the collection and testing of the particulate phase (PP), the gas-vapor phase (GVP), and a combination of both (PP+GVP), this method has limitations. For example, GVP is collected in PBS which has limited trapping of volatile or non-water-soluble compounds. The GVP fraction also has limited stability and must be used within 60 minutes of collection. Because of limited stability, GVP is only routinely tested for cytotoxicity using the NRU assay, but not for genotoxicity (MN assay) or mutagenicity (Ames assay). These limitations could be overcome with a method that allows collection of the PP and GVP together in a solvent with enhanced trapping and stability of GVP components. We evaluated the use of ethanol to collect PP and GVP components and compared it against the traditional HC collection using the NRU assay and CHO-K1 cells following HC guidelines. Reference 3R4F cigarettes were used. Extraction of PP in ethanol produced comparable results to DMSO-extracted PP (IC50 101.7 µg/mL vs. 87.6 µg/mL), however, collection of GVP in ethanol resulted in higher cytotoxicity when compared to GVP in PBS (IC50 40.2 µg/mL vs. 159.1 µg/mL) likely due to better trapping efficiency and stability of GVP components. The combination of PP+GVP collected in ethanol showed higher toxicity (IC_{50} 58.2 µg/mL) compared to PP (IC50 87.6 µg/mL) or PP+GVP (IC_{50} 110.3 µg/mL) collected under HC guidelines. The ethanol method allows for combined trapping of PP+GVP yielding a single whole-smoke extract, results in increased trapping of GVP components, and shows comparable or better cytotoxicity results in the NRU assay compared to the HC method. Ames, MN, and chemistry results are presented separately.

STPOST 47

Identification, discussion and recommendations for the optimal generation and use of *in vitro* genotoxicity assay data for tobacco and nicotine products

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The Institute for In Vitro Sciences is sponsoring a series of workshops to identify, discuss and develop recommendations for optimal scientific/technical approaches for utilizing *in vitro* regulatory genotoxicity assay data within and across tobacco and nicotine product categories. Workshops provide a unique opportunity for invited expert stakeholders to share experiences and to develop recommendations that may serve as a resource for developing optimal data to evaluate the toxicity of tobacco and nicotine products. It is envisioned that some of these recommendations would form the basis for the generation of guidance documents and/or serve as authoritative reference publications for optimal methodologies and data interpretation and to support regulatory submissions. During the first workshop (November 27-28, 2018) workgroup members identified important issues for using *in vitro* genotoxicity assays for evaluating tobacco and nicotine products and issues were triaged into three priority categories based on the amount of available information. This issue list serves as the basis for focused high-priority topics for subsequent workshops. The topic of the second workshop (June 4-5, 2019) was the generation of appropriate test samples for *in vitro* genotoxicity testing and the third workshop (September 2019) addressed optimal methods for cell exposure. Future workshops will tackle issues such as: (1) recommended cell types for *in vitro* cytogenetic evaluations; (2) recommendations for expressing exposure when comparing products within and among product categories; (3) recommendations for comparing toxicological responses within and among product categories; and (4) applying new or existing methods for assessing genotoxicity and other toxicological effects of tobacco and nicotine products using cells in culture. This presentation will provide a summary overview of the workshop series.

STPOST 48

Comparative study of *Salmonella typhimurium tester* strains TA1537, TA97 and TA97a in mutagenicity evaluation of tobacco products

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The Ames test has been conducted to examine the mutagenicity of tobacco products and to compare relative mutagenicity of cigarette smoke. The OECD TG 471 states that at least five tester strains should be used for the Ames test, and *Salmonella typhimurium* TA1537, TA97 and TA97a can be used interchangeably. It is well known that cigarette smoke shows clear mutagenic activity in some tester strains including TA1537, however, few studies have been reported using strains TA97 and TA97a. Thus, here, we compared strains TA97, TA97a and TA1537 in terms of their sensitivity to detect a mutagenic response and the discriminatory power to distinguish between different types of cigarette. The cigarette smoke condensate (CSC) derived from four types of test cigarette (i.e. 3R4F, 1R6F, 100 % Burley, and 100 % flue-cured) was subjected to the Ames test. Regarding sensitivity, in the presence of S9, all three strains showed clear, positive responses for all the CSCs. Especially a significant increase in revertant colonies was observed for TA97a at a lower dose than for the other two strains. In the absence of S9, TA97 showed a positive response to the CSCs, whereas the other two strains did not due to growth inhibition. Regarding the discriminatory power, in the presence of S9, the three strains provided consistent results in terms of the mutagenicity rank-order (i.e. Burley > 3R4F~1R6F > flue-cured) and the ability to discriminate statistically dose responses found in the different CSCs. We suggest that *S. typhimurium* strains TA97, TA97a and TA1537 have different sensitivities in detection of a positive mutagenic response, whereas the three strains are comparable in their ability to discriminate between different types of cigarette smoke. Further investigation is needed to understand the mechanism underlying the difference in sensitivity between the three strains in mutagenicity assessment of cigarettes.

STPOST 49

The CORESTA *in vitro* test battery for combustible tobacco products: update from the 2004 Rationale and Strategy Report

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In 2004, the CORESTA In Vitro Toxicity Task Force issued a rationale and strategy report, commonly known as “the CORESTA *in vitro* test battery guideline”. The goals of this guideline were to: 1) develop the rationale and strategy for conducting *in vitro* toxicity testing of tobacco smoke and 2) identify key procedures based on internationally recognized guidelines, adapted to accommodate the unique properties of tobacco smoke. The Task Force [now the Sub-Group (IVT SG) since 2015] performed a series of proficiency trials based on the guideline. Considering the time passed, the IVT SG has reviewed the guideline to: 1) re-evaluate the relevance of the initial rationale and strategy of *in vitro* testing of combustible tobacco products, 2) identify recent and comparable regulatory testing guidelines and examples in publications, and 3) provide a pragmatic summary of key features of each recommended assay. The endeavor revealed the continued usage and reference of the CORESTA *in vitro* test battery, especially where standardized and validated testing is required (e.g. regulatory submission), upholding that the overall strategy and rationale remain valid and relevant. Sometimes these standardized testing results are supplemented with newer and exploratory *in vitro* assays (e.g. air-liquid-interface testing with fresh whole smoke). However the CORESTA *in vitro* test batteries are continuously used in comparative product testing, such as evaluating the biological impact of changes in ingredients or product designs as part of a weight-of-evidence toxicity evaluation. In the updated 2019 guideline, the IVT SG recommends, where standardized *in vitro* toxicity testing is desired, the following test battery for combustible tobacco products: 1) cytotoxicity (neutral red uptake) assay with mammalian cells; 2) bacterial reverse mutation (Ames) assay in *Salmonella thyphimurium*; and 3) mammalian cell cytogenetics/mutation assays (the *in vitro* micronucleus assay, the mouse lymphoma assay, or the chromosome aberration assay). The IVT SG reiterates that the biological significance of the *in vitro* results needs to be evaluated in conjunction with all available chemical and exposure/dosimetry data, in the context of the overall product risk assessment.

STPOST 50

Altered lung barrier function is a physiologically-relevant biomarker of potential harm

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Cigarette smoking is a major risk factor for diseases including cardiovascular disease, lung cancer, and chronic obstructive pulmonary disease (COPD). One of the key functions of lung epithelial cells is to serve as a physical barrier against insults including cigarette smoke. Chronic smoking induces a state of chronic inflammation and oxidative stress, resulting in epithelial barrier disruption, which leads to increased lung permeability and culminates in tissue damage and remodeling, contributing to smoking-induced lung diseases. We evaluated lung barrier function in a single-center, ambulatory, clinical study. Lung permeability, measured as the half-life ($T_{1/2}$) of inhaled ^{99m}TC-DTPA, was assessed in 17 subjects consisting of six smokers (SMK), five moist snuff consumers (MSC), and six non-tobacco consumers (NTC). Half time clearance of ^{99m}TC-DTPA from the lungs was measured at baseline, and approximately 7 and 14 days later. Right lung images were captured with a gamma-camera after inhalation of ^{99m}TC-DTPA. Smokers, relative to MSC and NTC, exhibited significantly faster clearance of the inhaled probe (shorter $T_{1/2}$), indicating increased lung permeability. NTC and MSC had similar clearance times of the probe (longer $T_{1/2}$), suggesting that moist snuff use does not perturb lung permeability. The altered lung barrier function in smokers is a contributing factor to smoking-related diseases such as lung cancer and COPD, and may serve as a biomarker of potential harm related to tobacco use.

STPOST 51

Structural investigation of the tobacco specific urinary biomarker iPX

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Biomarkers of potential harm (BoPH) are tools to assess biological and pathophysiological effects associated with chronic tobacco product use, such as oxidative stress. Isoprostanes are widely recognized as BoPH for assessing endogenous oxidative stress. The objective of this study was to further characterize a novel and tobacco product-specific biomarker, designated as iPX, found in 24-hour urine samples of smokers, and moist snuff consumers (MSC), but not in the urine of non-smokers. Urine samples were screened for iPX by a three-step enrichment and chromatographic procedure. Initially, pooled urine samples were concentrated by solid phase extraction (SPE). After SPE, the iPX-enriched fractions were purified via HPLC, followed by a second chromatographic separation. The resulting fractions were analyzed in negative electro spray ionization (ESI) ion mode on a high-resolution QE Plus mass spectrometer. Urine from smokers and MSC was found to contain iPX peak(s) with characteristic fragment ions (m/z 115, m/z 139, and m/z 157) in the MS2 spectrum of the parent molecular ion (m/z 353.2333), indicating iPX has a molecular formula of $C_{20}H_{34}O_5$. Four isomeric peaks were separated chromatographically, all showing the characteristic ion patterns, and named as iPX-1, iPX-2, iPX-3, and iPX-4. The tentative structure and mass fragmentation pathways of the most abundant isomer, iPX-3, was proposed based on tandem high-resolution mass spectrometry and H/D exchange experiments. The results suggest that iPX-3 was transformed from the known isoprostane, iPF2a-VI, through ring opening and bond migration enzymatically or chemically (oxidative process involved). iPX-3 is present in undiluted smokers' urine at approximately 0.45 ng/mL and is suitable for larger scale liquid-liquid extraction using ethyl acetate. IPX-3 was also detected in the urine of MSC, indicating that it may be a tobacco product-specific metabolite. Additional work is necessary for structural characterization and exploring the metabolism and functions of iPX-3 in tobacco product consumers.

STPOST 52

A blood-based smoking-related gene expression signature using a machine learning approach

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Smoking is a leading risk factor in the onset of multiple forms of cancer, chronic obstructive pulmonary disease, and cardiovascular disease. At present, there is a limited understanding by which changes in gene expression profiles in blood or other tissues can be used to predict smoking status. In this study, we investigated whether a machine learning approach could provide an unbiased method to predict smoking status using microarray expression profiles obtained from the blood. Using multiple feature selection and classification methods, the most optimal algorithm that produced the best predictive model to determine smoking status was a combination of support vector machine (SVM), based on recursive feature elimination (RFE). The 16 gene signature from our machine learning model included not only three previously reported genes (*LNNR3*, *SASH1*, and *GPR15*), but also several newly identified genes including *GZMM*, which has been reported to be associated with lung adenocarcinoma. In addition, this gene signature has been validated by seven independent publicly available gene expression datasets. In summary, we show that machine learning analysis using expression profiling datasets from blood is useful in ascertaining smoking status and in developing novel biomarkers of potential harm.

STPOST 53

Nicotine kinetics and subjective effects of two tobacco heating products compared to cigarettes and a nicotine replacement therapy

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Recently, clinical studies have shown reductions in exposure to tobacco smoke toxicants when smokers of conventional cigarettes switch to a tobacco heating product (THP). Studies on nicotine pharmacokinetics (PK) and subjective effects of potentially reduced-risk products (PRRPs), such as THPs, relative to conventional cigarettes and other nicotine products may help understand the likelihood of switching success and provide data on potential abuse liability.

This study conducted in Verona, Italy (ISRCTN13439529), run in accordance with ICH-GCP, was a randomised, crossover study investigating nicotine PK and subjective effects. Subjects used the glo™ THP with consumables of two different nicotine yields (THP1.0 and THP1.1), a cigarette of subjects' usual brand, and a licensed nicotine replacement therapy (nicotine inhaler).

32 healthy smokers were recruited with each subject assigned a different product in accordance with pre-defined randomisation sequences over four PK periods following overnight nicotine abstinence. Subjective effects (product liking, urge to smoke a usual-brand cigarette, urge to use the study product, overall intent to use the product again) were also assessed at various timepoints during each PK period.

Based on C_{max} and $AUC_{0\text{-}240min}$, systemic nicotine exposure was greater for THPs than for nicotine inhaler, but lower than usual brand cigarette.

For the THPs median T_{max} (4 min) was closer to that observed for the cigarette (6 min) than for the nicotine inhaler (15 min). Product liking and overall intent-to-use again was greater for the THPs than for the nicotine inhaler, but lower than for cigarettes. Urge to smoke was reduced to the greatest extent when smoking a cigarette, and to the least extent when using the nicotine inhaler.

These findings demonstrate the glo™ THPs had a closer nicotine PK profile to conventional cigarettes than the nicotine inhaler, and that subjective effects of glo™ THPs were more positive than those for the nicotine inhaler.

STPOST 54

How did we move from smoking topography to vaping topography?

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The principle of measurement of the smoking puff analyser-manual (SPA-M) device is based on Bernoulli's Law applied through the thin layer coupled with the Perfect Gas Law. The issue with e-cigarettes is that the flow that comes through the hole is smaller than the diaphragm. That is why it is not possible to detect the difference of pressure (ΔP) through the cigarette holder. The easiest solution proposed was to "disturb" the flow in order to get a deflected flow and a non-zero ΔP between entry and exit of the cigarette holder's insert.

The aim of this study was to validate an adapter, in order to ensure that vaping topography is possible with an SPA-M device.

Three different models of obstacles were designed and manufactured to perform validation tests (a simple metallic bar, a holes ring and a ball).

The first test consisted in checking the volume of the puff to three different levels (42 ml, 55 ml and 80 ml) and to analyse the results of each obstacle in comparison with a soap bubble volumeter. The second test was to measure the pressure drop of e-cigarettes and to compare the results obtained by three adapters.

For the first test, no significant volume difference was recorded, even if for bigger volumes the holes ring adapter caused a slight decrease in volume. For the second test, the holes ring obstacle generated a significant difference, in contrast to the simple metallic bar and a ball, where the results were unchanged and remained in the acceptable tolerance range.

A special adapter design for e-cigarettes that includes a simple metallic bar allows SPA-M device to be used for vaping topography.

STPOST 55

Use behaviour patterns in Japanese novel tobacco vapour product (NTV) users

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Use behaviour patterns in users of novel tobacco vapour products (NTV) is important information in order to assess the public health impact of NTV in a real market situation. Therefore, we conducted online surveys (i.e. pre-survey and main-survey) to understand patterns of use behaviour of NTV (marketed under the name PLOOM TECH) users in Japan. In December 2018, a main-survey was conducted with a representative sample of 1973 NTV users (aged 21-69 years) in Japan. These participants were selected as per the quota method based on the distribution of NTV users in Japan, which was investigated in a pre-survey. We collected information about patterns of tobacco use behaviours (e.g. type of product used, daily consumption) before and after starting NTV use. In this survey, 18.9 % reported exclusive NTV use, 11.2 % reported dual use in combination with other tobacco vapour products or other tobacco products excluding cigarettes, 39.1 % reported dual use including the use of cigarettes, and 30.8 % reported multiple product use including cigarettes and other tobacco vapour products. Dual users of NTV and cigarettes (n = 701), who were exclusive cigarette smokers before starting NTV use, reported an average daily consumption of 16.2 cigarettes (95 % confidence interval [CI]: = 15.6-16.8) before and 12.9 cigarettes (95 % CI: 12.3-13.5) after starting NTV use. In conclusion, these findings provide the number of exclusive NTV users within NTV users in Japan. The results suggest the possibility that daily consumption of cigarettes is reduced when starting NTV use. However, as this survey was conducted only a few years since the launch of NTV in Japan, we assume gradual changes could occur in use behaviour patterns in NTV users over time. Therefore, further surveys monitoring future trends are needed.

STPOST 56

Perceptions of the relative harm of electronic cigarettes compared with smoking in the U.S.A.: analysis of the Population Assessment of Tobacco and Health (PATH) study data, 2013-2016

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Electronic cigarettes (e-cigarettes) have been characterised as significantly less harmful than smoked tobacco by an increasing number of public health authorities. Despite this, U.S. adults remain poorly informed about the relative risk of these products.

To quantify this, an analysis of the U.S. Population Assessment of Tobacco and Health (PATH) study was conducted. Available data from wave 1 (September 2013 to December 2014) and wave 2 (October 2014 to October 2015) of the study was analysed and presented at the Global Forum on Nicotine 2018. Here we update our findings following an analysis of the recently released data from wave 3 (October 2015 to October 2016).

This nationally representative survey of the U.S. adult population showed that over half believed e-cigarettes to be as harmful as or more harmful than conventional cigarettes. This tendancy has increased further over time (54 % in wave 1, increasing to 65 % in wave 2, increasing to 73 % in wave 3). Among current adult U.S. smokers, 43 % believed e-cigarettes to be as harmful as or more harmful than smoking in wave 1, increasing to 57 % in wave 2 and further increasing to 68 % in wave 3.

Misperceptions of the relative harm of e-cigarettes compared with conventional cigarettes is worsening in the U.S. and are particularly strong in adult smokers. It is likely that adult smokers may not even try an e-cigarette due to inaccurate beliefs about their relative harmfulness. Correcting these misperceptions may help more U.S. adult smokers to switch to less harmful nicotine e-cigarette products.

STPOST 57

Cross-cultural equivalence of the ABOUT™–Perceived Risk instrument in seven languages

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The risk of smoking has long been well understood by consumers. However, it is important to measure the perceived risks by consumers of novel smoke-free alternatives to cigarettes. The ABOUT™–Perceived Risk instrument was developed to allow the quantification of such perceived risks. The accumulated knowledge acquired through its use in international studies will provide meaningful information about the effects of risk perception on product use behaviour (e.g. initiation, cessation, switching) among current users and non-users. To directly compare findings from different countries, it is essential to examine the cross-cultural equivalence of the ABOUT™–Perceived Risk. This was the objective of this research. The translation process (i.e. linguistic validation) followed best practice guidelines and consisted of five successive qualitative steps: conceptual analysis of the original, forward translation, back-translation into English, cognitive interviews and external review, and proofreading. In addition, empirical comparability was established through psychometric evaluation of differential item functioning (DIF). To date, the qualitative linguistic validation has been successfully completed in six countries (France, Germany, Italy, Japan, Poland, and Russia). This included the cross-cultural harmonization of either semantic (meaning), idiomatic (practicality), or syntactic (grammar) issues. For example, in Japan, “earlier death” was changed to “die early” (semantic); literal translation of “being physically unfit” was not possible for an idiomatic reason; all items on the Addiction scale had to be structured with a subject (syntactic). Analyses of DIF have been conducted in Italy (N = 1623), Japan (N = 1618), and the U.S. (two samples, N_1 = 2020, N_2 = 1640), further supporting cross-cultural equivalence (ANOVA F-values between 0.02 and 2.73, df = 3, *p*-values ranging between 0.044 and 0.996, based on a random sample of n = 500). The equivalence of findings across studies is critical to best respond to the globalization of tobacco regulatory research. This research forms an initial evidence base for the appropriate international use of the ABOUT™–Perceived Risk.

STPOST 58

A consortium approach for consumer-reported outcome measures to assess tobacco- and nicotine-containing products

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In the tobacco space, consumer-reported outcome measures (CROM) are essential for understanding motivations, subjective effects, and behavior. Such measures have become particularly important with the advent of candidate modified risk tobacco products (cMRTP). Here we present the preparatory steps for a consortium approach initiated by the tobacco industry to develop, validate, use, and access CROM to assess tobacco- and nicotine-containing products (TNP), including cMRTPs. A CROM Task Force was formed within the CORESTA Product Use Behaviour Sub-Group. To refine goals, research questions, and scope of work, we reviewed 12 documents, including U.S. Food and Drug Administration (FDA) MRTP briefing package submissions (in the context of applications submitted by Altria Client Services, Philip Morris Products, Swedish Match, Reynolds), regulatory documents (FDA Premarket Tobacco Product and MRTP Applications, FDA Over-the-Counter label comprehension, EU Directive), review papers, and public health reports (U.S. Institute of Medicine, FDA Center for Tobacco Products activities). Data relating to self-report measures were extracted (i.e. concepts to be measured and methods recommended and/or used). The review revealed the need to propose a consensual definition of consumer-reported outcomes (CRO), categorize the concepts of interest measured to assess TNPs (including cMRTPs), and develop a common taxonomy and definition to qualify them. The review also highlighted unclear recommendations or best practices from guidance documents issued by different regulatory agencies on how each concept should be assessed. The Task Force will present a proposal for a definition of CROs, a draft concept taxonomy, and a summary of the information extracted relevant to each concept. The key outcomes presented here form the foundations of the CROM consortium. This work should facilitate a dialogue on requirements to develop common terminology, standards, and best practices for CROM in the tobacco space and emphasizes the need for more discussion between industry, academia, and regulatory body stakeholders.

STPOST 59

A novel repository for consumer reported outcome measures in tobacco research: development, implementation and evolution from 2016 to date

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The consumer reported outcome measures Repository is a measurement instrument knowledge base developed by Mapi Research Trust (MRT) for Philip Morris International (PMI) in June 2016. It enables PMI researchers to identify and select measures to assess tobacco-related consumer outcomes, to consult up-to-date information related to the measures of interest, and to localize instruments used in past and on-going in-house studies. This paper presents 1) the development and implementation of the Repository, and 2) the evolution of its content, structure, and functionalities from its implementation, to-date.

The Repository structure was established in two folders, an instrument folder and a study folder. A comparison was conducted between the initial implementation and the actual content, based on searches in the Repository, Quality Checks, and Internal Reports.

At the first Quality Check, in April 2017, the instrument folder comprised 58 instruments, with 44 focusing on tobacco behaviour (75 %). The study-folder comprised six studies. To date (April 2019), the Repository comprises 117 instruments, with two-third focusing on tobacco behavior, and 35 studies. Since its development in June 2016, four main functionalities have been implemented to the Repository: the possibility to upload additional instrument-related documentation, a full-text search functionality, automatic email alerts of instrument/metadata updates, and statistics on external licensing requests regarding instruments from the ABOUT™ Toolbox developed by PMI.

This unique initiative enables PMI researchers 1) to assess, select and use up-to-date and official versions (original and translations) of instruments in a standardized environment, and 2) to transfer them to the study operation team during the study set-up phase; 3) to share their own instruments with the scientific community; and 4) moreover, in tight collaboration with MRT, to implement new functionalities in the Repository, allowing the dynamic tool to meet PMI researchers' needs.

STPOST 60

Can we still observe a net health benefit due to the introduction of modified-risk tobacco products (MRTPs) should the illegal trade in counterfeit cigarettes increase? A model perspective.

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Since 2012 and the publication of the MRTP guidance by the U.S. FDA encouraging the use of computational modelling, many robust dynamic models have been developed that are projecting net health benefits in the whole population after introduction of modified-risk tobacco products.

The purpose of this additional exploratory analysis is to evaluate what could be the computational impact when varying the size of the market in illegal counterfeit cigarettes, given the potential for these products to increase risk of harm versus legal cigarettes.

We developed and ran an average population modelling having the capability to differentiate relative risks between the legal and illegal counterfeit markets in combustible cigarettes, as well as the effect of MRTP availability.

National Survey on Drug Use and Health (NSDUH) smoking prevalence and U.S. census bureau demographics data were combined into the model from 2010 and computed until 2030. Status quo scenario (i.e. without any MRTP) as well as introduction of MRTP scenario from 2020 were tested and compared.

While the wide range of scenarios is still showing a beneficial health impact in the long run after introduction of MRTPs, the analysis is showing that there are cut-off points in prevalence and harmful potential of the illegal trade counterfeit cigarettes beyond which no further benefits are observed (e.g. counterfeit cigarettes prevalence of 20 % being 20 % more harmful on average).

In conclusion, high prevalence of harmful illegal counterfeit cigarettes may have the potential to limit or even lower all foreseen benefits achieved through the introduction of MRTPs.

STPOST 61

Results of aging studies on e-liquids

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The objective of this research has been to understand the chemical changes that e-liquids may undergo with age. Aging may affect the sensory properties of the aerosols generated from aged e-liquids. Aging may also affect the toxicological properties of the aerosols. In this study, two methods of aging were compared. The first was long-term aging under ambient conditions with the samples aged in their original containers. The second was forced aging of aliquots of the samples for 8 days at 64 °C, in addition to ambient aging of the samples in their containers. Control samples for the accelerated aging study were kept in a freezer at a temperature of -22 °C before analysis. Aged and unaged samples were analyzed by liquid chromatography (LC, HPLC). At least three different LC conditions [reverse phase (RP) with conventional C18 columns as well as both RP and aqueous normal phase (ANP) conditions with Type-C silica columns with amide, bidentate C18, and phenyl hydride packings] using both isocratic conditions for use with an refractive index (RI) detector and gradient conditions for use with a UV detector with wavelength set at 195, 259, 280, or 347 nm. Peak identifications were made with known standards. In some samples, reduction of aromatic aldehydes through acetal formation was the biggest change. Evaluation of aged and unaged samples was also carried out with a glassmouth containing artificial saliva to compare transfer of flavor components of aged and unaged samples to the saliva. Aged samples showed less transfer of known flavorful components.

STPOST 62

Determination of 16 polycyclic aromatic hydrocarbons in cigarette mainstream smoke and filter by on-line coupled LC-GC/MS

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Polycyclic aromatic hydrocarbons (PAHs) are present as trace constituents in cigarette smoke. Many PAHs are well known carcinogens, International Agency for Research on Cancer (IARC) Group 1.

In this study, we present a novel fully automated on-line LC-GC/MS method, capable of simultaneously determining 16 PAHs (EPA 610 list) in mainstream cigarette smoke and filters. The method was used to determine the filtration efficiency of cigarette filters in commercial brands.

Two Y splitters and partially concurrent solvent evaporation (PCSE) are applied in the method that results in a pressure-free switch LC-GC interface. Prevention of detector pollution as well as solvent inhaling was achieved by using a backflush gas. Furthermore, solvent trapping reduced the loss of volatile compounds in samples during analysis.

The results showed that the levels of lower condensed PAH ring structures in mainstream cigarette smoke are much higher than those of the higher condensed PAH ring structures, such as benzo[a]pyrene (B[a]P). The levels of naphthalene (NAP), fluorene (FLU) and phenanthrene (PHE) were 634, 130 and 112 ng/cig., respectively, which in sum is more than half of the total absolute amount of all 16 investigated PAHs. The analytical RSD for most of the analytes was lower than 6 %. The results demonstrate that the investigated cigarette filters are highly selective for PAHs, although the PAHs have a wide boiling points range. However, the selectivity for the PAH filter retention decreased with the increase of the number of carbon atoms in the PAH molecule. The filtration efficiency of the investigated cigarette filters for the 16 PAHs was in the range of 36 % to 71 %.

Compared with methods currently applied to determine PAHs in cigarette smoke and filters (based on solvent extraction-SPE-GC/MS), our recently developed LC-GC/MS method is much simpler with respect to sample preparation, consumes less solvent and depicts high reproducibility as well as high sensitivity.

STPOST 63

Comparison of methods for measuring the particle size distribution of smokeless tobacco products

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Tobacco cut size is one of the product properties for smokeless tobacco products, which the Food and Drug Administration has proposed as a requirement for a Substantial Equivalence (SE) submission, (21 CFR Parts 16 and 1107). However, no guidance has been provided for a standard method to perform these measurements. Sieve analysis can been used to determine particle size distribution (PSD) for smokeless tobacco products; however, this technique is time consuming, labor intensive, and requires that moist products be dried prior to sieving. Dynamic image analysis (DIA) is a method used to measure particle size that incorporates a high-speed camera to capture images of particles, as they flow through a cuvette. Image analysis software is used to compute the PSD. DIA present distinct advantages over sieve analysis. DIA has greater resolution, since the bin sizes for the distribution can be set much more narrowly than with sieves. DIA offers a wide particle size range, limited only by the camera and optics in use, which for this work ranged from 1 µm to 20 mm. The image analysis algorithms allow for a variety of metrics to be applied to the distribution. In this work, the particle size is calculated as the diameter of a circle of equal projection (EQPC), and length of fiber through a direct connection of the two most distant points (LEFI). The EQPC method allows us to easily transform the data from a length mode to surface area or volume weighted distributions. We examine differences in PSD of four MST products including snus, snuff, fine-cut, and long-cut. A direct comparison between the weight-based sieve method and optical DIA methods is presented.

STPOST 64

Analysis of cylinder drying intensity on cut strips based on Enthalpy method

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Cylinder drying of cut strips is one of the key technologies during the cigarette manufacturing process, which involves complex mass transfer and heat transfer phenomena. In order to study the actual cut strip heating and heat distribution in the cylinder drying process, on the basis of obtained accurate temperature data of cut strips inside cylinder dryer, a thermodynamic model for cut strips during cylinder drying was established as viewed from the actual cut strip heating inside the cylinder dryer. The relationships of technical parameters of cylinder drying with heat for water evaporation in cut strips Q_v, heat for temperature rising of cut strips Q_s, total heat quantity Q and thermal efficiency coefficient η were investigated with canonical correlation analysis. The results showed that: (1) the established thermodynamic model well explained the actual cut strip heating and heat distribution in the cylinder drying process, and the variation coefficients of the four thermophysical parameters of cut strips under the same processing conditions were all less than 5.0 %; (2) the cylinder wall temperature in the first section and hot air temperature significantly positively correlated to the heat for water evaporation, the cylinder wall temperature in the second section significantly positively correlated to heat for temperature rising of cut strips, and the throughput of cut strips significantly negatively correlated to heat for temperature rising of cut strips. It is proved that the established thermodynamic model for cut strips during cylinder drying is feasible.

STPOST 65

Analysis of mainstream cigarette smoke constituents prioritized by the World Health Organization in a core collection of tobacco accessions: variability and correlations

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Among the different strategies proposed for tobacco regulation, the WHO study group on Tobacco Product Regulation (TobReg) has proposed mandating ceilings on smoke constituents. These constituents were selected according to their potential toxicity, their variability among brands and the potential for the constituents to be lowered with the up to date technologies.

To assess the potential use of the natural diversity of tobacco plants to reduce the proposed constituents, we evaluated the variability of different leaf and smoke constituents in a core collection of tobacco accessions, representative of genetic diversity available before intensive modern breeding. A panel of 145 tobacco varieties from the Imperial Tobacco collection was grown in the field with three repeats and a split-splot design. After curing, handmade cigarettes were prepared to analyse smoke constituents. A statistical approach, taking into account multiple factors linked to the growing environment and weight of tobacco actively burnt during puffing, was designed to identify potential differences between varieties.

Significant differences were found for some constituents between tested varieties, but for others, low variability and inverse correlation are major bottlenecks. The WHO strategy on ceiling cannot be implemented in the state of art, even if some elements can be treated individually.

STPOST 66

Collection and characterization of mainstream cigarette smoke condensates collected using a glass fiber filter and an ethanol containing impinger

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Traditional smoke collections for *in vitro* toxicology assays, including regulatory Health Canada (HC) testing, have utilized a multi-phase setup that traps particulate matter with a glass fiber filter followed by a liquid filled impinger for gas phase compounds. Dimethyl sulfoxide (DMSO) is used to extract particulate matter from the filter while the gas phase is collected in phosphate buffered saline (PBS). These solvents have been extensively used for the assays mentioned above but may not effectively trap the full range of compounds produced during cigarette combustion. Additionally, the separate collection of particulate and gas phases divides mainstream smoke into two samples. However, many compounds are present in both the particulate and gas phases so separation may not be ideal.

This study investigates an alternate smoke collection procedure that combines the particulate and gas phases together using ethanol instead of the traditional DMSO/PBS solvents. This alternate procedure traps particulate matter with a glass fiber filter in series with an ethanol filled impinger for gas phase compounds. The filter is extracted with the impinger contents to yield a single whole smoke condensate.

Mainstream 3R4F smoke was collected using ethanol and traditional HC methods. The filter pad and impinger contents were analyzed separately for the FDA abbreviated list of HPHCs. Particulate phase compounds like nicotine, benzo[a]pyrene, and TSNAs were similar in both methods. However, large differences were seen when comparing the trapping efficiency of ethanol versus PBS for volatile organic compounds (VOCs). The VOC content of PBS was found to be 6 µg/mL while ethanol was found to be 48 µg/mL, an 800 % increase. Ammonia, formaldehyde, and acetaldehyde increased by approximately 15 % when using the alternate trapping procedure. The stability of the extracts was also determined for both methods. The impact of the condensate collection method on the HC toxicology assays will be presented separately.

STPOST 67

A comparison of quartz filter collection versus electrostatic precipitation collection in e-cigarette aerosol samples

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The determination of metals in e-cigarette aerosol has been routinely performed by collecting the aerosol on quartz fiber filter pads. The trapped aerosols are digested using acid dilution and analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The quartz filters have similar performance to standard Cambridge filters. The major problem with using quartz filters, is that they contain detectable levels of many of the metals of interest and, even more problematic, the amount of metals varies between filters and filter lots. Each new lot of filters must be evaluated for background levels to establish method limit of detection (LOD). Even with background subtraction, the quartz filter method has elevated LODs and increased variability near the method LOD.

In order to reduce background levels and improve method LOD we evaluated the use of an electrostatic precipitation (EP) unit to collect the aerosol. The EP unit has long been established in the collection of cigarette smoke (Health Canada Official Method T-109).

In this study we validated the use of a 20-port EP system for the determination of metals in e-cigarette aerosol; focusing on trapping efficiency, analyte recovery, and background contamination. We will present findings that show a decrease in background contamination and an improvement in the detection limits of the EP method over the quartz filter method. The method LODs were lowered by an average of 72 %. For example, Chromium for the filter collection method had an average method LOD of 103 ng/collection while the LOD for the EP method was 5 ng/collection. The EP system offers an additional improvement since each EP unit has the ability to collect up to 3.5 g of aerosol collected mass (ACM) while the filters are limited to maximum of ~0.8 grams of ACM. The ability to collect additional ACM significantly reduces method LOD on a per gram basis.

STPOST 68

Comparative levels of carbonyl delivery between mass-market cigars and cigarettes

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The recent 2016 deeming of cigars by the U.S. Food and Drug Administration (FDA) has led to an increased interest in cigar science, including ways to accurately measure the harmful and potentially harmful constituents (HPHCs) found within mainstream cigar smoke. At present, there are a limited number of standardized methods available for the evaluation of HPHCs in mainstream cigar smoke, except for nicotine and carbon monoxide. This study sought to investigate carbonyl delivery in commercially available cigars and cigarillos and compare them to levels found in cigarettes. First the standard cigarette method, CORESTA Recommended Method No. 74 (CRM 74), was optimized for cigar smoking, including evaluation of the trapping efficiency and the stability of the carbonyl-hydrazone adducts due to the increased smoke time required for cigar collection. The optimized trapping solution was then applied in a survey of the carbonyl delivery in commercially available cigars and cigarillos for comparison to published cigarette data. Smoked under CRM 64 conditions, cigars were found to yield similar levels of formaldehyde to those found in commercially available cigarettes (20.2 ± 11.7 vs. 22.1 ± 13.5 µg/cig respectively). Greater levels of acetaldehyde (2133 ± 470 vs. 365 ± 176.5 µg/cig), acrolein (52.7 ± 23.7 vs. 33.4 ± 17.0 µg/cig) and crotonaldehyde (42.4 ± 14.7 vs. 14.7 ± 6.8 µg/cig) were observed in cigar mainstream smoke when compared to cigarettes collected under conditions prescribed by ISO standard 3308. Furthermore, cigarettes smoked under the Health Canada Intense smoking regime delivered higher levels of formaldehyde (20.2 ± 11.7 vs. 74.6 ± 24.0 µg/cig), acrolein (52.7 ± 23.7 vs. 120.5 ± 14.9 µg/cig) and crotonaldehyde (42.4 ± 14.7 vs. 51.5 ± 8.7 µg/cig) emissions as compared to cigars smoked under the CORESTA regime, while acetaldehyde was found to be higher in cigar emissions (2133 ± 470 vs. 1234 ± 147 µg/cig).

STPOST 69

Within brand and across brand content and variability of nicotine and tobacco-specific nitrosamines in eight commercial U.S. cigar brands

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The recent 2016 deeming of cigars by the U.S. Food and Drug Administration (FDA) has led to an increased interest in cigar science, including ways to accurately measure the harmful and potentially harmful constituents (HPHCs) found within cigar tobacco. However, there are few published studies on HPHCs in cigar filler and even less is known about the cigar to cigar variability of HPHCs in cigar filler tobacco. In this study we attempted to quantify the variability of nicotine and TSNAs (NNN, NNK, NAT, NAB) in cigar filler on a per cigar basis, from both machine-made and premium cigars. Eight total brands were analyzed.

Cigars were individually conditioned, weighed, and ground. Tobacco from each cigar was analyzed in triplicate for nicotine and NNN, NNK, NAT, and NAB content (by GC-FID and LC-MS/MS, respectively). Analytical results were reported on a per gram basis and corrected to a per cigar basis using the original cigar weight.

On a per gram basis, the level of nicotine present in the products ranged from 0.75 to 1.80 % with an average variability of 9.5 % for the analysis of 10 cigars per brand. The amount of nicotine per cigar ranged from 17.9 to 232.6 mg with an average variability of 12.3 %. Premium (long-leaf) cigars showed the highest amount of nicotine variability at 20.2 %.

On a per gram basis, the total amount of TSNA present in the products ranged from 7.8 to 55.9 µg/g with an average per brand variability of 23.7 %, for the analysis of 10 cigars per brand. The amount of total TSNA per cigar ranged from 18.4 to 585.5 µg with an average per brand variability of 24.2 %. Individual TSNA variation ranged from 14.4 to 61.3 % across all brands tested.

STPOST 70

Structure and diversity analysis of microbial communities in cigar products by high-throughput sequencing technology

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To investigate the structure and diversity of bacterial and fungal communities in cigar products, the bacterial 16S rRNA and fungal internal transcribed spacer (ITS) regions in 10 representative cigar samples were sequenced by high-throughput sequencing technology. The abundance of sequences, alpha diversity, structure of microbial communities and species clustering were analysed based on sequencing data. The results showed that: (1) The diversity of bacteria and fungi in cigar samples were abundant. A total of 360 bacterial genera and 49 fungal genera were identified, and the highest diversities of bacterial and fungal communities were observed in the samples of Brand C and E, respectively. (2) *Firmicutes* and *Ascomycota* were the dominant phyla, *Staphylococcus*, *Acinetobacter*, *Pseudomonas* and *Aspergillus genera* were the dominant genera in the cigar samples. (3) Significant differences were observed in the diversity and structure of bacterial and fungal communities among these samples. Cluster analysis suggested that the distribution of microbial communities in the samples of Brand H and I from the same country was similar. This study analysed the microbial community composition in cigars, provides a guideline on the isolation of beneficial strains to improve the quality of cigars, and has important theoretical significance and potential application value.

STPOST 72

Optimising carbon footprint of international meetings

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It is broadly acknowledged that global warming is intimately influenced by the extracted carbon from the earth, and transferred to the atmosphere mainly as carbon dioxide (in addition to other greenhouse gases converted into CO_2eq). Considering the anticipated economic, environmental and social impacts of a global temperature increase, a number of political initiatives have been taken since the Rio de Janeiro summit in 1992. Many regulations invite organisations to report their carbon emissions. Some countries even apply tax payment per ton of CO_2 emitted. Such policies aim at increasing transparency and encouraging behaviour changes to reduce carbon emissions, and ultimately mitigate the impact of global warming on populations.

People travelling to international meetings is one of many emission sources. Such events are important in many cases, but emissions can be reduced by making an informed choice of the meeting locations. To support this goal, a web interface tool has been developed to estimate carbon emissions. Participant locations are the input parameters; flying distances are calculated and converted into CO_2eq considering each individual location as a potential meeting location. The process consists in converting locations into GPS coordinates using geolocation application programming interface, then estimating distances and converting them into CO_2eq by using an acknowledged approach developed by the French Environment and Energy Management Agency. The output is a graphical representation helping meeting organizers make an informed decision.

This poster will present the principles of calculations, the functionality of the tool and examples illustrating its potential benefits.

Note: Not travelling individually will not prevent the plane from flying. But fewer travellers will eventually mean fewer planes. Behaviour changes of a small group of individuals are usually not enough, however, population behaviour is a sum of individual behaviours, and global changes will come if individuals change first.