ABSTRACTS OF PRESENTATIONS MADE AT THE

2015 CORESTA JOINT MEETING OF THE

SMOKE SCIENCE AND PRODUCT TECHNOLOGY STUDY GROUPS

JEJU ISLAND, SOUTH KOREA

(in alphabetical order of first authors)
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Study to evaluate selected questions of subjective measures of individuals' motivations for and use of tobacco and nicotine products

Pre-market data are required to estimate population uptake of e-cigarettes and may be used as a component of a dynamic population model (DPM). To develop tools to collect such data, a suite of questionnaires of subjective measures of individuals' motivations for and use of tobacco and nicotine was collated. The main objective of this pilot study was to test subject burden and understanding of a selection of the questionnaires and to assess their effectiveness for use in pre-market assessment. The secondary objective was to determine changes in responses following a short period of e-cigarette use.

The study was conducted by the Centre for Drug Misuse Research in Glasgow, U.K. Thirty-seven subjects aged between 19 and 64 years who were smokers of at least ten cigarettes per day, and had never used but were willing to try e-cigarettes, were recruited. Subjects completed a web-based questionnaire including questions about cigarette consumption, smoking history and perceptions of e-cigarettes prior to first use.

Each subject was provided with an e-cigarette and cartridges to use in their normal environment for a week and asked to record their daily e-cigarette use and daily cigarette consumption in a diary. After a week the subjects completed a follow-up questionnaire assessing their perceptions and experiences of using the e-cigarette.

Subjects indicated good comprehension of the questionnaire instruments and many subjects reported cognitive and behavioural effects of e-cigarette use. For example, daily cigarette consumption decreased with more frequent use of the e-cigarette, and the intensity of urges to smoke significantly decreased following the week of use.

The questionnaires were suitable to collect pre-market data and data may be useful as input for a DPM. The questionnaires need to be validated with larger subject numbers and different populations and additional items are required to collect data from never and former smokers.

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Characterization of an electronic device that heats tobacco using an *in vivo* rodent inhalation exposure system

Devices that heat, rather than combust tobacco, have been reported to deliver an aerosol which is chemically and biologically less complex than cigarette smoke. In this study, a nose-only inhalation exposure system was constructed for an electronic device that heats tobacco (Test) and for conventional cigarettes (3R4F: Ref), and their exposure atmospheres were evaluated for acute responses in rats. Modified Canadian Intense puffing regimen was used and the aerosols at nose port were characterized over one hour by measuring Wet Total Particulate Matter (WTPM), particle size, and selected constituents (propylene glycol [PG], nicotine, benzo-a-pyrene (BaP), carbonyls and carbon monoxide [CO]). Spatial and temporal stability among exposure ports were acceptable. Aerodynamic particle sizes were ~2.1 (Test) and 0.7 µm (Ref) and the aerosol concentrations were overall reproducible within the targets (200 to 1200 µg/L WTPM). Test aerosols had no measurable CO and BaP, substantially lower nicotine and carbonyls, but higher PG compared to Ref aerosols. During 3-day exposures, male rats (5/group) were exposed to air (Control), Test (800 & 1200 µg/L WTPM), or Ref (1200 µg/L WTPM). Abnormal clinical signs were limited to some Ref rats. Post exposure blood carboxyhemoglobin was high only for Ref (38%) while plasma nicotine was slightly higher for Ref compared to Test. Microscopically, there were only minimal lesions in larynx (epiglottis) and trachea (1-2/5) of Test-High, while the respiratory tract lesions (nose, larynx [epiglottis/lateral wall], trachea, and lung) were in greater severity and incidence (1-4/5) in Ref-High. In summary, overall consistent with the previous chemistry and *in vitro* results, test aerosol from a tobacco-heating device showed substantially reduced biological activities in rats.

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Analysis of selected carbonyl compounds in e-aerosols and e-liquids using pentafluorobenzyl hydroxylamine derivatization and gas chromatography-mass spectrometry

An improved gas chromatography-mass spectrometry (GC-MS) method using o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride (PFBHA) derivatization has been developed for the analysis of carbonyl compounds in e-aerosols and e-liquids. In addition to formaldehyde, acetaldehyde, acetone, propionaldehyde, acrolein, methyl ethyl ketone (MEK), butyraldehyde, and crotonaldehyde, this method has also been validated for the determination of glycoaldehyde (a hydroxycarbonyl), dicarbonyls (glyoxal, methylglyoxl, 2,3-butanedione), and methyl vinyl ketone (MVK).

For e-cigarette aerosols, carbonyls were collected by passing the aerosol through a 44 mm glass fiber filter pad into a cryogenic trap (≤ 70 °C). The pad was extracted with the trapping solution. 1 mL of the extract was diluted with water and derivatized with PFBHA at room temperature for 24 hours. Three solvents were evaluated for trapping efficiency: methanol, isopropanol (IPA), and acetonitrile. All three demonstrated excellent trapping efficiency with no impact on the derivatization. However, significant amounts of acetone and MEK were found in methanol and IPA making these solvents unsuitable for this application. The PFBHA derivatives were then extracted into toluene and analysed by GC-MS using selected ion monitoring (SIM) mode.

For e-liquids, a 0.5 g sample was diluted with water and derivatized with PFBHA using the same process as e-aerosols. However, for some flavoured e-liquids, up to 20 times more PFBHA was required to complete the derivatization of the unsaturated carbonyls and 2,3-butanedione (diacetyl). In order to ensure a sufficient amount of PFBHA had been added, fortified matrix test samples had to be prepared.

This method exhibited good linearity (R^2>0.99) and specificity. The accuracy and precision of the method was evaluated using fortified commercial e-liquids and e-aerosols. For all investigated carbonyl compounds, recoveries ranged from 82 to 117% with precision between 2 and 16%. Most of the target compounds could be quantified to levels below 0.1 µg/g for e-liquids, and 1.0 µg/collection for e-aerosols.

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Method for alkaloid determination in tobacco that is highly selective, sensitive and suitable for regulatory reporting

Smokeless tobacco manufacturers have been required to annually report concentrations of nicotine in smokeless tobacco products (STP) sold in the United States to the Centers for Disease Control and Prevention (CDC) since 1999. The CDC protocol requires nicotine measurements conducted by gas chromatography with a flame ionization detector (GC-FID). Since the CDC protocol specifies a non-selective detector, new matrices and matrices with known interferences often require that calibration be performed by standards addition to accurately determine nicotine concentrations. The objective of the work was to validate and compare a more rapid, sensitive and selective method using GC with mass spectrometry detection (GC-MS) to the CDC's GC-FID protocol. Statistical analysis (e.g. Schuirmann's two one-sided tests (TOST) approach) determined that the two methods can be considered equivalent for the quantitation of nicotine in STPs and are thus both suitable for regulatory reporting. The GC-MS method had a broader calibration range (0.8-50 mg/g) compared to the CDC protocol (3.0-60 mg/g) and had no matrix interferences due to the selectivity of MS detection. The GC-MS method demonstrated a more than a tenfold increase in sensitivity. This method was validated for the quantitative analysis of nicotine as well as three additional alkaloids (nornicotine, anabasine, anatabine) in STPs, leaf tobaccos, cigarette filler, pipe tobacco and cigar filler.

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Effect of laboratory conditions on e-cigarette aerosol collection

Smoking machines were first developed to generate smoke from tobacco cigarettes for the purpose of comparing cigarette tar and nicotine yields under consistent conditions. The International Organization for Standardization (ISO) specifies the atmosphere for the conditioning and testing of tobacco products in reference document ISO 3402:1999E. Conditioning is typically conducted for 48 hours and requires an atmosphere of 22 ±1 °C and 60±3% relative humidity (RH). The testing atmosphere requires 22 ±2 °C and 60±5% RH. No standardized environmental conditions or smoking regimes exist for e-cigarette aerosol collection. Therefore, the purpose of this work was to evaluate the effect of laboratory environmental conditions on the collection of e-cigarette aerosols using a consistent puffing regime. While temperature can typically be controlled in most laboratories, RH cannot. Therefore, RH was the primary focus of this investigation. Commercial e-cigarettes were puffed using a square wave profile for four seconds, 55 cc puff volume, and 30 second puff interval on a 20 port linear smoking machine. Twenty puffs were collected on conditioned Cambridge filter pads (CFP) and the aerosol mass (AM) collected was evaluated for total mass and concentration of nicotine, menthol, propylene glycol, glycerin, and water using gas chromatography with flame ionization and thermal conductivity detectors. Aerosol collection was conducted at 22 ±2 °C with a %RH of 40, 60, and 80. Differences in analyte concentrations at the various RHs will be discussed.

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The flavour delivery characteristics of twin capsule filter in cigarette designs

Sales of conventional cigarettes are decreasing while sales of cigarettes containing capsules are increasing. Demand for twin capsule cigarettes is increasing in the Korean market due to the flavour. When twin capsules were applied in one cigarette, mainstream smoke flavour delivery was evaluated. As ventilation levels and position of capsule were changed, flavour delivery also changed. This research presents the maximum flavour delivery in mainstream smoke. NTM (Non Tobacco Material) was designed by ventilation levels and positions of the capsule within the cigarette. The study design included two ventilation levels (0% and 80%) and two positions of the capsule within the cigarette (mouth section and tobacco section). Combinations of these two materials were made of four cases (0%-mouth, 80%-mouth, 0%-tobacco, 80%-tobacco). In case of low ventilation, a large amount of flavour was delivered by capsule break in tobacco section. In case of high ventilation, plentiful amount of flavour was delivered by capsule break in mouth section. The results were proved by thermal image analysis, sensory evaluation and CFD programs. Which position of capsule break was good for higher amount of flavour delivery in case of different ventilation levels? High ventilation was mouth section and low ventilation was tobacco section.

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Sub-chronic inhalation toxicity study of cigarette smoke from the 3R4F reference cigarettes in rats

The 3R4F reference cigarette has been developed to replace the 2R4F reference cigarette due to diminishing stock of 2R4F. The toxicity data of 3R4F is limited. In this study, the effect of mainstream cigarette smoke of 3R4F was evaluated in compliance good laboratory practice (GLP) guidelines under the International Organization for Standardization (ISO) machine smoking conditions. Male and female Sprague-Dawley rats were exposed to mainstream cigarette smoke at 50, 100 or 200 µg total particulate matter (TPM)/L for six hours per day, five days per week, for 13 weeks. Mortality, clinical signs, body weights and food consumption were observed during exposure periods. Clinical chemistry, haematology, blood carboxyhemoglobin, urine nicotine and its metabolites, gross pathology and histopathology were also determined. In males, body weight was significantly decreased at 200 µg TPM/L and food consumption was significantly decreased at above 100 µg TPM/L. However, body weight and food consumption did not show significant decrease in females. All smoke-exposed groups had equivalent increases in blood carboxyhemoglobin, urine nicotine and its metabolites relative to the control group. Histopathological changes, such as increased alveolar macrophages, pigmented, thickened alveolar wall and hypertrophy/hyperplasia, were observed in lungs at above 50 µg TPM/L. These changes were related to the increase of absolute or relative lung weights. Exposure of cigarette smoke related changes were also observed in nasal cavity, larynx and trachea in histopathological examination. It was concluded that the respiratory tract was adversely affected via inhalation exposure of cigarette smoke. These results may be used as the basic data for comparison of the tobacco products toxicity.

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The process of CORESTA cooperation

The vision of CORESTA is "to be recognised by our members and relevant external bodies as an authoritative source of publically available, credible science and best practices related to tobacco and its derived products."

To pursue this vision, CORESTA develops analytical methods, produces technical, study and reference reports, and publishes guides and recommendations on good practice and usage. CORESTA documentation is available from its website at www.coresta.org and reflects the work done within the Association through cooperation.

The steps leading to this work and the related outputs needed to be formally structured and documented in order to ensure sustainable improvements. A CORESTA Standards Task Force was then launched in 2012 to streamline the cooperation process and ensure that all steps were properly marked and reported to ease the follow-up of the on-going work and further archiving.

The poster will present the flow diagram that describes this process, and the responsible entities, starting from a new work item proposal up to the documents produced and made publically available.

Further perspectives on the future work undertaken by the CORESTA Standards Task Force and the corresponding impacts on the way of working will be commented.

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Analysis of aerosol by low temperature pyrolysis GC/MS method: a simple method to assess aerosol composition

Heat-not-burn (HnB) tobacco products comprise an aerosol tobacco substrate heated by a heating system whose design depends on the HnB product These products are typically heated at a temperature below 300-350 °C, which is sufficient to release nicotine and some tobacco aromatic components, but not high enough to generate significant levels of pyrolysis and combustion products often involved in cigarette smoke toxicity.

The objectives of the developed method are to analyse aerosol composition and assess how different aerosol substrate designs affect it.

Pyrolysis gas chromatography mass spectrometry (Py-GCMS) was used to instantly heat the sample and characterise aerosol emission through mass spectrometry after separation by a capillary column without second reaction generation. The effect on aerosol emission of parameters such as temperature, duration of heating and sample form was tested. Aerosol deliveries increase with temperature and heating duration. The aerosol emission is higher with powder vs sheet form samples. Components of thermal degradation increase with temperature and heating duration. Main components of HnB tobacco aerosol analysed are humectants, nicotine, nicotyrine, aromatic components such as phytol acetate.

The nicotine emission level depends on temperature: it is low below 160 °C and not statistically different between 180 °C and 350 °C. Nicotine emission depends also on humectant levels.

Nicotyrine, a minor alkaloid found in plants and cigarette smoke rather come from tobacco itself than thermal degradation according to literature on thermal degradation of nicotine.

In conclusion, this method allows screening of the potential of different substrates to generate aerosol in standardized heating conditions representing the ones of various HnB products. It can provide interesting indications on the relative amounts of the various analytes present in the aerosol. Nevertheless, this type of approach is not sufficient to evaluate the absolute level of each analyte, as it neither takes into account the amount of substrate heated, nor the dynamic of aerosol flows within the final HnB product design.

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Comparison of CORESTA Recommended Methods with the simultaneous determination method of volatile organic compounds and carbonyls in mainstream cigarette smoke

There are several methods to analyse constituents in mainstream cigarette smoke. Some methods have already been validated and others are under development. The simultaneous determination method of volatile organic compounds (VOCs) and carbonyls was published (Uchiyama, 2013). On the other hand, CORESTA has developed CORESTA Recommended Methods (CRMs) No. 70 for VOCs and No. 74 for carbonyls.

In this study, we compared the simultaneous determination method with CRM70 and CRM74 at the same laboratory. In the simultaneous determination method, VOCs and carbonyls which passed through a glass fibre filter pad were trapped by carbon adsorbent in a cartridge instead of the impingers. VOCs were eluted by first elution with carbon disulphide and carbonyls were eluted by second elution with methanol. VOCs were analysed by gas chromatography-mass spectrometry (GC-MS), and carbonyls were analysed by high-performance liquid chromatography (HPLC) after derivatisation. As a result, formaldehyde measured by the simultaneous determination method was less than the one measured by CRM74. One possible reason is that formaldehyde dissolves in water on a glass fibre filter pad which collects particle constituents and water in mainstream cigarette smoke.

Repeatability of the methods was investigated, and the good repeatability was shown to these methods. Trapping efficiency of the simultaneous determination method with double cartridges was investigated, too. Almost 100% of VOCs and carbonyls except formaldehyde were trapped by the first cartridge.

As an additional experiment, formaldehyde was analysed by the simultaneous determination method without a glass fibre filter pad. Even without a glass fibre filter pad, the amount of formaldehyde was lower than the one measured by CRM74.

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Using residual length data to estimate the rate of self-extinguishment of banded cigarettes

For non-banded lower ignition propensity (LIP) cigarettes the residual length of extinguished cigarettes has been shown to be useful to more precisely estimate the rate of self-extinguishment compared to just counting self-extinguished cigarettes (ISO 12863). In practice, however, cigarette papers with bands are much more common on LIP cigarettes. The present study investigates, if residual length data of banded cigarettes may also help to provide a more accurate estimate of the rate of self-extinguishment.

Of six different banded LIP cigarettes with self-extinguishment rates between 75% and 100% the rate of self-extinguishment and the residual length were measured with three replicates of 40 cigarettes for each cigarette design. The rate of self-extinguishment was estimated from a statistical model based on residual length data and compared to ISO 12863 data. The results show that practically no improvement with respect to the accuracy of the estimate can be achieved. These results agree with predictions obtained from simulation. As banded LIP cigarettes extinguish at the bands, but as the band position is random, residual length data of banded cigarettes contains variability, which does not provide additional information about the rate of self-extinguishment. This deteriorates the accuracy of the estimate. To improve the estimate, the rate of self-extinguishment was estimated from the number of the band at which the cigarettes extinguished. Again estimates obtained from a statistical model were compared to measured results and to simulations and a reasonable agreement was found. It was found that the standard deviation of the estimated rate of self-extinguishment can be reduced by about five to ten percent for self-extinguishment rates of 75-100%. Overall it has to be concluded that for banded LIP cigarettes residual length data contains little additional information about the rate of self-extinguishment, but still offers some limited potential to provide a better estimate of the rate of self-extinguishment than counting extinguished cigarettes.

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New highly sensitive and selective method for carbonyl determination in e-cigarette aerosols

Low levels of thermal degradation products such as carbonyls (formaldehyde, acetaldehyde, acrolein, and crotonaldehyde) have been reported in e-cigarette aerosols. The collection techniques and analytical methodologies used for the quantification of carbonyls in e-cigarette aerosols have been adapted from methods developed for tobacco cigarettes. These methodologies typically use HPLC-UV and are often not sensitive enough to detect the low levels of carbonyls found in e-cigarette aerosols (e.g., LOQ ~0.3 µg/puff). These methods are also subject to interference from e-cigarette flavor systems resulting in a potential for false positive identifications or incorrect quantification of carbonyls. Therefore, the objective of this work was to develop and validate a rapid, selective and sensitive method optimized for the analysis of carbonyls in e-cigarette aerosols using UPLC-MS. For this work, e-cigarette aerosols were trapped in sequential 20-puff collections following a puff regimen of 4 second duration, 55 mL volume, 30 second interval and square wave profile. The collection apparatus involved a 5-port linear smoking machine fitted with a 44 mm Cambridge filter pad followed by an impinger containing acidified 2,4-Dinitrophenylhydrazine. This optimized method showed high trapping efficiency, an LOQ of 0.016 µg/puff and an instrument run-time of only four minutes. Six leading commercially available e-cigarettes were evaluated (five devices each) to confirm that the method was fit-for-purpose. All commercial products tested contained formaldehyde and in most cases, the levels were well below those observed in conventional tobacco cigarettes (less than 3 µg/puff). However, for some commercial products, levels above tobacco cigarettes were detected with the highest at 14.1 µg/puff.

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Testing, standards and Good Manufacturing Practices

AFNOR[1] recently published the world's first voluntary standards for testing electronic cigarettes and e-liquids. This framework makes it much easier for manufacturers and consumers to know which methods to use when testing e-vapour products and how to assess and compare data. Gaps still remain due to the fact that these standards are based on existing technology, and technological advancements in e-vapour devices may, at some point in the future, imply new testing standards. While these are recreational nicotine-delivery products and not medical devices, existing pharmaceutical practices can still be useful in defining the testing methodology framework for developers/manufacturers to ensure that an ad-hoc assessment is run on each new technology or product. Flexibility in such practices should be allowed so that alternative methods can be introduced to suit specific devices. The objective of this presentation is to review existing testing methods, and to discuss how a light approach to pharmaceutical product development could help e-vapour developers establish best practices. Such an approach also aims at ensuring the alignment between a product's objective and the tests, methods and controls deemed necessary for applying to any new technology created in line with the Electronic Nicotine Delivery Devices.

[1] AFNOR: The French Standardization Agency is an international services delivery network that revolves around four core competency areas: standardization, certification, industry press and training. As the French representative of the European and international standards organizations, AFNOR aims to promote innovation, excellence in performance and sustainable development of companies and civil society.

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Development and validation of a portable puffing topography analyser

Puffing topography data (puff volume, duration, number and inter-puff interval) are central to understanding how cigarettes are used by smokers and a number of commercial devices are available to record puffing topography. Puffing topography studies conducted in a central location have been observed to influence smokers' behaviour and therefore development of portable devices that enable measurement of smokers' puffing topography in their everyday environment is required to provide data that are reflective of actual use-behaviour.

The objective of the project was to develop and validate a portable puffing topography analyser able to record and store smokers' puffing topography data in their everyday environment.

The portable smoke analyser (PSA) was developed using the operating functionality of our laboratory based Smoking Analyser Number 7 (SA7) equipment (British American Tobacco). Topography data are recorded at a frequency of 25 Hz and stored on a micro-SD™ memory card. Six devices were validated in terms of accuracy of puff volume and duration using three replicate measurements of 16 puffing regimes repeated over three days, to produce a total of 864 measurements.

Puff volumes were within ±1.0 mL of the pre-set volume across the range 25-80 mL, for 854 of the 864 total measurements recorded. Puff durations were within ±0.2 s of the durations recorded by the SA7 for 859 of the total measurements.

Following completion of laboratory based validation we have conducted a field study to compare the PSA and SA7 in their ability to record smokers' puffing topography, with the PSA being used in both central location and home use testing.

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Analysis of nitrosamine content, moisture, and pH in pouched smokeless tobacco products: a comparison of constituents in the intact product, tobacco and pouching material

The purpose of this study was to determine if water content, pH and select chemical constituents differed in the pouching material versus the tobacco of portioned smokeless tobacco products. In this study, nineteen different pouched moist snuff and snus smokeless tobacco products where analyzed for volatile and tobacco specific nitrosamine content in the tobacco, the pouch material and in the intact product. Sample pH and % oven volatiles were also determined for all samples. We found that % oven volatiles were higher in the intact product versus the tobacco alone by ~0.1% to 5.8% while the pH of the tobacco alone was generally higher than the intact product. The most pronounced differences between intact product, tobacco material, and pouching material were observed in nitrosamine content. We found that nitrosamine distribution between the tobacco and pouching material varied by product type, e.g. moist snuff versus snus, and that the relative distribution of the nitrosamines between the tobacco and pouching material was consistent within different products for a given product type. We will present data illustrating the differences in nitrosamine distribution, pH, and % oven volatiles in the intact product, tobacco alone, and pouching material and discuss practical implication for product testing.

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Effect of power level on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols

The study objective was to determine the effect of power applied to the atomizer of refillable tank-based e-cigarette (EC) devices. Five different devices were evaluated, each at four power levels. Aerosol samples were collected using a 55 mL puff with a four second duration. Aerosol results will be reported for each puff block as mass/puff and normalized for the power applied to the coil in mass/watt. The range of aerosol produced on a per puff basis ranged from 2.5 to 34.4 mg, and, normalized for power applied to the coil, ranged from 0.38 to 1.38 mg/watt. Aerosol samples were also analysed for the production of formaldehyde, acetaldehyde, and acrolein at each power level. The amount of formaldehyde, acetaldehyde, and acrolein produced per puff ranged from 0.04 to 74.4 µg, 0.05 to 56.1 µg, and <0.02 to 12.9 µg, respectively. The amount of formaldehyde, acetaldehyde, and acrolein produced per mg of aerosol produced was from 0.009 to 9.49 µg, 0.004 to 7.16 µg, and <0.002 to 1.65 µg, respectively. These results were used to estimate daily exposure to formaldehyde, acetaldehyde, and acrolein from EC aerosols, and were compared to estimated daily exposure from both consumption of cigarettes and workplace exposure limits. Two of the devices produced aerosol that exceeded aldehyde workplace exposure limits and the estimated exposure due to combustible cigarettes usage. The other three devices produced aldehyde amounts below both the estimated exposure due to combustible cigarettes usage and a workplace exposure limit.

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Impact of smoking cessation on the metabolomic profile of former smokers

Smoking is known to cause several diseases. However, the underlying pathophysiological mechanisms are still not completely understood. In order to prevent these diseases it is of paramount importance to identify biomarkers helping to decipher the root cause of smoking induced perturbations in biochemical pathways.

Gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS) has evolved as a powerful tool for untargeted metabolomic profiling. It combines the separation power of GC with structural information obtained by high resolution mass spectrometry. GC-TOF-MS has been successfully applied in a previous study at ABF in order to identify differences in the metabolome between smokers and non-smokers under strictly controlled dietary conditions.

The purpose of the current study was to analyse the changes in the metabolome in different body fluids (blood, saliva, urine) of former smokers who decided to quit smoking. A human study with 60 healthy smokers was conducted in order to investigate the impact of smoking cessation at different time points on the metabolomic profile. The subjects stayed at the clinical research organisation for 24 h (8 a.m. to 8 a.m.) at time point (TP) 0, when they still smoke. Dietary intake as well as the biological sample collection procedure (plasma, urine, saliva) was performed under strictly-controlled conditions. TP 0 was used in the following as baseline/reference TP. The same conditions and procedures were repeated after smoking cessation for 1 week (TP 1), 1 month (TP 2) and 3 months (TP 3). In order to check the compliance with smoking abstinence, urine and saliva samples were collected and analysed for cotinine at increasing intervals.

The fully validated GC-TOF-MS method revealed differences in the plasma metabolome of smokers still smoking and after having quit. This presentation will share the first results on how the plasma metabolome changed over a period of three months of smoking abstinence.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 22

A novel method for the determination of propylene glycol and glycerine from various biological matrices – suitable biomarkers for e-cigarette consumption

Despite the popularity e-cigarettes have gained worldwide, very little rigorous research has been performed in terms of product control and the effects of these devices on human health. The systematic and objective assessment of the actual dose by suitable analytical methods is a pre-requisite for the toxicological evaluation of this new product category. Particularly, the systematic evaluation of the quantitative assessment (dosimetry) of the main e-liquid components (nicotine, propylene glycol (PG) and glycerine (G)) as well as the uptake into the human body via the route of inhalation is currently almost completely missing.

We developed and validated a bioanalytical method for the quantification of PG and G in various human body fluids (plasma, urine, saliva). The method combines the separation power of gas chromatography in conjunction with the high specificity provided by MS detection. Method validation according to FDA guidelines showed excellent results in terms of sensitivity, reproducibility and robustness. Moreover, the use of minimal sample volume along with a straightforward sample extraction procedure allows a time- and cost-efficient analysis of these compounds in large clinical studies.

The method was applied to samples derived from an e-cigarette pilot study. This poster presentation will share the outcome of PG and G analysis in body fluids derived from an e-cigarette pilot study and will discuss their potential as biomarkers of exposure to e-cigarettes.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 29

Smokeless tobacco particle size: insufficient science for regulation

Tobacco regulatory policies worldwide should be grounded in science-based evidence. Documents obtained through the U.S. Freedom of Information Act (FOIA) indicate the Food and Drug Administration (FDA) is internally recommending that tobacco particle size be incorporated into regulatory actions (e.g. substantial equivalence determinations). This is based on the supposition that smokeless tobacco cut styles differentially impact constituent delivery to the consumer.

Cut style descriptors typically used with U.S. moist snuff smokeless tobacco products include terms such as "fine cut" or "long cut." Consumer information regarding their difference is typically gained from product labelling and general appearance rather than precise measurements, and there is no industry standard for what constitutes a particular cut style. FDA's recommendation suggests that smokeless tobacco particle size is a product characteristic that must be measured and reported in the context of market clearance applications, despite the lack of any formal agency guidance or regulation on the matter.

Available scientific data does not give appropriate or relevant information regarding the significance of smokeless tobacco cut styles, and there is currently very limited data that examines whether a difference in cut style has any meaningful significance beyond that recognized by the consumer. The only study of which we are aware that examined two products matched in nicotine and pH but differing in cut style (fine cut vs. long cut) showed no difference in nicotine uptake. Therefore, a pilot study was conducted to analyze the physical characteristics of moist snuff tobacco in order to determine the feasibility of generating data that might be useful for regulatory and reporting purposes.

Four commercially available moist snuff tobacco products were purchased at retail and analyzed using a variety of traditional particle testing techniques. Analytical results including surface area and particle porosity were used to evaluate methods for characterizing smokeless tobacco particle size with differing cut style descriptions, and the results will be discussed in this presentation. These data will also be used to assess whether it is appropriate to use currently existing methodology to evaluate tobacco products and discuss its significance in both a scientific and regulatory context.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STW04

E-cigarette and advanced personal vaporizers: current leading technologies and trends and the importance of materials and design on performance

E-cigarettes and vaporizers have the potential to be the greatest harm reduction tool introduced in recent memory. Since the introduction of e-cigarettes technology has continuously improved to meet changing consumer demands.

This session of the workshop will cover the history of e-cigarettes and vaporizers from the early devices to modern, high power and temperature controlled tank based atomizers. Topics covered will include basic e-cigarette/vaporizer design, key milestones in the industry, and current industry leading technology with a focus on cutting edge next generation devices.

Details will be provided on the differences between disposable/rechargeable "cigalike" devices versus power regulated and mechanical vaporizers. This will include a discussion of high wattage and temperature controlled devices. Additional topics will include real world examples of how the choice of coil and tank material and general device design features impact device performance.

The talk will close with an overview of the future direction of devices with a focus on product research and development cycle its impact on product timelines and product innovation.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 37

Study of adsorption materials in cigarette filter by inverse gas chromatography

Inverse gas chromatography (IGC) method was introduced to determine the thermodynamic and kinetic parameters between specific gas molecules and column packing materials, which played an important theoretical role in understanding the selective adsorption of different adsorption materials in cigarette filters for smoke components. The retention time of probe molecules with different polarities on the surfaces of active carbon, silicon dioxide, graphene, ion exchange resin, molecular sieve, etc., was determined by gas chromatography-inverse gas chromatography (GC-IGC) system with double column ovens. The surface physicochemical parameters of the above adsorption materials were obtained by physicochemical calculation methods. The data of this experiment showed that: 1) Although the interactions between active carbon and several probe molecules were not the strongest, active carbon still presented better adsorption effect due to many active sites on its surface. 2) There was a large amount of oxygen atoms on the surface of silicon dioxide and ion exchange resin, not only simple physical adsorption but also chemical adsorption occurred on their surfaces. 3) The surface of graphene oxide was Lewis base, it possessed a stronger adsorption capacity for organic acid; while the surface of reduced graphene oxide was Lewis acid, it possessed a stronger adsorption capacity for organic base. 4) Molecular sieve presented a certain selective adsorption effect due to its special pore structure and surface composition. Taking advantage of ion molecule reaction-mass spectrometer (IMR-MS), the adsorption laws of some smoke components upon the solid adsorption materials in cigarette filter were validated. The results of this experiment are helpful to understand the strength of interactions between smoke components and adsorption materials from a molecular level and provide a reference for the research and development of novel adsorption materials for cigarette filter.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 23

A single step solid-phase extraction method for GC/MS analysis of aromatic amines in mainstream cigarette smoke

As stated in the Federal Register (Vol. 77, No. 64) Docket No. FDA-2012-N-0143, Aromatic Amines (AAs) are included in the "Established List of the Chemicals and Chemical Compounds Identified by FDA as Harmful and Potentially Harmful Constituents [HPHCs] in Tobacco Products and Tobacco Smoke". To date, no standardized method for AA determination in mainstream cigarette smoke has been developed. The two previously reported techniques for AA determination require either two or three solid phase cartridge extractions and the use of a solid phase extraction (SPE) manifold because of the complexity of the matrix and the trace amounts of AAs in cigarette smoke (ng per cigarette). These multistage techniques are time consuming and difficult to automate. The purpose of this work was to develop a simplified and automated extraction technique for three AAs (1-aminonaphthalene, 2-aminonaphthalene and 4-aminobiphenyl) in mainstream cigarette smoke without compromising analyte recovery. The method uses solid-phase mixed-mode cationic exchange cartridges (e.g., Waters Oasias® MCX or Phenomenex Strata™-X-C), is compatible with the RapidTrace® automation, eliminates multiple classes of interferences and samples can be analyzed by gas chromatography with mass spectrometry (GC/MS). Recovery of AAs was evaluated using labeled internal standards and ranged from 48.2 to 52.4% for 1-aminonaphthalene, 2-aminonaphthalene and 4-aminobiphenyl with the cigarette smoke matrix and ranged from 77.7 to 85.0% without the sample matrix. Recovery yields were consistent with the previously proposed methodologies.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 04

Determination of diacetyl in e-vapor products using gas chromatography and mass spectrometry

While diacetyl is approved for food use, the United States National Institute for Occupational Safety and Health (U.S. NIOSH) has suggested it may be associated with respiratory disease when heated and inhaled. NIOSH has defined safety limits for occupational exposure to diacetyl. Farsalinos and co-workers recently (2015) investigated 159 sweet-flavored e-vapor refill formulations where they observed that 110 contained measurable amounts of diacetyl with many exceeding NIOSH limits. Farsalinos and co-workers used a modified method developed for the analysis of carbonyls in mainstream cigarette smoke to quantify diacetyl in e-vapor formulations. This method was validated for e-vapor formulations and involved derivatization using 2,4-dinitrophenylhydrazine (DNPH) followed by high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector. The purpose of this work was to develop a more sensitive and selective method for the analysis of diacetyl using gas chromatography and mass spectrometry (GC/MS). This method does not require derivatization, uses a labeled internal standard, and the MS is operated in the selected ion monitoring (SIM) mode to maximize selectivity and sensitivity. All requirements for method validation were met such as linearity, accuracy, precision, limits of detection (LOD), and limits of quantitation (LOQ). For example, the coefficient of determination is greater than 0.990, the calibration ranged from 0.1 µg/g to 16.2 µg/g of e-cigarette formulation and the recovery ranged from 90 to 110%. The method is suitable for potential regulatory reporting and the quality control purposes that may be needed in this product category.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 50

Study on mechanical filtration through tobacco columns: the effects of cut filler shape and size distribution

There have been several reports on tar and nicotine filtration through tobacco columns. Using tar and nicotine for the measurement of mechanical filtration through tobacco columns is not appropriate because tar and nicotine filtration includes the effect of condensation on cut-filler. Previous studies have evaluated not only mechanical filtration, but also condensation on cut-filler. Therefore, the behaviour of mechanical filtration through tobacco columns remains unclear. Since tobacco columns have specific shaped cut filler and a wide range of particle size distribution, this may be the cause of the specific filtration behaviour. Understanding the effects of shape and size distribution on mechanical filtration contributes to the improvement of the prediction accuracy of mechanical filtration efficiency.

In the present study, mechanical filtration through tobacco columns has been investigated using PSL (Poly Styrene Latex) standard particles to compare actual filtration efficiency with predicted efficiency by theoretical and/or empirical equation for spherical packed beds. Furthermore, the effect of cut-filler shape as well as of cut-filler size distribution on mechanical filtration have been investigated with a size shape factor determined through pressure drop measurements and a distribution factor determined through sieve measurements.

Since the effect of diffusion in tobacco columns was lower and the effects of interception as well as inertia were higher compared to spherical beds, our results imply that a partially faster flow could occur in tobacco columns compared to packed beds with uniform granular size. In addition, filtration efficiency was shown to correlate with size shape factor ($r=0.894$, $p<0.05$) and distribution factor ($r=0.683$, $p<0.15$). In that respect, the size shape factor has exhibited a higher coefficient of determination ($n=6$) compared to the distribution factor. This suggests that the size and shape of cut-filler should be considered into the filtration through tobacco columns in order to improve prediction accuracy.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 24

UPLC-MS/MS for high-throughput analysis of aromatic amines in cigarette smoke

Aromatic Amines (AAs) are included in the "Established List of the Chemicals and Chemical Compounds Identified by FDA as Harmful and Potentially Harmful Constituents [HPHCs] in Tobacco Products and Tobacco Smoke" (Federal Register (Vol. 77, No. 64) Docket No. FDA-2012-N-0143). To date, no standardized method for AA determination in mainstream cigarette smoke has been developed. Previously reported techniques for the quantitative analysis of trace amounts of AAs in cigarette smoke (ng per cigarette) included gas chromatography with mass spectrometry (GC-MS) involving multistep solid phase extractions (SPE) or high performance liquid chromatography with multistage mass spectrometry (HPLC-MS/MS) with liquid-liquid extraction. The purpose of this work was to evaluate a higher throughput approach using ultra-performance liquid chromatography (UPLC) with MS/MS with a single step SPE clean-up. This method demonstrated applicability to all AAs on the FDA HPHC list and was validated for the three AAs on the current abbreviated HPHC list. All requirements for method validation were met such as linearity, accuracy, precision, limits of detection (LOD), and limits of quantitation (LOQ). For example, the linearity was demonstrated with a coefficient of determination of greater than 0.990 for the calibration ranges of 1.5 to 150 ng/cigarette for 1-aminonaphthalene, 0.75 to 75 ng/cigarette for 2-aminonaphthalene, and 0.6 to 30 ng/cigarette for 4-aminobiphenyl under the ISO smoking regime.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 06

Influences of shredded stem on cigarette properties

Shredding is a process of forcing tobacco stems between counter-rotating toothed blades in a mill where they are stripped lengthwise into thin, fibrous particles. Three types of stems were prepared, namely expanded shredded stem (ESS), expanded cut stem (ECS) and expanded cut rolled stem (ECRS). The impact of the three different stem treatments on mainstream smoke emissions and main physical characteristics of the cigarette as such were investigated. The results showed that: 1) Cigarette containing ESS (ESS cigarette) delivered more tar, nicotine and phenol, less CO and HCN comparing with cigarette containing ECS or ECRS. 2) When the proportion of stem material in cigarette blend was 20%, the deliveries of tar, nicotine and phenol in the smoke of ESS cigarette increased by 6.3%, 11.2% and 10.8%, while the deliveries of CO and HCN reduced by 11.6% and 3.4%, respectively, compared with ECS cigarette. 3) At the same inclusion rate of the respective blend material, the pressure drop and ventilation rate of ESS cigarette were lower, while its firmness was higher than those of ECS and ECRS cigarettes. 4) With the increase of shredded stem length, the CO delivery, pressure drop, ventilation rate and the cigarette ends fall out of ESS cigarette decreased, while the deliveries of tar and nicotine did not change. 5) Drying manner would influence the shredded stem. Compared with cylinder drier, the shredded stem dried by the flash evaporation drier increased the pressure drop, ventilation rate and firmness of cigarette and decreased the deliveries of tar, nicotine and CO in mainstream cigarette smoke.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 25

Application of linear models to link cigarette yields delivered under ISO and intense regimes

Since it was first requested to measure and to report tar and nicotine in a limited number of countries, observation is made of an increasing trend for more testing and reporting requirements. From a single regime and two smoke yields, recent recommendations include nowadays two smoking regimes and up to 93 tobacco and smoke constituents.

Considering the increase of the number of data generated and the similarities between certain smoke constituent formations during combustion, it is worth to explore possible correlations between emissions. For this purpose, 90 smoke constituents have been analysed under ISO and intense regimes for 23 different brands as well as 36 tobacco constituents and 17 cigarette design parameters; in total 5359 data were available for statistical evaluation.

A comprehensive search for the best subsets of up to five explanatory variables for predicting ISO smoking regimes in linear regression have been performed using an efficient branch-and-bound algorithm. Best models of all sizes have been selected using different classical criteria such as correlation coefficient, Akaike Criteria (AIC) or Bayesian Criteria (BIC).

It was then observed that many yields are predictable from a limited number of variables and consequently that a reduced number of tests could have been carried out without loss of information on product characteristics.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Jeju, 2015, abstr. IG 01

Association mapping in a collection of tobacco reference cultivars. Step One: Variability of smoke constituents

Recent developments of sequencing technologies and computational methods have given the opportunity to detect natural variation underlying complex traits in crops. For this purpose, association mapping can be used to identify the link between tobacco genes or molecular markers to smoke constituents. Such association can support the development of new tobacco varieties suitable for future potential regulatory constraints.

In our study, a panel of 161 tobacco varieties, composed of flue-cured, Burley, dark air-cured and Oriental types, was grown in open field. Because of its large genome, RNA-Seq based sequencing was chosen to capture differences of gene expression together with SNPs variation in the 161 varieties. Three different tissues at two growth stages were used to do a comprehensive analysis of the transcriptome. After curing, cigarettes were made with each variety and were mechanically smoked according to the Canadian Intense smoking conditions. The mainstream harmful or potentially harmful constituents (HPHCs) listed in the FDA abbreviated HPHC list[1] were determined using in-house and internationally recommended methods.

Taking into account multiple factors linked to the growing environment and weight of tobacco actively burnt during puffing, our first investigations showed significant differences of several smoke constituents between varieties, thus demonstrating the potential of association mapping for the development of future varieties. For some constituents, differences were however not significant. A description of the approach and preliminary results obtained from the smoking of a sub-group of flue-cured varieties is reported here.

[1] U.S. Food and Drug Administration, 2012. Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act. http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297828.pdf

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 21

Evaluation of cigarette smoke-induced initiation and promotion potency in Bhas 42 cell transformation assay

Chemical carcinogenesis is known to be a multistage process consisting of initiation, promotion, and progression. There are many *in vitro* assays for detecting initiation (genotoxic) potency, which are adopted by regulatory authorities. In contrast, there are few validated *in vitro* assays for detecting promotion and/or progression potencies despite a significant number of carcinogens being considered to act via non-genotoxic mode of action. In light of this, many industries continue to largely depend on results from animal carcinogenicity studies. Bhas 42 cell transformation assay (CTA) is a simple *in vitro* assay for predicting carcinogenicity of test chemicals by measuring morphologically transformed foci in v-Ha-ras-transfected BALB/c-3T3 cells. The assay consists of two components; initiation assay and promotion assay, and both assay protocols have been validated internationally. The purpose of this study is to evaluate the initiation and promotion potency of cigarette smoke total particulate matter (TPM) and gas vapour phase (GVP) in the Bhas 42 CTA. Kentucky reference 3R4F TPM or GVP were tested in the initiation or promotion assay according to the Organisation for Economic Co-operation and Development (OECD) draft test guideline. Results from the initiation assay indicated that neither fraction gave positive responses up to a significant cytotoxic dose. In the promotion assay, 3R4F TPM but not GVP induced a significant increase of transformed foci. We then investigated the assays potential to discriminate the promotion activities of TPM collected from combustible and heated tobacco products. As a result, the promotion potencies of these products were ranked separately in proportion to the toxicant levels included in the FDA abbreviated list of HPHCs. Furthermore, the results were consistent with that from the *in vivo* skin-painting assay. These results suggest that the Bhas 42 CTA is a useful tool to assess the promotion potency of tobacco products, and could potentially be used to replace the *in vivo* skin-painting assays.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 26

Kinetic analysis of changes in concentrations of tobacco constituents during heating

Heat treatment of tobacco represented by heating and steaming is of crucial importance in the tobacco industry because it influences the quality of cigarettes such as their aroma and taste. The rates of these changes depend on the temperature and the moisture content of the tobacco. The objective of this study was to identify the change rate of some constituents related to aroma and taste during heat treatment. A sample vessel packed with a filler of flue-cured tobacco was put into a through-flow heating unit. The air at 60% or 80% relative humidity had been adjusted beforehand to a prescribed temperature in a range of 333 K to 353 K, and the flow rate of the air had been adjusted to 3 m/s. After the prescribed time, all of the filler was taken out of the unit. The constituents of the filler were extracted with several organic solvents and the yields were determined by gas chromatography. The concentration of the constituents decreased with increasing temperature, and the concentration of nicotine decreased with increasing humidity of the air. The changes in concentrations could be approximated to pseudo first-order reactions. Moreover, the temperature dependence of the rate constant could be expressed by the Arrhenius equation, which allows to obtain the activation energy and frequency factor as parameters of the equation. The curves of the concentrations calculated with the parameters were in agreement with each experimental value under various heating conditions. Therefore, it was concluded that the simplification and the parameters of the reaction are valid.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 01

Tobacco regulations in Korea

Tobacco was introduced to Korea via Japan in 1618. However the modern tobacco industry of Korea started in 1945, after its independence from Japanese rule which had lasted for 36 years from 1910. The Korean government established a "Monopoly Bureau" in the Ministry of Finance in 1948. The Monopoly Bureau controlled tobacco cultivation, cigarette manufacturing and sales of tobacco products in Korea until it was succeeded by the "Office of Monopoly" in 1955, a separate government organisation for national tobacco business. Although the Korean government maintained the tobacco monopoly system until 2002, it began tobacco regulation policy in 1976 by adopting the first health warning on cigarette packs. From that time, diverse tobacco regulation policies including sales restriction, restriction of advertisings, health warnings, labelling and restriction of smoking have been implemented in Korea. Recently the Korean government raised cigarette prices by 80% to cut down smoking prevalence and implemented LIP mandating regulation which came into force on 22 July 2015 to reduce fire accidents caused by cigarette butts. Furthermore, a new regulation introducing pictorial warnings on cigarette packs is under discussion in parliament. There have been endless controversies surrounding tobacco regulations in Korean society, especially cigarette price hikes which provoked a bitter dispute between smokers and the Korean government. Regardless of socio-economic status, most of Korean smokers still consider cigarette price hikes as a means of increasing national tax income rather than an effective no-smoking policy as the government claims. Controversy and strong opposition to current tobacco regulations in Korea indicate that social consensus and science-based regulations are critical factors for successful tobacco regulations.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 52

Characterization of tobaccos and mainstream smoke from machine-made mass-market cigarillos and filtered cigars

Cigars are included in the US Food and Drug Administration's (FDA's) proposed deeming regulations. Mass-market filtered cigars are apparently a priority for FDA because their appearance is similar to 100 mm filtered cigarettes, their low-cost relative to the price of cigarettes and most other tobacco products, and some are highly flavored and alleged to have increased youth appeal. Until recently, there was little contemporary information available on these products other than reports we have made at past meetings of CORESTA and other organizations. While several articles on these products have been published recently (Pankow et al., *N. Engl. J. Med.*, 2014; Caruso et al., *Nicotine Tob. Res.*, 2015; Pappas et al., *J. Anal. Toxicol.*, 2015), none of them have dealt with smoke deliveries and harmful and potentially harmful constituents (HPHC) in mainstream smoke. Tobacco product regulation requires a thorough knowledge of product chemistry. To provide such information we initiated a study that included tobacco chemistry and smoke chemistry of four products: a traditional European-style cigarillo (A) and three 100-mm filtered cigars (B, C, and D). Products were purchased at retail in the Atlanta, GA, area in early 2015. Products (180 cigars each brand-style) were sent to a commercial laboratory for tobacco and smoke analyses (ISO). That laboratory used the 3R4F Kentucky reference cigarette as a laboratory control. Remaining products were reserved for other testing. Some of the results (mg/cigar or mg/cig) obtained (order A, B, C, D, 3R4F) include: tar 36.4, 22.1, 18.4, 19.8, 8.47; nicotine 1.22, 0.878, 0.612, 0.621, 0.93; CO 42.5, 31.6, 36.4, 40.9, 10.9; and (ng/cigar or ng/cig) 4-AMP 11.4, 2.96, 3.32, 3.23, 1.8; NNN 553, 375, 348, 409, 98.9; NNK 329, 366, 333, 349, 88.3. Other smoke analytes followed similar trends except for ammonia, acrolein, and formaldehyde. Data on tobacco analytes will also be presented.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 29

An e-cigarette smoking machine for non-routine analyses

Over the past few years, several commercial laboratories have established capabilities for testing the aerosols generated by e-cigarettes. These laboratories have used test procedures and instrumentation that are likely to become generally recognized methods for the analysis of aerosols generated by e-cigarettes. However, the technology associated with the design and manufacture of e-cigarettes and the formulation of e-liquids is rapidly changing, and new analytical techniques are needed for understanding the underlying science of current and future generations of e-cigarette products. In some cases, these techniques require or will require downstream analytical techniques that are not compatible with existing smoking machines because of space requirements and/or the need for reagents that may interfere with the proper operation of conventional smoking machines. Consequently, we have designed, built, and tested a four-port e-cigarette smoking machine for non-routine analyses of e-cigarette aerosols. We have expanded on the machine design we employed for the measurement of e-cigarette aerosol temperatures (CORESTA Congress 2014, ST 41) using features from constant-vacuum smoking machines designed by A. B. Canon and reported in 1976 by J. R. Newsome et al. (http://industrydocuments.library.ucsf.edu/tobacco/docs/gllb0093) and first reported in 1963 (*Tob. Sci.* 1965 9:102-110). We will report the basic operating parameters of our machine and its use for aerosol stream-visualization, aerosol derivatization (for example, persilylation of TPM with BSTFA/TMCS), and GC-MS characterization of the derivatized and underivatized aerosols generated under normal and so-called "dry-puffing" conditions.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 12

Estimation of mouth level nicotine exposure using filter analysis

Mainstream smoke yields determined by a machine-smoking method cannot adequately predict exposures experienced by human smokers. In this work, a filter analysis was used to estimate mouth level nicotine exposures of smokers. The objectives were to estimate mouth level concentration versus measurement of nicotine in the butts of smoked cigarettes, and to investigate the relationships between mouth level nicotine estimates and yields derived whilst machine-smoking.

388 smoked cigarette butts of five commercially available cigarette brands (ISO tar 5~6 mg) were collected. For method calibration purposes, the five cigarette brands were machine smoked under ISO. Thereafter, the nicotine content in the butt filter and the Cambridge filter pad was determined and mouth level nicotine concentration was estimated. Through that, the relationship between the mouth level nicotine estimates from smokers and nicotine content analysed by machine-smoking was established. Mouth level nicotine concentration could be estimated by analysis of nicotine in the smoked filter butts. The results of the mouth level nicotine estimate were similar in four brands and those of the fifth brand were higher than for the other four. Furthermore, it was found that under the ISO regime, machine derived nicotine yields were higher for all five investigated brands compared to the mouth level estimates for nicotine derived from the filter butt analysis.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 17

The quantitative analysis of major constituents from e-cigarette liquid to aerosol using two types of e-cigarettes and the examination on its aerosol delivery

In recent years, the use of e-cigarette products has increased globally, generating increased interest in better understanding the composition and emissions from these products. The purpose of this study is to examine the delivery of some specific constituents from the e-cigarette liquid to its formed aerosol in the process of atomisation in e-cigarette device. More detailed, excipient, nicotine, water, trace metals and carbonyl compounds in both e-cigarette liquid and its formed aerosol were quantitatively analysed and compared along with aerosol volume measurement, accordingly the delivery ratio of some constituents were observed. The analysis was conducted according to CORESTA Recommended Method (CRM) No. 81 (e-cigarette smoking conditions) using two types of device, commercial refillable tank device and disposable cig-like device, when the composition of e-cigarette liquid were intentionally varied to validate its effects. According to some results, formed aerosol volume of tank device was 6 mg/puff, which was three times higher than disposable device, 2 mg/puff, on average, although it was different to some extent according to samples. The delivery amount of nicotine and menthol in aerosol showed a proportional relationship with respect to that incorporated in e-cigarette liquid, respectively. Also, the ratio of nicotine and menthol incorporated in e-cigarette liquid to its total weight and that delivered to total amount of generated aerosol were found to be comparatively similar levels, respectively.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 28

Process optimisation of ultrasonic vibration-assisted enzymatic digestion of cellulose and pectin in tobacco stems

Tobacco stems contain greater amounts of cellulose, hemicellulose, lignin, and pectin compared to lamina. For this reason, stems have a sensory deficit compared to lamina. The aim of this work was to establish an optimised model, via quadratic polynomial stepwise regression, for the digestion of cellulose and pectin. A $L_9(3^4)$ orthogonal experiment with the four factors including (1) pectinase (0.1-1.0%), (2) cellulase (0.1-1.0%), (3) ultrasonic power (200-400 W) and (4) ultrasonic time (30-40 min) were utilised. Furthermore, the microstructure of treated and untreated tobacco stems under the optimum digestion conditions were analysed by scanning electron microscope (SEM). The results showed that the digestion rates of pectin and cellulose were significantly affected by the four factors mentioned above. The contribution of the four factors to the rate of digestion of pectin was 26.166%, 28.649%, 18.188%, 26.997%. The contribution of the four factors to the rate of digestion of cellulose was 11.550%, 11.550%, 25.671%, 51.228%, respectively. Two quadratic polynomial models were found to be sufficient to describe and predict the digestion rate of pectin and cellulose. The optimal cellulase, pectinase, ultrasonic power and ultrasonic time were 0.9%, 1.0%, 400 W, and 41 minutes, respectively. Under these conditions, the digestion rates of pectin and cellulose were 47.28% and 38.67%, respectively. Compared to a predicted value, the deviations were 2.54% and 3.81%, which confirms the validity of the model for predicting digestion rates of pectin and cellulose. Moreover, the physical characteristics of the treated tobacco stem under the optimum digestion conditions were thinner compared to the untreated stems. The results of this study demonstrated that the proposed model can be successfully applied to optimising the digestion rates of pectin and cellulose.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 27

Analysis of process intensity on tobacco moisture retention based on uniform design

Tobacco moisture retention provides essential information about moisture adsorption behaviour and physical characteristics of cigarette formulating, which also influence cigarette aroma and smoking quality. Effect of the intensity of the casing process on flue-cured tobacco strips was analysed based on uniform design. Isosteric heat, equilibrium moisture content and the monolayer moisture content derived from Guggenheim-Anderson-de Boer (GAB) model of tobacco strips were studied to determine the moisture retentivity with different casing process parameters. Quadratic polynomial regression model and contribution analysis were carried out for understanding correlations between blast temperature, processing time, steam flow compensation and processing capacity and the monolayer moisture content. It was shown that the casing process intensity does affect the moisture adsorption behaviour of flue-cured tobacco and the GAB model was capable of describing the full shape of the water adsorption isotherms of tobacco strips. The synthetic evaluation demonstrated that medium process intensity significantly improved tobacco moisture retention than that with high/weak process intensity. The weak moisture retentivity can probably be attributed to high blast temperature and large steam flow compensation. The study confirmed that medium casing process intensity was the most effective means to improve moisture retention of tobacco strips, thus providing a reliable technical tool for cigarette formulation design and refined processing.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 40

Plasma perforation of tipping paper: selected benefits for cigarette consumption

Plasma perforation represents an advanced technology for the generation of pre-perforated tipping paper. During the perforation process within an inert gas environment, a so-called low-temperature plasma triggers local micro-evaporation events on the Tipping Paper surface provoking the formation of small perforation holes with a high hole density. The stabilities of hole parameters, air permeabilities and cigarette properties like the degree of filter ventilation and open draw resistance are significantly higher with plasma perforation than with the standard techniques of electrostatic and laser perforation. Cigarettes made with plasma perforated tipping paper are more efficient in ventilation rates and smoke yields reduction than conventionally perforated cigarettes due to a more homogeneous air flow through the ventilation zone and larger diffusive contributions to the dilution process. The first target of the present study is to demonstrate the capability of plasma perforation to optimise significantly the carbon monoxide/tar and nicotine/tar ratios of specifically designed cigarette samples. In this context, quantitatively determined diffusion capacities of the respective tipping paper qualities are related to smoke analysis results for scientific confirmation. The second part of this contribution reveals the effect of plasma perforated tipping paper on the sensory properties of cigarette smoke. This is realised by carrying out a survey with a professional smoker panel. The findings confirm that plasma perforation is a smart way to improve the compliance with regulatory targets requested by the tobacco industry and to enhance the physiological perception of the natural taste of cigarettes.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 02

Influence of microwave treatment on the combustibility of tobacco and its kinetic analysis

Microwave treatment can promote the expansion of tissue structure of tobacco leaves and improve their combustibility. To study the structure and combustibility of tobacco leaves treated with microwave for different durations, scanning electron microscope combining with simultaneous thermal analysis was employed in this study. The kinetics at volatile component evolution stage and coke combustion stage during tobacco combustion were analysed. The results showed that: 1) The structure of tobacco leaves expanded gradually with the increase of microwave treatment duration. The tissue of tobacco leaves expanded abundantly and the cross-sectional width of tobacco leaves reached its maximum when the duration of microwave treatment was 55 seconds. 2) The combustion characteristic index, Sn value, of treated tobacco leaves in N_2 or N_2/O_2 atmosphere increased first and then decreased with the increase of microwave treatment duration, and Sn value reached its maximum when the duration of microwave treatment was 55 seconds. It indicated that microwave treatment improved the combustion and pyrolysis performance of tobacco leaves, however the combustibility of tobacco leaves deteriorated once the duration of microwave treatment was longer than 55 seconds. 3) The main thermal weight loss phases of combustion reaction and pyrolytic reaction of tobacco leaves were analysed with Coats-Redfern method and Malek method, and their kinetic analysis was conducted by F1 kinetic model. The full tissue structure expansion and best combustibility of tobacco leaves have been achieved by microwave treatment for optimal duration, which would provide a theoretical reference for the design and development of tobacco products.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 19

Are chemical constituents exhaled in a room where e-vapor products are used?

A controlled clinical study was conducted to determine potential constituents in the atmosphere where e-vapor products and cigarettes were used.

Levels of nicotine, propylene glycol (PG), glycerol, 15 carbonyl compounds (including formaldehyde and acrolein), 12 volatile organic compounds, and 4 trace metals were measured using ISO or EPA methods. Exhaled breath (6 analytes), room air (34 analytes) and surface samples (3 analytes) were investigated. The products used were MarkTen® 2.5% Classic (M10), a Prototype GreenSmoke® 2.4% (GS), Ego-T Tank with subjects' own e-liquids (Tank) and subjects' own conventional cigarettes (CIG). Exhaled breath samples (EBS) were collected at baseline (sham use) and with test products from 37 subjects (23 males and 14 females). Room air measurements were made in a controlled exposure chamber (EC, 113 m^3 with 2.25 air changes/hour). Products (M10 and GS) were used under controlled conditions (10 puffs/person, once/30 minutes for 4 hours) and *ad lib* use (all four products used once/hour for 4 hours). Baseline measurements (without product use) were made in the EC for a 4-hour period, and background measurements were conducted for selected constituents.

Room air levels of nicotine, PG and glycerol, under both controlled and *ad lib* use, were several-fold below the current published limits for workplace exposure to airborne contaminants. Room air formaldehyde levels from M10, GS and Tank systems were similar to the background and baseline. Most of the other constituents measured were below the limit of quantification during M10, GS and Tank use. Significant levels of most constituents were observed during CIG use.

Under the study conditions, for the e-vapor products tested, the few chemical constituents exhaled are several-fold below the permissible limits. The results from surface sample measurements suggest that third-hand exposure to nicotine is unlikely.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 25

Migration of 126 pesticide residues in cigarettes during smoking

Pesticide residues could be an important index in the health risk assessment of cigarettes. The transfer amount of pesticide residues into smoke depends on the physical and chemical properties of pesticides. In order to investigate the distribution rules and transfer rates of pesticide residues in cigarettes during smoking, 126 pesticide standards were added to cigarettes at different concentrations, and the transfer rates of these pesticides into mainstream smoke (MSS), sidestream smoke (SSS), ash and butt were determined by sidestream smoking machines under the ISO standard smoking with liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS) and chromatography (GC-ECD) methods. The results showed that: 1) The transfer rates of suckercides into MSS, SSS and butt were 13.16%, 21.74% and 10.27%, respectively; those of organochlorine pesticides, herbicides and pyrethroids were 8.68%-10.11%, 15.72%-17.28% and 5.65%-6.74%, respectively; and those of organophosphorus insecticides, fungicides, heterocyclic insecticides and carbamate insecticides were 1.70%-5.26%, 1.90%-6.11% and 1.22%-4.55%, respectively; 2) With the total transfer rate of 22.96%, the transfer rate of these pesticides into sidestream smoke was the highest, followed by that into mainstream smoke, that into butt was the lowest, while none of these pesticide residues was detected in ash.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STW03

E-cigarette product regulation and standardisation

E-cigarettes have been introduced and seen substantial sales growth in a number of markets. As a result, regulators, manufacturers, consumer groups and public health scientists have become interested in the quality, safety and performance of these products. The extent of regulation ranges from limited controls, as consumer products, pharmaceutical/medical regulation to prohibition and product standards vary widely.

This presentation will provide an overview of current product regulation in the major markets, the different stakeholders in the standardisation process and current status of activity in standardisation and other bodies, for example, the request by the Conference of the Parties to the FCTC for the formation of an expert group on e-cigarettes, New Work Item Proposals in ISO TC126 Tobacco and Tobacco Products on an analytical puffing machine and analytical methods for nicotine and other constituents in emissions, the progress by CEN TC437 on Electronic cigarettes and e-liquids, the publication by Association Française de Normalisation (AFNOR) of three voluntary standards on devices, liquids and emissions, the publication by the British Standards Institution of Publicly Available Specification 54115, and the contribution being made by the CORESTA E-cigarette Task Force.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. IG 02

Update on the progress of the Framework Convention on Tobacco Control

The last Conference of Parties of the Framework Convention on Tobacco Control (FCTC) met in October 2014, and made decisions on taxation, product content and disclosure, electronic nicotine and non-nicotine delivery systems (ENDS/ENNDS), alternative products, liability and trade matters. These decisions will influence the industry's future research.

For product content, further work will address constituents, and also disclosure, testing and measuring cigarette emissions and contents and include them in future guidelines. Cigarette characteristics such as slim size, filter ventilation, flavour delivering capsules may be the object of next guidelines. Dependence and liability of all tobacco products will be monitored. The WHO will assess if Standard Operating Procedures for nicotine, TSNA and B[a]P in cigarettes are applicable to other tobacco products.

For ENDS/ENNDS, Parties were recommended to consider banning or regulating. The WHO was invited to consider analytical methods for contents and emissions, and to prepare a report on ENDS/ENNDS potential role in quitting tobacco usage and impact on health and tobacco control efforts.

In terms of alternative crops to tobacco, the main policy options and recommendations approved covered the inclusion of tobacco growers in policy development and the liability of the tobacco industry for health, social and environmental damages in tobacco production and in the supply chain.

In terms of trade facilitation, Parties of the FCTC were encouraged to cooperate in exploring legal options to minimise the risk of the industry making undue use of international trade and investment instruments to target tobacco control measures, and to take into account public health objectives when negotiating trade and investment agreements.

In terms of liability, the FCTC secretariat will study procedures for settling disputes on the interpretation or application of the convention, the kind of disputes that may be subject to such procedures and the interaction of the procedures with other dispute settlement mechanisms.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 36

Comprehensive analysis of proteins in tobacco leaf

In the last two decades, proteomics has been a vastly expanding field of research. Proteomics aims at the comprehensive and comparative measurement of the proteins present in a sample, e.g. plants. Major developments in separation and analytical sciences have substantially extended protein identification rates and protein quantification accuracies. Proteins, which constitute about 10% of the dry weight of tobacco leaf, are known to affect the flavour of cigarettes. Understanding tobacco protein profiles and the changes in the functions of curing, storage and aging might broaden our understanding in the tobacco flavour.

The purpose of this study was to develop a comprehensive analytical method for proteins in tobacco leaf.

Following protein extraction, solubilisation and in-solution proteolysis, the resulting peptides mixtures were analysed by two-dimensional reversed-phase liquid chromatography coupled to high resolution quadrupole time-of-flight mass spectrometry (Q-TOF). Fifteen peptide fractions, sampled in an off-line manner from a reversed-phase column operated at high pH, were subsequently analysed by nano-LC Q-TOF MS/MS under acidic conditions. Proteins were identified by the Spectrum Mill platform.

About three hundred different tobacco specific proteins were identified representing a four-fold increase over the one dimensional approach.

Differentiating protein profiles were observed depending on tobacco curing type. As an example, proteins related to photosynthesis were particularly abundant in Oriental leaf types, a direct result of sun exposure.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 31

Ignition propensity of cigarettes according to ISO 12863 using two different substrates

There are two standards available to determine the ignition propensity of cigarettes: ASTM E.2187-09 and ISO 12863. One of their main differences is that ISO 12863 allows the use of other substrate materials as an alternative to Whatman No. 2 filter paper as long as such substrates are equivalent. The target of this study was to determine if an alternative substrate, LIPCan filter paper produced by Tervakoski of delfortgroup, would be a proper candidate for a substrate. These filter papers comply with the requirements in section 7.3.2 in ISO 12863. Four different lower ignition propensity cigarettes with two different styles, king size and super slim, were used in this study. The cigarettes were tested according to ISO 12863 on Whatman No. 2 and LIPCan filter paper. The results show that every cigarette brand passed the test according to ISO 12863 and ASTM E.2187-09 on Whatman No. 2 and LIPCan filter paper.

In conclusion the results show that within the typical variation of the test according to ISO 12863 LIPCan filter paper is equivalent to Whatman No. 2 as no statistical difference between the results obtained with two substrates could be found at statistically significant levels of 95% or 99%.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 13

Influence of machine-based puffing parameters on aerosol and smoke yields from contemporary nicotine inhalation products

A study was conducted to investigate the effect that changing the puffing parameters used to generate an aerosol from an electronic cigarette had on the mass of aerosol collected as well as the yields of the major chemical constituents of the aerosol; propylene glycol (PG), glycerol, nicotine and water.

The study examined a range of puff volumes and durations, as well as investigating the effect of the puff profile, using two puff actuated electronic cigarettes – one disposable and one rechargeable.

The results of this study were compared with previous studies looking at the effect of puffing parameters on the mass of aerosol delivered by commercial cigarettes (CCs) and an electrically controlled Tobacco Heating Product (THP).

For the e-cigarettes tested, there was no significant difference in aerosol mass and major constituents between 3s rectangular and bell-shaped puff profiles. Whilst puff volume did not significantly affect the mass of aerosol from e-cigarettes or the THP, puff duration did. For the THP, puff frequency also had an effect on total aerosol mass. By contrast, for CCs, both puff volume and puff frequency significantly influenced smoke yields whilst puff duration did not.

Puffing parameters have wide-ranging effects on smoke and aerosol yields that differ depending on the product and which correlate with the individual heating mechanisms of different devices. In combination with a growing body of information on how consumers use these new nicotine and tobacco products, this knowledge can help to design more appropriate standardised testing procedures.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 08

Effect of puff duration and puff volume on e-cigarette aerosol collection

Smoking machines were first developed to generate smoke from tobacco cigarettes for the purpose of comparing cigarette tar and nicotine yields under consistent conditions. Smoking machines have been used worldwide to verify cigarette designs and ensure regulatory compliance for decades. Two standardized machine smoking protocols frequently used for regulatory reporting are the International Organization of Standardization (ISO) smoking regime and the Health Canada Intense (HCI) smoking regime. Both protocols call for specific puff volumes (35 and 55 cc), puff profile (bell shaped), puff durations (2 seconds), interval between puffs (60 and 30 seconds) and percent ventilation blocking (0 and 100%). There are no such standardized methods for collecting e-vapor product aerosol. In 2013, a Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) E-Cigarette (ECIG) Task Force (TF) was formed to address this and other relevant topics regarding e-cigarette testing and has made commendable progress. The purpose of this work was to evaluate the effect of puff volume (within the constraints of standard linear smoking machines) and duration (2 to 5 seconds) on e-cigarette aerosol mass (AM) collection. This information would be useful to both the CORESTA ECIG TF as well as relevant regulatory bodies. It was observed that puff volume has little effect on AM while puff duration plays a key role in the amount of AM collected. Larger puff volumes do appear to create some evaporative loss particularly with longer puff durations. For single device designs, the puff duration has a linear increase on AM from 2 to 5 seconds.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 30

U.S. pharmacopeia dissolution technique for the determination of nicotine and flavor release from smokeless tobacco products

Smoking machines were first developed to generate smoke from cigarettes for the purpose of comparing cigarette tar and nicotine yields under consistent conditions. Smoking machines with standardized puffing protocols have been used to measure the components in cigarette smoke for decades. There are no standardized methods for measuring the release of components in smokeless tobacco. The objective of this work was to develop a standardized dissolution technique for smokeless tobacco products using a SOTAX CE7 Smart USP-4 flow-through dissolution apparatus. The flow-through dissolution apparatus was configured for off-line collection of USP artificial saliva (no enzymes) with a pH of 6.8. The flow rate was 4 mL/minute, temperature was held at 37 °C and fractions were collected (e.g. every 4 minutes) for 60 minutes. The dissolution fractions were analyzed by GC/MS for flavor and nicotine release from pouch moist smokeless tobacco (MST) and snus products. Rates of release showed a quadratic distribution and a faster release of nicotine and flavors for pouch MST compared to snus. This technique demonstrated excellent reproducibility and can be applied to measure a variety of constituents that are released from smokeless tobacco for comparative and regulatory reporting purposes.

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Was there a temporal increase in cigarette nicotine yield-to-content ratios in products reported to the Massachusetts Department of Public Health?

Since 1997, Philip Morris USA (PM USA) and the other major US cigarette manufacturers have been required to provide the Commonwealth of Massachusetts with smoke nicotine yields and nicotine in filler for high volume brand families. In 2014, researchers from the Massachusetts Department of Public Health (MDPH) published a paper concluding, among other things, that "average [nicotine] yield-to-content ratios of recent years were markedly higher than those of earlier years."

We explored the PM USA data submitted to MDPH between 1997 and 2013 examining the finding that the nicotine yield-to-content ratio had increased. For the years 1998-2008, the filler nicotine of PM USA products was tested using CORESTA Recommended Method (CRM) No. 35. From 2009 to the present, the filler nicotine for the PM USA products was measured by the CDC test method.

It is well known that CRM35 is sensitive to secondary alkaloids in addition to nicotine, and has been found to result in higher values than nicotine-specific methods similar to the CDC method. Testing on 3R4F cigarette filler showed 13% lower results for the CDC method than was measured for 3R4F at the time of manufacture when tested with CRM35. A lower measured value for filler nicotine makes the denominator in the nicotine-to-content ratio smaller, and therefore makes the ratio larger.

We conclude from our investigation that changing the test method from CRM35 to the CDC method explains most, if not all, the observed increase in average nicotine yield-to-content ratios for PM USA cigarettes.

This investigation illustrates the difficulties in making inferences of product time trends, particularly when analytical methodology changes during the period under investigation.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 51

Puff-by-puff menthol delivery in flavoured cigarette

Menthol is the most widely used flavour in cigarettes and other consumable products. There are several methods for applying menthol to the cigarette filter: direct injection of molten menthol (A), menthol solution (B), mentholated thread (C) and menthol capsule (D).

In this study we compare puff-by-puff menthol delivery to smoker using various menthol application methods. A, B, C and D filters were compared keeping menthol amount, pressure drop and other key physical parameters constant. Cigarette sample with filter (D) was smoked in two regimes: capsule crushed before smoking (D1) and another way in the middle of smoking (D2). In addition, we have tested a filter containing two menthol capsules: one crushed just before smoking and second one in the middle of smoking.

Cigarettes with the above described filters were made using CM7 tobacco column. The smoke was collected on Cambridge filter pads using the ISO smoking regime with some machine adjustments, to get puff by puff results. Menthol analyses were made using Agilent 5973N GC-MS.

This study provides very practical and insightful data into flavour delivery dynamics in ever so popular mentholated cigarettes including brand new double capsule filter.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 18

Environmental chamber evaluation of emissions from electronic cigarettes

At present, limited data exists for second-hand emissions from electronic cigarettes. Deficiencies of existing studies include: poorly controlled environments, lack of validated analytical methods for emissions, and incomplete background characterization.

To address these deficiencies, R.J. Reynolds Vapor Company sponsored a study in a 28 m^3 environmental chamber to evaluate the emissions from electronic cigarettes (VUSE Original, VUSE Menthol, and a market sample [blu, NJOY]) relative to the leading menthol and non-menthol cigarettes and relative to a non-smoking blank. Each product cohort consisted of 11-12 subjects. For each test product, five smoking/vaping and five non-smoking/vaping blank test sessions were conducted. Four randomly-selected subjects from a cohort participated in each session. Test sessions consisted of a 10 min background collection period and a 10 min smoking/vaping period (non-smoking/vaping for blank sessions). Subjects then exited the chamber and a 120 min sample collection period followed. Airborne concentrations of 25 commonly measured second-hand smoke (SHS) constituents were determined using methods validated according to the principles of good laboratory practices. The analyte list included: formaldehyde, acetaldehyde, nicotine, glycerol, propylene glycol (PG), benzene, toluene, PM 2.5, and other selected SHS markers and SHS constituents.

By design, test conditions resulted in analyte concentrations that were much higher than those expected in normal smoking environments. Despite the elevated concentrations, most electronic cigarette analyte concentrations either were not detectable in emissions from the electronic cigarettes or were not statistically significantly different from blank sessions. Glycerol concentrations were generally higher than combustible cigarettes. PG was increased or decreased relative to combustible cigarettes depending upon the product comparison. Nicotine concentrations were also higher than the blank, but reduced by 88-99% relative to combustible cigarettes. The results demonstrate that consumption of the most commonly used “cigalike” electronic cigarettes will have a negligible impact on indoor air quality in most environments.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 09

Influence of vaping topography on the retention rate of nicotine following use of e-cigarettes

The "quantity of nicotine exhaled" by an e-cigarette user is the most important factor influencing bystander exposure to nicotine. To that end, it is important to determine the quantity of nicotine that is retained by the e-cigarette user and not exhaled. Here we investigated the influence that vaping topography (i.e. mouth-hold versus inhaling) has upon the retention of nicotine following e-cigarette use in experienced volunteers.

Using a smoking machine, the mainstream aerosol from e-cigarettes containing different concentrations of nicotine were first evaluated by GC-FID to determine the relationship between quantity of nicotine delivered per puff and puff duration. The e-cigarettes were then freely vaped by volunteers through a cigarette holder attached to a smoking topography analyser which recorded puff volume and puff duration. This allowed the quantity of nicotine delivered to the volunteer during each puff to be determined. A Proton Transfer Reaction Mass Spectrometry (PTR-MS) machine, calibrated for nicotine, was then used to determine the quantity of nicotine exhaled following each use of the e-cigarettes and subtracting this figure from the estimated delivery enabled the retention rate to be calculated.

Our main finding was 98% the nicotine was retained by the volunteers when the e-cigarette aerosol was inhaled, regardless of puff volume or puff duration. In contrast, when the e-cigarette aerosol was held in the mouth only (i.e. no inhalation), a reduced but still a substantial quantity of nicotine, was retained. The novel experimental protocol presented here may also be used to determine the retention rates of other chemical components known to be present in e-cigarette aerosols.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 10

Method development and validation for the quantification of metals in liquids and aerosol of e-cigarettes

Due to the rapidly increasing popularity of e-cigarettes, several studies were carried out to characterise this new type of product. E-cigarettes produce a condensation aerosol by heating an e-liquid invariably consisting of a mixture of propylene glycol and glycerine containing a proportion of nicotine and flavourings. The presence of trace metals in e-liquids or e-vapours has been previously reported[1] which is probably due to transfer from the device to the e-liquid.

In this study, a method was developed and validated for the quantification of trace concentration levels of tin, copper, aluminium, nickel, iron, silver, and chromium in e-liquids and e-vapour aerosols. For the analysis of the e-liquids, an aliquot of the liquid was digested and oxidised in a Teflon pressure vessel using nitric acid and hydrogen peroxide. An aliquot of the digested solution was subsequently analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

For the analysis of e-cigarette emissions, a cigarette smoking machine was used to generate aerosol, and particulates in the aerosol were collected by electrostatic precipitation. The precipitate was dissolved in nitric acid and an aliquot of this solution was subsequently analysed by ICP-OES.

The quantification of results was carried out using Yttrium as an internal standard. The regression coefficients of the applied calibration curves for each compound were calculated better than 0.999. For the e-liquid, the limits of quantification (LOQ) ranged from 0.4 µg/g (iron, copper) to 4.5 µg/g (tin), and for emissions from 0.003 µg/10 puffs (copper, iron) to 0.04 µg/10 puffs (tin).

In this presentation, the fully validated method including further validation parameters, e.g. precision, repeatability, recovery and potential contamination sources, e.g. from equipment used by sample preparation, will be discussed.

[1] M. Williams, A. Villarreal, K. Bozhilov, S. Lin, P. Talbot; PLOS ONE; Volume 8; Issue 3, 2013.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 21

PAHs in mainstream smoke – challenges for analytical methods?

The Conference of Parties (CoP) to the WHO FCTC and the U.S. FDA have specified toxicants in mainstream cigarette smoke that are of regulatory interest, including Polycyclic Aromatic Hydrocarbons (PAHs). Currently, benzo[a]pyrene has been prioritised by the CoP and the U.S. FDA list of 18 harmful and potentially harmful constituents (HPHCs) for regulatory data submission. However, in the U.S. only the full FDA HPHCs list must be reported including all 16 PAH substances in due course.

The measurement of PAHs in mainstream smoke is technically challenging because of their relatively low abundance (parts per billion), numbers of isomers and the consequent need for high chromatographic and detection selectivity. Their determination requires not only thorough optimisation of the extraction and clean-up strategy but also the application of appropriate instrumentation to give sufficient selectivity and sensitivity.

The presented study discusses some challenges associated with optimisation of targeted analytical methods for determination of PAH substances in mainstream cigarette smoke. As an example, an analytical approach comprising stable isotope dilution MS, extraction using Accelerated Solvent Extraction (ASE), a dual Solid Phase Extraction (SPE) clean-up and a comparison of three different GC/MS systems (high resolution magnetic sector (GC-HRMS), triple quadrupole (GC-MS/MS) and single quadrupole (GC-LRMS) mass analysers) is shown. Practical aspects of method development as well as advantages and limitations of all three systems are discussed. GC-HRMS was demonstrated to be the most sensitive instrument for quantitative analysis of all PAHs present at sometimes (ultra) low concentration levels in mainstream cigarette smoke.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STW01

Electronic cigarette use and harm reversal: emerging evidence in health and disease

The growing popularity of e-cigarettes (ECs) proves that many adult smokers are keen to use an alternative to combustible cigarettes to reduce tobacco consumption or quit smoking and to relieve withdrawal symptoms. Data from internet surveys and clinical trials have shown that ECs may help smokers in quitting or reducing their tobacco consumption. Although smokers switching to regular EC use are likely to gain significant health benefits, direct confirmation is not available and it will take a few decades before improvements in individual and population health outcomes due to the regular use of e-vapour products is firmly established. Nonetheless, early changes in health outcomes can be detected in smokers switching to e-vapour products. Acute investigations do not appear to support negative health outcomes in EC users and initial findings from long-term studies are supportive of a beneficial effect of EC use. This paper presents evidence that EC use can reverse harm from tobacco smoking, and focuses on reductions in biomarkers used as proxy for risk prediction in respiratory, cardiovascular and metabolic disease. The emerging evidence that EC use can reverse harm from tobacco smoking should be taken into consideration by regulatory authorities seeking to adopt proportional measures for the e-vapour category.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 12

Determination of flavours and their metabolites in e-liquids and biological fluids – identification of biomarkers of exposure to prominent e-cigarette flavours

E-cigarette use, also referred to as vaping, is increasing worldwide as these products are becoming a preferred alternative for conventional cigarettes among smokers. Almost all e-cigarette liquids (e-liquids) contain flavour chemicals. Although the majority of the flavours used are `generally regarded as safe´ (GRAS) food additives, very little is known about their metabolic fate after inhalation. Thus, concerns are raised by regulatory authorities on the potential health risks caused by flavour additives in e-cigarettes. As a pre-requisite for further toxicological evaluation of this new product category, novel analytical methods for the determination of the most prominent flavour compounds are needed to investigate the uptake into the human body via the route of inhalation.

We developed and validated bioanalytical methods for the quantification of menthol, a frequently used flavour additive, and its major metabolite, para-menthane-3,8-diol, in various human body fluids by means of GC-MS and LC-MS/MS, respectively. Method validation according to FDA guidelines showed excellent results in terms of sensitivity, reproducibility and robustness. Moreover, a multi-analyte method based on headspace GC-MS has been developed for the simultaneous determination of the most abundant flavours in e-liquids and human body fluids. The straightforward sample preparation procedure allows a time- and cost-efficient screening of those compounds in clinical studies.

The methods were applied in a pilot e-cigarette study. This poster presentation will share first quantitative data on menthol, its metabolite para-menthane-3,8-diol and further flavour components in e-liquids and body fluids analysed in this pilot study. Additionally, their potential as biomarkers of exposure to e-cigarettes will be discussed.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 48

CelFX™ carbon technology: puff-by-puff profile

CelFX™ carbon filter technology has been shown to reduce vapor phase smoke components by 40-90% in prior work[1]. In order to expand the understanding of this filtration performance, this work will evaluate the puff-by-puff machine profile to assess the filter performance on individual puffs. It is known that nicotine or tar delivery from the first puff is different than the last puff. This work will seek to assess the effect on that puff profile from presenting the carbon to the smoke in a much more efficient and higher loading format. Carbonyls and volatile compounds will be tracked and compared against a cellulose acetate filter control (Kentucky 3R4F cigarettes).

[1] CelFX™ Matrix Technology Super Slim Filter comparison with commercial carbon filter, ST31, CORESTA Congress 2014, Quebec.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 18

Particle breakthrough comparison of commercial carbon filters with CelFX™ carbon/acetate filters

Previous works have evaluated cigarette filter carbon particle release from various filter designs. The recently introduced CelFX™ Matrix Technology offers high loadings of carbon, with low pressure drops and has not been studied for the particle release effect. This study will compare a filter with a CelFX™ segment against cigarette filter designs containing either a carbon-on-tow segment or a cavity segment. In all cases, a cellulose acetate segment is used as the mouth end. This study focuses on particle release from 0.125 micron to 5 micron. This particle range encompasses the respirable particles as listed by Organization for Economic Cooperation and Development (OCED) guidelines. Initial data shows CelFX™ carbon filters have significantly less particle release than a cavity filter for 0.125-5 micron size particle range. Commercial carbon-on-tow filters will also be evaluated.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 35

Direct extraction of Cambridge filter pad holders

Quantification of cigarette smoke constituents, using a smoking machine designed in accordance to ISO 3308 is the basis for research and development within the tobacco industry.

The loss by the incomplete removal of total particulate matter (TPM) and therewith the precipitated smoke constituents from a classical Cambridge filter pad (CFP), using a conventional filter holder is often discussed. To minimise these loss of analyte quantities and the impact of ageing processes and contamination possibilities of the collected TPM, a new filter pad holder has been developed, as an integral unit. It allows the complete flushing and direct extraction without opening the holder and separate handling of the filter pad. Earlier published studies have already shown that direct extraction techniques do have an impact on the deliveries and repeatability rates of smoke constituents. The new application simply has the advantage that the filter pad holder can be used directly for mechanical extraction and rinsed out without any further instrumentation.

Correspondingly, first results showing differences in nicotine and water deliveries will be presented. Especially for the determination of higher molecular and unstable substances it could be of further interest to operate with an enclosed system.

Last but not least, the system as it is designed gives advantages related to occupational health and safety requirements as the operator does not handle open solvents anymore.

Besides the technical description, the paper will share some first analytical data showing different amounts in nicotine and water deliveries and give an outlook into further automated handling.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 10

A new *in vitro* testing module for the cytotoxic evaluation of e-cigarette vapour

The e-cigarette market has recently been booming, and e-cigarettes are often described as "reduced-risk" nicotine products or alternatives to combustible cigarettes. However, no regulations for e-cigarettes are currently in force, so that the quality and safety of e-liquids is not necessarily guaranteed. There are two major ways to analyse e-cigarette vapour: chemically and biologically. Whereas chemical evaluations are mostly restricted to known toxic components, biological analysis gives information about effects triggered in the human body after inhalation. For the generation of relevant data, a system is needed which is able to produce stable data. Therefore, the smoking machine has to produce e-liquid vapour of reproducible quality, which can then be used to expose human bronchial epithelial cells directly at the air-liquid interface. A new flexible compact version of smoking machine and exposition module set-up is introduced. The suitability of the system is demonstrated by presenting dose-response curves for normal human bronchial epithelial cells after direct cigarette smoke and e-cigarette vapour exposure at the air-liquid interface. The low standard deviation of the results confirms the robustness of the system and proves the ability to generate reproducible data. In summary, the *in vitro* testing module represents a platform for the generation of stable data to evaluate the toxicological potential of e-cigarette vapour.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 42

The effects of surface properties of tipping paper on the oil resistance function for the capsule-containing filter

The needs for cigarette products with various flavour capsules have increased for the uniqueness and impacts of flavours. These trends for capsule-containing cigarettes have required capsule-containing filters, oil resistant filter plug wrappers without porosity and the online perforation equipment for tipping paper. However, the production capabilities of online perforation equipment for tipping paper have been limited because of the increased capsule-containing cigarette products. Therefore, the new functional materials have been discussed for the replacement of oil resistant filter plug wrappers and for the increased facility-overloads for online perforation equipment because all of the products with capsule-containing filters need to use online perforation equipment.

In this research, a new type of tipping paper with oil resistant properties has been developed for the replacement of oil resistant filter plug wrappers and to improve facility-overloads of online perforation equipment for tipping paper in the factory. The oil resistant tipping paper was manufactured by the application of polymers and corona treatments. The effects of the surface properties of tipping paper such as surface energy, paper composition and smoothness on the oil resistance properties after capsule breakage were evaluated. The coating of oil resistant polymers onto tipping paper decreased the surface energy from 65 mN/m to 30 mN/m and increased the oil resistant properties from No. 3 to No. 12 expressed as the degree of oil resistance. The corona treatment of polymer-coated sides of tipping paper increased the surface energy from to 30 mN/m to 36 mN/m and improved the speeds of cigarette production.

As expected, the new oil resistant tipping paper improved the oil resistant properties after capsule breakage and decreased the overloads for the online perforation equipment in the factory.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 05

Quantitative screening of potential contaminants in e-cigarette formulations: ethylene glycol and diethylene glycol

The U.S. Food and Drug Administration (FDA) evaluated two commercial e-cigarettes and a nicotine replacement therapy inhaler in 2009 (DPATR-FY-09-23). Ethylene glycol (EG) and diethylene glycol (DEG) were included in this evaluation as potential impurities in e-cigarette formulations. DEG was found in one e-cigarette cartridge in the study (quantities were not included). The U.S. Pharmacopeia (USP) discusses permissible levels of EG and DEG in polyethylene glycol and glycerin (<0.1%), the major components of most e-vapor product formulations. The USP only provides non-selective methods for the analysis of these potential contaminants in USP grade propylene glycol and glycerin. These methods are subject to potential interferences caused by flavor systems found in e-cigarette formulations. Therefore, the purpose of this work was to develop and validate a sensitive and selective method specifically for the quantitative screening of e-vapor formulations for EG and DEG. The method developed uses gas chromatography with mass spectrometry (GC/MS). All requirements for method validation were met such as linearity, accuracy, precision, limits of detection (LOD), and limits of quantitation (LOQ). The linearity was demonstrated with a coefficient of determination of >0.995 for the calibration range of 10 to 800 µg/g of e-cigarette formulation.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 46

Comparison of the *in vitro* toxicity of mainstream smoke from three different types of cigarettes using a direct cell air-liquid exposure system

Cigarette smoke is a complex mixture of thousands of chemicals distributed between the particulate and vapour phase from mainstream cigarette smoke, which together are termed "whole smoke". Generally, *in vitro* toxicity of the cigarette smoke has been determined for the total particulate matter (TPM) prepared by collection on the filter and gas/vapour phase (GVP) by using impingers with a buffer solution. Recently, a whole smoke exposure system that directly exposes the cell air-liquid interface to freshly generated cigarette smoke has been introduced to test the *in vitro* toxicity of cigarette smoke.

In this study we compared the *in vitro* biological activity of whole smoke from three cigarettes with different tar and nicotine contents using a smoke direct exposure system consisting of a smoking machine and a chamber exposing cellular cultures to whole smoke at the air-liquid interface. Smoke was generated at various dilutions (1:1 - 1:10, smoke:air) under two different smoking condition (ISO and Health Canada Intense [HCI]). The cytotoxic activity of cigarette smoke was assessed by a "WST-1" assay using the CHO cell line and the mutagenicity activity was measured by a modified "bacterial reverse mutation assay" using standard tester strains (TA98 and TA100).

In our work, the cytotoxicity and mutagenicity of whole smoke from three different types of cigarettes under ISO and HCI smoking regimes are compared. Furthermore, the contribution of the vapour phase of whole smoke to the cytotoxic and mutagenic activity is determined.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 14

The influence of slim cigarette market share on smoking prevalence

Slim cigarettes were defined in the 2012 draft EU-Tobacco Product Directive (EU-TPD) as cigarettes with a diameter of less than 7.5mm. Slim cigarettes are marketed in various countries around the world and have been growing as a share of the global cigarette market even as the overall cigarette sale volumes in most markets have been falling. Allegations that slim cigarettes may negatively impact tobacco control efforts led the European Commission to propose a slim cigarette ban in 2012. After considerable debate among legislators and EU Member States, the ban on slim cigarettes was ultimately excluded from the final version of the EU-TPD of April 2014.

Oxford Economics investigated whether there is any association between the preferences for slim cigarettes measured as slim cigarettes market share and smoking prevalence rates to determine whether these allegations are justified. To do this, data on the slim cigarettes market share, ranging from 0% to 70%, and smoking prevalence rates, ranging from 7% to 42%, from the years 2012, 2006 and 1996 were compiled for a core sample of 95 countries. To illustrate the cross-country differences in preference for slim cigarettes, in 2012 high market shares for slim cigarettes were found in Eastern Europe and Russia ranging generally between 20% and 40%, Korea 39%, and Indonesia 37%, while many other countries, including Germany (1.1%), Finland (0.3%), and the United Kingdom (0.4%), had a very low slim cigarettes market share.

First, raw correlations between the market share of slim cigarettes and smoking prevalence rates were examined, followed by multivariate cross-country regressions where various confounding factors were controlled for, such as regional and cultural dummy variables as well as socio-economic factors such as income per capita, education, price, affordability, and tobacco control index. This analysis was performed for the overall, male and female smoking prevalence.

Oxford Economics found no evidence that the preference for slim cigarettes measured as the market share of slim cigarettes was associated with greater smoking prevalence. Although raw correlations between the slim cigarettes market share and smoking prevalence were sometimes positive and statistically significant, this result disappeared when potential confounding factors were fully controlled for. Rather, the cross-country variation in smoking prevalence was substantially explained by a number of regional and cultural dummy variables, as well as socio-economic factors, such as income per capita.

Oxford Economics concluded that policy measures aimed at restricting the sales of slim cigarettes are therefore unlikely to be effective at reducing smoking prevalence.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 05

Smoke analysis of fine-cut tobacco (Part 2) – challenges for testing Make-Your-Own tobacco

The set of three ISO standards within ISO 15592 specify methods for sampling, conditioning and determining nicotine and "tar" in mainstream smoke of fine-cut tobacco. The method requires four articles to be prepared and smoked using two weights of tobacco corresponding to two diameters, and two types of wrappers with different burning properties. It provides a 2×2 'matrix' of four different measured values for nicotine and "tar" for each tobacco. The first part of our study was focused on the calculation of three matrix points from measuring just one point. This second part focuses on practical considerations for applying this method to the determination of nicotine and "tar" in Make-Your-Own (MYO) tobaccos, and especially the challenges to test such highly expanded tobaccos.

It is well known that laboratories can face difficulties in performing the measurement of some matrix points especially for the testing of MYO blends. Herein, we present a study to identify the key challenges for MYO tobacco to comply with the ISO standard.

The first main challenge comes from incorporating a relatively large volume of the targeted tobacco weight within a fixed tube volume leading to very high pressure drops of the smoking articles, especially for the 5.2 mm diameters. Routine smoking machines have a maximum limit for pressure drop. When this is exceeded, the puff volume is no longer compliant with the corresponding ISO standard.

The second challenge is the rod filtration effect. Due to the compression of tobacco, yields for MYO are no longer correlated with the amount of tobacco per FCSA: increasing tobacco weight per FCSA involves a decrease of tar and nicotine yields.

The ISO standard 15592 was initially designed for RYO tobacco and its applicability has never been fully assessed for other products such as MYO and this requires further attention.

Reference: B. Teillet et al., Smoke analysis of fine-cut tobacco - a predicting model and other challenges (Part 1), ST86, CORESTA Congress 2014, Quebec.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 28

The comparative assessment of cigarette smoke with an *in vitro* BALB/c cytotoxicity test using altered vapour phase delivery products

The objective of this study was to evaluate a modified air-liquid interface BALB/c 3T3 cytotoxicity method for the assessment of smoke aerosols *in vitro*.

The functionality and applicability of this modified protocol was assessed by comparing the cytotoxicity profiles from eight different cigarettes. Three reference cigarettes, 1R5F, 3R4F and CORESTA Monitor 7 were used to put the study into perspective and five bespoke experimental products were manufactured, ensuring a balanced study design. Manufactured cigarettes were matched for key product characteristics such as nicotine delivery, puff number, pressure drop, ventilation, carbon monoxide and blend, but significantly modified for vapour phase delivery, via the addition of two different types and quantities of absorptive carbon.

The results demonstrate cytotoxicity for all products tested, with clear and statistical differences between the balanced experimental products when compared to the control. In fact the assay was able to distinguish between all vapour phase altered and reference products, in a statistical manner.

This study has further characterised the *in vitro* vapour phase biological response relationship and confirmed that the biological response is directly proportional to the amount of vapour phase toxicants available in cigarette smoke, when using a Vitrocell® VC 10 exposure system. This study further supports and strengthens the use of aerosol based exposure options for the appropriate analysis of cigarette smoke induced responses *in vitro*. This may be especially beneficial when comparing aerosols generated from alternative tobacco aerosol products, particularly those with reduced vapour phase toxicants, which are not assessed using standard *in vitro* techniques.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 39

Dead volume and impinger capture: will machine design change puffing conditions?

Minimising dead volume between cigarette and capture pad smoking machines has been shown to be critical in achieving total capture of total particulate matter (TPM) especially when smoking intensively. Less attention has been paid to dead volume in smoking experiments where the volatile component of mainstream smoke is captured in liquid impingers.

The objective of this study is to determine the impact of dead volume on the pressure drop of the capture system and how this changes the combustion conditions of the cigarette.

This was examined using two types of linear smoking machine (SM450 and SM450i with dead volumes of 19.73 and 11.26 ml respectively) and a puff capture device that could record the profile parameters as experienced by the test piece.

It was found that profile shape of puffs when impingers are added change from the "normal" smoking condition. Comparing no impinger with systems of increasing dead volume it was found that peak flow is reduced by 13% and 16% respectively, asymmetry increased for the largest dead volume from 1:1 symmetry to 5:4 asymmetry and that the peak maximum occurred at 1 s, 1.25 s and 1.35 s respectively.

The consequences for analytical measurement are discussed in the context of "peak clipping" and combustion temperature, notably that the elongation of peaks due to the elasticity of the drawn smoke present particular problems with some machine designs that utilise a single engine. Reducing the dead volume between smoking article and impinger system from traditional systems has a positive impact on the puff shape and peak velocity.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 30

Systematic errors in ventilation measurement and their resolution

Calibration of ventilation measurement equipment is an "ideal" process designed to remove differences between a transfer standard and the real world. However when real measurements are made by instruments there is no practical method of making an inline flow measurement without disturbing the ventilation measurement as this introduces additional resistance into the flow path. In practice calibration with a transfer standard is not sufficient to eliminate measurement errors and limits the accuracy of ventilation measurements.

The objective of this study was to investigate the impact of pressure drop (PD) in the ventilation measurement path and the nonlinearity that this introduces. This was then related to flow modelling of the transfer standard which shows the cause of the observed sensitivity as a function of flow. A further objective is to develop schemes that minimise these errors.

It was found that inducing a small balancing PD in the reference path of approximately 1 mmWG, using a non-linear multipoint interpolation for calibration and constant referencing to three points on the interpolated curve significantly improves accuracy of measurement. Improvements in accuracy were shown across the range, the magnitude of the improvement being dependent upon the actual ventilation being measured. Typical improvements (absolute value) between the standard and new system would be 0.5% at 28% ventilation, 1% at 47% ventilation, 1.1% at 60% ventilation, 0.6% at 70% ventilation and 0.1% at 93% ventilation. Increasing the PD of the ventilation standard exacerbates the observed problem although in practice this should not happen with real samples.

The improved instrument design, which takes into account the flow modelling of the transfer standard and reference path PD, can give more accurate ventilation measurements.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 14

Use of the TK6 human lymphoblastoid cell line for the assessment of the genotoxic potential of complex mixtures

There has been a significant focus over recent years to evaluate the relevance of *in vitro* micronucleus (IVM) data obtained with cell lines of different origin and different status regarding the p53 checkpoint. Those evaluations and additional experimental approaches indicate that the p53 potent human cell line TK6 may be a suitable biological tool for reducing false positive results during genotoxicity testing of single chemical compounds.

The present study was initiated to implement the IVM test with TK6 cells in Imperial Tobacco Group (ITG) labs using established positive controls (i.e. cyclophosphamide A [CPA], methyl methane sulfonate [MMS] and vinblastine) as a first step. The main goal was to investigate whether the TK6 cell line could also be a useful tool for the assessment of complex mixtures like cigarette mainstream smoke condensate (total particulate matter (TPM)). The latter was generated using the CORESTA monitor test piece CM7 under ISO smoking conditions. The evaluation of the microscopic preparations was performed with an automated slide scanning system controlled by the Metafer4® software (Metasystems). All testing was performed in the absence of cytochalasine B.

For both single positive controls as well as TPM it was observed that the efficient expression and detection of micronuclei in TK6 cells required an extended recovery period after treatment which is in accordance with literature data. The micronucleus background frequencies for TK6 cells were slightly higher than those obtained with V79 cells. However, in experiments performed with TPM and single substances statistically significant dose dependent 3 up to 8 fold increases over background levels with similar dose response characteristics as for the V79 cell line were found. The results indicate that the TK6 cell line could be a suitable and meaningful alternative to the rodent p53 deficient cell line V79 for tobacco product assessment. Subsequent validation experiments are in progress to determine the level of variability of micronucleus frequencies in TK6 cells.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 45

Cigarette smoke as a source of exogenous and endogenous antioxidants in smokers: novel findings

Cellular oxidative stress, derived from imbalance between the production of reactive oxygen species and the efficacy of the antioxidant defence, can be a direct and/or indirect consequence of cigarette smoking. Pro-oxidant properties of cigarette smoke are accounted for by the abundance of smoke oxidants, whose origin may be of free-radical as well as of molecular (peroxides, electrophilic carbonyls, etc.) nature. Oxidants may be additionally generated in a smoker through biochemical transformations of relatively persistent smoke chemicals (e.g. benzo[a]pyrene, aromatic amines). The antioxidant potential of the smoke is scantily addressed in the literature. However, one should take into account that any reactant in oxidation processes may exhibit both oxidant and antioxidant propensities depending on the reaction conditions. Such a bimodal behaviour is unique to a number of smoke chemicals. Thus, hydroquinone, catechol and numerous phenolic and polyphenolic reactants may act as both antioxidants (through scavenging free radicals) and pro-oxidants (through generation of oxygen-contained free radicals) responsible for oxidative damage of biomolecules. As we have recently shown, smoke constituents indeed exhibit at the same time both pro-oxidant and antioxidant activities (Palmina et al., 2014. *Aust. J. Chem., 67*, 858-866) because of a dual role of the smoke reactants in oxidation processes. Apart from this mechanism, we have studied the possibility of the antioxidants generation directly in smokers. For that purpose, we have developed a model system consisting of a cigarette-smoke extract, peroxidase and amino acids. Using such a system, we have demonstrated that under physiological conditions the oxidation of the smoke tar and its individual components (e.g. catechol) in the presence of H_2O_2, peroxidase and glycine affords the products whose antioxidant potential is much higher than that of initial, unoxidised, chemicals.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 07

Which parameters are suitable to evaluate e-vapour products puffing behaviour?

The growing popularity of e-vapour products (EVP) carries with it an increasing interest in the examination of vaping behaviour.

As part of a product stewardship programme to evaluate and assess e-vapour products prior to placing them on the market, a randomised, parallel group, multi-centre study was conducted[1].

Puffing topography was evaluated during the confinement period of this clinical study. Two flavour variants were provided and 40 subjects were randomised to either the EVP arm or conventional cigarette (CC) arm at a ratio of 3:1 respectively.

This method uses a non-invasive measurement device (SODIM, SPA/M) which can be adapted to the mouth end of a CC and EVP. This instrument is portable and was given to volunteers at day 0, day 2 and day 5 of the study for a four hour period.

A total of 5667 puffs were processed. Puffing topography parameters such as puff volume, inter puff interval, puff duration, and peak flow were averaged for each subject per time point and evaluated using descriptive statistics.

In addition to puffing topography and comparison between EVP and CC, we will describe how the puff device was initially validated; including accuracy, precision and robustness of the SPA/M device against smoking machine (sin, triangle or square wave).

We will also address the following questions: Do we need to pay attention to potential bias coming from the adaptor to properly record the flow rate? How to properly set up the training phase? Does the puff have a specific shape?

[1] Tan Walele, ST20, CORESTA SSPT2015

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 03

The challenges associated with emission ceilings based on multivariate quantile

In 2012, we evaluated and presented at the CORESTA Congress[1] the probable impact of emission ceilings as proposed by the joint working group of the WHO Study group on Tobacco Product Regulation (TobReg)[2] using experimental data and we demonstrated the necessity of considering the correlation between all of the analytes for which a ceiling is to be applied. Indeed, if only one analyte is considered in isolation the relation order is total and the decision depends on the position of a single numeric value with respect to a threshold. In this case, the most important issue is the determination of a suitable threshold according to various criteria based on different considerations ranging from toxicology to method/product variability. But when several analytes (dimensions) are involved, the relation order becomes partial making the problem imprecise and complex and raises the question: what is the best regulatory approach to limiting the quantities of multiple analytes together? We refer to this problem as the multiple-ceiling (MC) problem. The MC problem will require the evaluation of several related fields of decision-mathematics, depending on which approach would be chosen. Indeed, there are, and remain, several alternative approaches, the choice of which ultimately depends to some extent on what specific considerations regulators want to base their decision.

The objective of this presentation is to introduce the MC problem and to discuss the technical limitations.

[1] X. Cahours, T. Verron, S. Purkis, S. Colard (2012). Product compliance mapping. CORESTA Congress (Sapporo, Japan)
[2] World Health Organization (2008). The scientific basis of tobacco product regulation, second report of a WHO Study Group (TobReg). WHO Technical Report series 951.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 20

Responsible practice in e-vapour products (EVP) product stewardship

Imperial Tobacco has developed a comprehensive product stewardship programme to evaluate and assess its e-vapour products prior to placing them on the market. Experience and knowledge of tobacco products has been harnessed along with expertise from the fields of pre-clinical and clinical assessment. In addition, different guidelines covering both established and developing regulatory frameworks have been consulted on and applied, for example International Conference on Harmonisation (ICH) and Organisation for Economic Co-operation & Development (OECD) guidelines as well as the FDA's guidance on Modified Risk Tobacco Product Applications.

We are active in U.S. and European industry and scientific forums dedicated to developing standardised test methods for the evaluation of e-vapour products. This includes the CORESTA E-cigarette Task Force which is developing test methods for measuring chemical constituents in e-liquids and aerosols.

Our product stewardship approach has been discussed with relevant stakeholders and has received endorsement from a competent authority. We apply this approach to all of our products, whether developed or acquired, and this has resulted in several significant improvements prior to launch. Our presentation(s) will focus on the following areas; 1) pre-clinical assessment, 2) evaluation of existing clinical studies and 3) post-market stewardship practices.

Material hazards associated with e-vapour products, the influence of device designs and e-liquid on aerosol properties, stability and *in vitro* biological endpoints will be the main focus of discussion for pre-clinical assessment. We will also discuss our existing clinical data in relation to the pharmacokinetic and pharmacodynamics effects and compare this with published studies on specific subpopulations (experienced vs naïve) involving use of different devices and the potential for these products to aid in quitting or reducing smoking. Finally, various approaches to post-market stewardship including long-term clinical trials and biomarker analysis will be reviewed.

Our approach will provide reassurance to both consumer and regulators on the responsible stewardship and quality of e-vapour products. Imperial Tobacco welcomes the opportunity to participate in forums such as CORESTA to drive improvements and shape future product and testing standards.

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Computational fluid dynamics simulation of cigarette smoke particulate phase retained by cigarette filter

A computational fluid dynamics model was developed to simulate the flow and retention of cigarette smoke aerosol particulate phase in an acetate filter whilst using nicotine as a surrogate for the particulate phase of the mainstream smoke. A volume averaging method was applied to simplify the physical model of filter. Commercially available software, ANSYS FLUENT 13.0, was used to simulate the distribution of smoke particulate phase (nicotine) in the cigarette filter at different time points of the puff (puff duration: two seconds, under ISO conditions). At the end of the smoking procedure, the axial and radial distribution of the volume fraction profiles of nicotine in the filter, as well as the filter filtration efficiency, were obtained. To validate the effectiveness of the developed model, the simulation results in this work were compared with experimental data. The results showed that: 1) The axial distribution of the nicotine retention in the cigarette filter calculated by simulation was that the concentration of nicotine decreased gradually from the tobacco column side to the mouth end of the filter, which is in good agreement with the experimental data. 2) In radial direction, both experimental and simulated concentration of nicotine in the cigarette filter exhibited a reducing tendency from the centre to the periphery of the filter, however, the simulated concentration increased near the periphery of the filter. 3) The relative deviation of filter filtration efficiency to nicotine between simulated and experimental results was 21%.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 44

Optimisation of *in vitro* standard testing of e-vapour and heated tobacco products

The risk assessment of tobacco products includes a number of biological *in vitro* test assays which have become *de facto* standards over the last decades. These test methods are designed to assess specific toxicological end points and are able to discriminate the biological activity of cigarettes with different condensate levels and tobacco blends. Moreover, the *in vitro* setup provides the opportunity to investigate the specific biological activity from exposure to different portions of the smoke (i.e. whether whole smoke, condensate or gas vapour phase).

In recent years the industry has become focused on the development of potentially 'Reduced Risk Products'. Initial attempts to reduce 'tar' and targeted substances from the gas vapour phase by selective trapping were followed by the development of products with tobacco heating technology and, most recently, electronic-vapour products (EVP).The concomitant assessment using accepted and adapted *in vitro* methods is the key to demonstrate the reduced *in vitro* toxicity of such products.

Due to their composition the toxicological assessment of particulate matter especially from EVPs poses a challenge for standard test systems. Direct exposure of cells to the freshly generated smoke/aerosol at air liquid interface (ALI) seems to be the most practicable alternative approach. Due to the low toxicity of vapour produced by EVPs the sensitivity of *in vitro* test systems towards gas components is of crucial importance and their effects must be recognised.

In order to fulfil all the requirements the NRU assay, Ames test and IVM assay were optimised and representative results will be presented:

i) In the NRU assay the HepG2 cells were seeded on a collagen matrix for exposure to aerosol under ALI conditions in a newly constructed smoke exposure machine (SEIVS).
ii) Ames test procedure was optimised for smoke/vapour and their gas phase components in particular.
iii) A positive vapour control was adapted for IVM following ALI exposure. Additionally p53-competent human B lymphoblastoid cell line TK6 was introduced as a "better predictive" alternative to V79 hamster cells in the IVM assay.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 06

Determination of gas-phase carbonyls in e-cigarette aerosol using a sorbent tube (EPA TO-11A) vs. an impinger collection

The aerosols generated from e-cigarettes are primarily composed of fine particles of liquid and gas phases of the vaporized e-liquid. Low levels of thermal degradation products such as carbonyls (e.g. formaldehyde, acetaldehyde, acrolein, and crotonaldehyde) have been reported in e-cigarette aerosols. A rapid, selective and sensitive method specific to measuring carbonyls in e-cigarette aerosols using ultra performance liquid chromatography-mass spectrometry (UPLC-MS) has been developed. This method was optimized for aerosol collection using a 44 mm Cambridge filter pad (CFP) followed by an impinger containing acidified 2,4-Dinitrophenylhydrazine (DNPH) to capture both liquid and gas phase carbonyls, respectively. While the use of CFPs and impingers are common for traditional cigarette smoke collection techniques, environmental air sampling techniques typically involve the use of sorbent tubes (e.g. DNPH impregnated silica) for the collection of gas phase carbonyls as described in the U.S. Environmental Protection Agency (USEPA) Compendium Method TO-11A. Therefore, this collection regime was evaluated as an alternative to the traditional impinger approach for gas phase collection. It was demonstrated that both methods are suitable for the collection of gas phase carbonyls in e-cigarette aerosols and they show equivalent trapping efficiencies. For 20 puff collections, it was observed that approximately 70% of the formaldehyde is trapped in the liquid phase on the CFP and approximately 30% is trapped in the gas phase by either the sorbent tube or the impinger. The sorbent tube collection had one major limitation. The tubes had inconsistent packing densities which could restrict air flow, thereby altering the puff volume. While there are no puff volume issues using the impinger method, sorbent tubes must be pre-selected based on packing density prior to aerosol collection.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 20

Research on differences of deliveries of components in mainstream cigarette smoke under two smoking regimes

In order to research the differences of deliveries of components in mainstream cigarette smoke under two smoking regimes from different angles, the samples of nine Virginia type cigarettes and 11 blended type cigarettes of different tar yields (1.0-13.3 mg) were smoked by smoking machine under ISO and Health Canada Intense (HCI) smoking regimes. The differences of deliveries of components in mainstream cigarette smoke were analysed. First, the results demonstrated that from the point of single cigarette, the deliveries of components determined under HCI regime were significantly higher than those under ISO regime; when comparing the cigarettes of high/moderate tar yield, the differences of deliveries of components between ISO and HCI regimes were greater for the cigarettes of low tar yield. Secondly, from the point of per milligram of nicotine, the deliveries of particle components, including N-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, benzo[a]pyrene and phenol, determined under the two regimes were almost the same; the deliveries of gas components, including CO, carbonyl compound and HCN, in the mainstream smoke of low tar (<6 mg) cigarettes determined under HCI regime were higher than those under ISO regime; however, those of high tar (>6 mg) cigarettes determined under the two regimes were similar. Lastly, from the point of per milligram of tar, the deliveries of most particle components determined under HCI regime were slightly lower than those under ISO regime; however, the deliveries of gas components in the mainstream smoke of low tar cigarettes determined under HCI regime were higher than those under ISO regime.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. STPOST 03

Development and application of FT-NIRs model for online monitoring of the quality of cut tobacco in primary processing line

Near infrared (NIR) spectroscopy is especially suitable for online monitoring of the productive process due to its fast and non-destructive detection feature. The online monitoring of cut tobacco is important for cigarette industrial enterprises to guarantee the consistency of product quality. Common practice is to use online NIR spectra and chemical contents of corresponding samples to establish online quantitative prediction models. However, it will be a huge burden to establish and uphold the models in case of multipoint monitoring. Moreover, the difference between instruments and the problem of instrument ageing will confine the working life and range of the established models. The objective of this study was to develop a simple method, which is helpful to conveniently acquire online NIR models for monitoring cut tobacco. A rotating plate was designed to simulate tobacco conveyor, so that standard samples and their spectra in different conditions were obtained for model transferring. Then a chemometric method, Spectral Space Standardization (SST), was successfully utilised to transfer the offline prediction models of tobacco powder into online models of cut tobacco. Hereby, the online monitoring of cut tobacco quality was achieved by examining tobacco components in virtue of the obtained online models. Experimental results of tobacco combustion heat showed that the average prediction relative error (APRE) decreased from 6.5% to 2.7% after model transferring, which was in close proximity to that of the offline model (2.0%). It indicated that SST could eliminate the systematic difference between offline and online spectra of a sample, and the prediction accuracy of online models was almost the same as that of offline models. No more than 20 standard samples are needed for model transfer, so model transfer can be finished in one day, which enables the proposed method to be applied to any section of the tobacco primary processing line or tobacco threshing and redrying line.

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Study on reduction of harmful components in mainstream cigarette smoke with electric-heating atomisation technology

A novel cigarette smoking device was designed by adopting electric-heating atomisation technology for the purpose of reducing the harmful components in mainstream cigarette smoke. The smoking device was composed of a cigarette holder and an electronic atomisation module, which comprised an atomiser, a battery, an airflow sensor, etc. During smoking, negative pressure was formed inside the smoking device, the airflow sensor was activated by inhaled air, then the atomiser was connected to the battery. The solution in the atomiser was atomised and mixed with mainstream cigarette smoke, and harm reduction was realised via the interaction of atomised aerosol and mainstream cigarette smoke. To investigate the harm reducing effect of the smoking device, ten cigarette samples and one reference cigarette were inserted in the smoking device after the mixed solution of glycerol and propylene glycol was added into the atomiser, and smoked by a smoking machine under the ISO smoking regime. The deliveries of ammonia and phenol in captured mainstream cigarette smoke were determined and compared with those of cigarettes smoked without using the smoking device. The results showed that by using the smoking device adopting electric-heating atomisation technology, the delivery of ammonia in mainstream cigarette smoke was reduced by 54.4%-67.8%, and phenol was almost removed from mainstream cigarette smoke completely; which may improve the harm reduction effect. By adopting a novel cigarette harm reducing technology other than traditional technologies, the smoking device is simple in structure and convenient in operation. Furthermore, the device also features flavour enhancing and moisture retaining performances through adding various functional solutions, and its application prospect is good.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 49

Selective reduction of hydrogen cyanide and crotonaldehyde in cigarette smoke with cholinium lysine ionic liquid

In order to selectively reduce deliveries of hydrogen cyanide (HCN) and crotonaldehyde (CRA) in cigarette smoke, 20 different natural amino acids were screened by a smoke test with 0.3 mmol/cig of amino acid used for cigarette with a small connection device between cigarette filter and smoking machine. Lysine, which is rather cheap and determined to be effective in the reduction of HCN and CRA ($P<0.01$), was selected for the synthesis of cholinium lysine ionic liquid ([Ch][Lys]). Then [Ch][Lys] was adsorbed on fumed silica and made into [Ch][Lys]/SiO_2 (60-80 mesh fine particles), which was characterised by ^{1}H-NMR, FT-IR, SEM and N_2 adsorption. The performance of [Ch][Lys]/SiO_2 in terms of its sensorial impact on smoking quality as well as on the reduction of HCN and CRA was investigated. The results showed that: (1) When [Ch][Lys]/SiO_2 was used on cigarette at 0.3 mmol/cig, the deliveries of HCN and CRA in mainstream smoke reduced by 83% and 30%, respectively, which was more effective than using lysine. (2) The reduction of HCN and CRA in mainstream smoke is linearly correlated to the dosage of [Ch][Lys]/SiO_2 within the range of 0-0.3 mmol/cig. (3) When [Ch][Lys]/SiO_2 was added to a dual-filter at 0.08 mmol/cig, the deliveries of HCN and CRA were 27% and 10% lower than the control respectively; whereas the deliveries of tar, nicotine and other typical harmful components, such as carbon monoxide (CO), ammonia (NH_3), 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), benzo[a]pyrene (B[a]P) and phenol changed by less than 5%. The addition of [Ch][Lys]/SiO_2 in the filter did not significantly impact the sensory performance of the respective cigarettes ($P>0.05$), which was confirmed by a sensorial analysis against the reference (without adsorbent). It was concluded that [Ch][Lys]/SiO_2 could selectively reduce the deliveries of HCN and CRA in cigarette smoke w/o having a negative impact on the sensorial properties of cigarette smoke.

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CORESTA Meeting, Smoke Science/Product Technology Groups, Jeju, 2015, abstr. ST 47

Molecular epidemiology of smoking behaviours among smokers of cigarettes with different tar yields

Smoking behaviour is a comprehensive action with various smoking characters affected by sociological factors and cigarette itself. To investigate the smoking behaviours of smokers of different tar yield cigarettes, molecular epidemiology, including epidemiological survey and biomarker determination, were adopted to study smoking behaviours and their influencing factors. On the basis of cross-sectional design, 435 adults (aged > 18 years) were categorised into four groups: non-smoker, low-tar, moderate-tar and high-tar groups. The urine samples were collected from each group. The metabolites of eight biomarkers, nicotine, tobacco specific nitrosamines, polycyclic aromatic hydrocarbons, 1,3-butadiene, acrylonitrile, acrolein, crotonaldehyde and benzene, in urine samples were determined by HPLC-MS/MS. The results showed that: 1) The differences of age, gender, education, occupation and income reached statistically significant levels ($P<0.05$) among different groups, while no significant statistical difference ($P>0.05$) was observed in the factors of marriage status and body mass index. 2) There were no significant differences in cigarette number ($P=0.572$, >0.05), puffing interval ($P=0.443$, >0.05), depth of inhalation ($P=0.934$, >0.05), butt length ($P=0.854$, >0.05), nicotine dependence ($P=0.149$, >0.05) among different groups. The number of cigarettes smoked by a smoker did not change significantly ($P>0.05$) when the smoker switched to higher or lower tar yield cigarettes. 3) The metabolite levels of nicotine, tobacco specific nitrosamines, crotonaldehyde, acrolein, 1,3-butadiene, acrylonitrile and benzene in the urine of smokers in low- and moderate-tar groups were significantly ($P<0.05$) lower than those in high-tar group; however, the metabolite levels of polycyclic aromatic hydrocarbons did not differ significantly ($P>0.05$) among different groups. It was indicated that smoking behaviours were affected by age, gender, education, occupation and income; the tar yield of cigarette was not a main influencing factor of smoking behaviours. The exposure level of smokers of low tar yield cigarettes to hazardous compounds is lower than that of moderate or high tar yield cigarettes.

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On-line puff resolved analysis of e-cigarette vapours and heat-not-burn tobacco products

Photo ionisation-time of flight mass spectrometry (PI-TOFMS) has been established for on-line, puff-resolved characterisation of cigarette smoke. For example many toxicants, such as butadiene, acetaldehyde, naphthalene, phenol or polycyclic aromatic hydrocarbons (PAH), can be detected with single puff-resolution in cigarette smoke. A special PI-TOFMS system, based on a special vacuum ultraviolet (VUV) -lamp for PI and integrated with a smoking machine, is commercially available (LM2x-Photo-Tof, Borgwaldt KC, Hamburg, Germany) and suited for many industrial routine and research tasks. The more sophisticated laser-PI-TOFMS technology allows a parallel use of different PI technologies (resonance enhanced multi photon (REMPI) or single photon ionisation (SPI)) in conjunction with conventional electron ionisation (EI). This instrument was applied for highly sensitive on-line detection of smoke and emission aerosol constituents as well as vaporisation and thermal break down products from e-cigarettes (eCig) and heat-not-burn (HnB) tobacco smoking devices. Different HnB- (electrical tobacco heating, charcoal-tip and a tobacco extract containing-pod system) and eCig-systems were investigated. While the composition of the eCig aerosols in general is rather simple, the HnB aerosol is more complex. However, most toxicants are dramatically reduced in HnB aerosol if compared to cigarette smoke. The puff-resolved release of nicotine, aerosolising compounds (glycerol and propylene glycol), flavour compounds (e.g. vanillin or menthol) and toxicants (benzene, butadiene etc.) of different HnB and e-cigarette devices have been investigated using different smoking regimes (e.g. ISO, HCI). Large differences in puff release profiles of the relevant compounds are obtained for the different devices. Furthermore the smoking regime has a strong influence on the release dynamics as well. The results obtained from the HnB- and eCig-devices are finally compared to findings obtained from standard reference cigarettes.

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