ABSTRACTS OF PRESENTATIONS MADE AT THE

2013 CORESTA JOINT MEETING OF THE

SMOKE SCIENCE AND PRODUCT TECHNOLOGY STUDY GROUPS

SEVILLE, SPAIN

(by alphabetical order of first authors)

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 06

Particle size distribution of electronic cigarette aerosols and the relationship to Cambridge filter pad collection efficiency

The relatively volatile nature of the particulate matter (PM) fraction of e-cigarette aerosols presents an experimental obstacle with regard to particle size distribution measurements. This is particularly true for instruments requiring a high degree of aerosol dilution prior to measurement. To illustrate, we have shown that average particle diameters determined by a high-dilution, electrical mobility method are in the 25-50 nm range and total particulate matter (TPM) mass calculated based on the suggested diameters are orders of magnitude smaller than those determined gravimetrically. This discrepancy is believed to result from extensive evaporation of PM at the dilution level conditions of the electrical mobility analysis. Also presented are the results of a study for which e-cigarette particle size distribution measurements were made in an undiluted state by a spectral transmission procedure. When the undiluted e-cigarette aerosol was examined, particles in the 210-380 nm count median diameter range were observed, with particle number concentrations in the 10^9 particles/cm^3 range. These measurements are comparable to those observed in prior studies of tobacco burning cigarette smoke aerosols. Per puff TPM mass for the e-cigarettes calculated from the aerosol size distribution parameters measured by spectral transmission were in good agreement with replicate determinations of TPM mass by gravimetric filter collection. In light of this observation, capture of e-cigarette generated TPM by fibrous filters is predicted to be a highly efficient process over the expected range of filtration flow rates. Supporting experimental data are provided demonstrating this to be the case. Additional data are presented providing some insight into the particle/vapor partitioning of the primary components of e-cigarette aerosols. This information should be useful both as a description of the aerosol properties of the material produced by these widely used devices and as input data for studies of inhaled aerosol dynamics.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 27

Analysis of selected carbonyl compounds in tobacco products by using pentafluorobenzylhydroxylamine derivatization and gas chromatography-mass spectrometry

A reliable method for the routine analysis of trace carbonyl compounds, including formaldehyde, acetaldehyde, acetone, propionaldehyde, acrolein, methyl-ethyl ketone (MEK), butyraldehyde, and crotonaldehyde, in tobacco products is presented. One gram of tobacco sample was spiked with a mixture of three isotope-labelled carbonyls used as internal standards, and extracted with water. An aliquot of the aqueous extract was derivatized with o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride (PFBHA). The PFBHA derivatives of the carbonyls were extracted with hexane and analysed by gas chromatography-mass spectrometry (GC-MS). The accuracy and precision of the method were evaluated using spiked matrix samples, including the Kentucky reference 3R4F cigarette filler and the CORESTA smokeless reference products CRP1, CRP2, CRP3, and CRP4. All investigated carbonyl compounds, with the exception of acrolein, demonstrated excellent recoveries (87-114%) and precision (0.2-7.6%) for the different spiked tobacco products. Acrolein, when spiked directly on the product, was found to be unstable. The linear range of the method was from 0.08 to 27 µg/g and detection limits ranged from 0.03 to 0.08 µg/g, dependent on the compound.

The method was applied to analysis of Kentucky reference 3R4F cigarette filler and the four CORESTA smokeless reference products. Formaldehyde (0.300-6.45 µg/g) and acetaldehyde (0.849-18.4 µg/g) were detected in all tested reference products. Acetone (0.197-3.31 µg/g) and propionaldehyde (0.156-1.07 µg/g) were found in the 3R4F, CRP1, CRP2, and CRP3. Levels of MEK, butyraldehyde, and crotonaldehyde were found to be below the method detection limit for all tested reference products. Using this method, storage conditions (storage time, container, and temperature) were found to impact the yields of carbonyls detected in reference tobacco product samples (3R4F, CRP2, CRP3) being both compound and sample matrix dependent; concentrations of formaldehyde in all tested products gradually increased as the storage time increased, while the concentrations of acetone in 3R4F samples dramatically decreased as the storage time increased.

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Prediction of smoke exposure from smoking time

We have recently shown that cigarette smoke yield (TNCO) depends linearly on the difference between the time of smouldering and the time of smoking using several machine smoking regimes (CORESTA 2012), with the filter ventilation open or blocked. It is obvious that the smoker's exposure increases when the intensity of smoking increases, i.e. when the smoking time decreases. However, from our previous observations, we wanted to know whether human smoking yields could also be predicted through the measurement of human smoking time. For this purpose, a smoking behaviour controlled study was carried out to compare the human nicotine smoking yields obtained by both filter tip analysis and the cigarette burning time model. The results of our study show that i) smoke exposure, defined here as nicotine human smoking yield, can be assessed by measuring the smoking time and also ii) this smoke exposure is a linear function of the smoking time whatever the smoking behaviour.

In this presentation, the experimental set up and the results will be discussed as well as limitations and perspectives.

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Factors driving short term smoking compensation

Smoking compensation, defined as a modification of smoking behaviour, i.e. increase in puffing intensity, has been studied extensively. It has often been stated that compensation, generally occurs following a switch to a lower tar and nicotine yielding cigarette and a simplistic assumption that compensation is driven by the smokers' wish to regulate their nicotine intake to a constant value. Cigarette smoking constitutes a complex series of behavioural events which occur each time a puff is taken and inhaled. In this poster, using results from several internal studies, systematic changes are shown to occur in the puffing topography and human smoking yields when various physical properties of cigarette are modified. Our results suggest that during the course of smoking a single cigarette, nicotine yield is not a major controlling factor in puffing behaviour. On the other hand, physical parameters of the cigarette, such as draw resistance, can have a significant impact on puffing behaviour and consequently on exposure. Other factors will come into play regarding the longer term decisions taken by smokers on their subsequent choice of product and smoking behaviour.

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A quantum dot-labelled immunoassay for assessing the *in vitro* genotoxicity of cigarette smoke based on quantitative detection of γH2AX

Phosphorylation of histone H2AX (γH2AX) is a sensitive marker of activated DNA damage involving the formation of double-strand breaks. A novel quantum dot-labelled immunoassay was developed for profiling the genetic toxicity of cigarette smoke based on the quantitative detection of γH2AX expression in A549 cells. After stimulation, cells were fixed and permeabilised in the wells of 96-well plates for the measurement of γH2AX in whole cells, which eliminated the need of preparing cell lysates. A high-affinity mouse anti-γH2AX antibody and a normalisation antibody that recognised the total H2AX regardless of phosphorylation status were used for detecting the γ-H2AX and total H2AX in cells separately. Then two specific quantum dots-labelled secondary antibodies were added and combined specifically with the primary antibodies. The quantum dots with different excitation were used as sensitive markers for the existence of analytes with higher laser efficiency, larger stokes shift and excellent photostability. The fluorescence of detecting γH2AX is normalised to that of the total H2AX in each well for the correction of well-to-well variations. Due to the unique size-dependent optical properties of QDs, the fluorescence readout signal of this method was 5-fold higher than that of the traditional immunofluorescence assay which uses organic dye labelled-secondary antibody. A dose-dependent effect of cigarette smoke exposure on γH2AX expression was investigated and the contributions of cigarette smoke condensate and vapour phase components to the genetic toxicity of cigarette smoke were also discussed by comparing the γH2AX levels in stimulated cells.

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Do cigarette smoke yields from a single smoking regime fit with current regulatory objectives?

Many regulations worldwide require the reporting of tar, nicotine and carbon monoxide (TNCO) and set limits on their yields measured following the ISO smoking regime (ISO3308, 2012). The intention of FCTC Art. 9 is to characterise and monitor cigarettes, and in the USA, FDA has to make testing data publicly available in an understandable and not in a misleading way. The introduction or recommendation for an additional more intense smoking regime with filter ventilation blocked has been made within this regulatory context. However, this raises a number of analytical issues and does not make the data less misleading. On the basis of a cigarette burning model presented previously (CORESTA 2012), investigations were conducted on 10 products with different designs in order to understand the burning process when different machine smoking regimes were applied. The relationship between yields and the difference between smouldering and smoking time, with filter ventilation open or blocked, is described by a straight line passing through the origin for all designs tested. These studies showed that a single smoking regime would fit with the FCTC Art. 9 and FDA purposes. The reporting of i) ISO TNCO yields with puff numbers, ii) filter ventilation and iii) cigarette dimensions, characterises the products as well as data from two smoking regimes, and it provides valuable data for cigarette monitoring. In addition, the association of the burning time derived from the number of puffs with the yields would be appropriate to communicate understandable and not misleading data.

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Compensation of the effects of non-standard temperature and pressure when measuring diffusion capacity

Although the diffusion capacity of cigarette papers is routinely measured and instruments for this measurement have been available for several years, the repeatability and reproducibility of diffusion capacity measurements are not yet fully satisfactory. One reason for the high variation in diffusion capacity data are deviations in temperature and pressure from standardised conditions, especially within the measurement chamber of the instrument. Deviations in temperature and pressure will have an influence on the gas flow rates, the measured CO_2 concentration and the diffusion processes inside the measurement chamber. An evaluation of these effects on a theoretical basis shows that they cause relative errors in the diffusion capacity of several percent for temperature deviations of about 5 degrees or pressure deviations of about 5 kPa. Usually, errors are larger for cigarette papers with high diffusion capacity. The individual effects may add up to a relative error of more than 20%. As a mathematical model of diffusion and convection processes in the measurement chamber is often used in instruments to calculate the diffusion capacity from the measured CO_2 concentration, these errors can be reduced by modifying the existing model and including temperature and pressure effects. The dependence of model parameters on temperature and pressure was derived from data available in the literature. The modified model is evaluated and it is shown that the errors can be substantially reduced. An important conclusion is also that a meaningful statement of diffusion capacity values should include temperature and pressure during measurement.

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Variation in toxicant yields from selected products

The WHO Study Group on Tobacco Product Regulation (TobReg) have recommended setting simultaneous ceilings on mainstream smoke emissions of nine toxicants expressed as a ratio to nicotine. The proposed ceilings are recommended to be based on the market median (NNN, NNK) or 125% of the market median (remaining seven chemicals). Toxicant measurements on products, and their compliance to a proposed ceiling, is likely to be affected by analytical and product variability over time. In order to contextualise measurement data, it is important to understand the variation of smoke emissions from commercial cigarette products across time.

A study was conducted to provide information on the variability in mainstream smoke yields of these nine chemicals, and their ratios to nicotine, as measured under the Health Canada intense regime, from three large volume commercial cigarette products from a single market (Germany). Tobacco blend chemistry and cigarette physical measurements were also conducted. The three cigarette products were sampled monthly over ten consecutive months in 2010-11. Control data from the 3R4F Kentucky Reference cigarette was also collected at the same interval.

Two main sources of variation were investigated – analytical variability and cigarette product variability. The mean variation for each chemical of interest as a ratio to nicotine will be presented along with the expanded uncertainty (k=2) as an estimate for a 95% confidence limit. This within laboratory 'tolerance' around measured values for each chemical of interest was found to reach up to 40% in this study. A practical lower limit of variation for a reasonably controlled analysis was identified. Also identified were chemicals which demonstrated larger than average analytical variation and would benefit from improvement in analytical methods.

In summary, measurement of the nine chemicals recommended by TobReg for regulatory ceilings was demonstrated to be affected by significant level of variation.

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The statistical benefit of performing GLP bioanalysis using assays that have reduced variability

Since the tobacco industry is moving into the area of regulated bioanalysis, we have noted that there is confusion surrounding the type of regulatory standards required for analytical methods to be used for testing tobacco constituents and biomarkers in biofluids. This presentation will focus on demonstrating the benefits of performing GLP bioanalysis by discussing the following bioanalytical topics:

1. GLP – when is a study required to follow GLP guidelines and what constitutes a truly GLP study
2. The importance of assuring sample integrity
3. Critical elements of GLP method validation:
   a. Selectivity
   b. Sensitivity
   c. Carry-over and Contamination
   d. Accuracy & Precision
   e. Stability
4. GLP standards are the cornerstone of bioanalytical chemistry. These principles are very different from clinical chemistry and GMP assays and include:
   a. Value of standards
   b. Relationship between standards and quality control samples
   c. Importance and timing of proper chromatographic integration
   d. Batch acceptance criteria
   e. Reporting sample concentrations
   f. Importance of incurred sample reproducibility

The benefit of performing GLP analysis for tobacco constituents and biomarkers is that the improved analytical precision which has a direct impact on statistical analysis. Specifically, more precise analytical methods allow studies to dose fewer subjects to achieve the same statistical power.

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Validation of the VitroCell VC10 smoke exposure system for *in vitro* assays

The VITROCELL VC10® smoke exposure system offers multiple platforms and air liquid interface (ALI) exposure to mimic *in vivo*-like conditions for assessing the toxicological impact of smoke in *in vitro* assays. The validation plan to ensure the system and corresponding assays were fit-for-purpose consisted of equipment qualification, pre-validation method development and method validation for neutral red uptake (NRU), sister chromatid exchange (SCE) and Ames assay (TA98, TA100). Parameters assessed for establishing the experimental model consisted of 1) optimization of mammalian and bacterial cell growth at ALI in static and flowing air conditions; 2) investigation of pH changes during exposure; and 3) determination of smoke concentrations and appropriate positive controls for all assay types. In each validation protocol, at least six experiments were performed. Smoke airflows of 10, 8, 6, 4 or 2 L/min and air-control were used for evaluating the assays, and acceptance criteria were established accordingly. NRU acceptance criteria were established to be 1) coefficient of variance is <15% in optical density (540 nm;OD_{540}) values between chamber replicates; 2) the positive control elicits >50% decrease in NRU relative to the air control; 3) mean OD_{540} of all air control replicates is >0.2 and 4) viability values above and below the IC_{50} are required. Acceptance criteria for the Ames assays were set as follows: 1) ALI control counts fall within specified ranges for TA98 and TA100; and 2) positive controls induce ≥2.0 fold increases in revertant numbers over the ALI control. SCE assay acceptance criteria were set as follows: 1) the average number of SCE/cell in the incubator and air controls is within a specified range; 2) at least one positive control treatment exhibits ≥ doubling in the average number of SCE/cell over the incubator and air controls; 3) ≥15 cells must be scored for at least two chamber replicates and 4) data must be generated from at least three non-zero concentrations. Through critical function and assay assessments via the installation, operational, performance and validation protocols, the VC10 was deemed fit-for-purpose and has been shown to induce exposure-related changes in cell survival, sister chromatid exchange and bacterial mutagenesis. Collectively, these criteria support the validity of the use of the VC10 system with several *in vitro* genetic toxicological assays.

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A modified Cambridge filter holder and extraction equipment and methodology to more accurately quantify water in high water content aerosols

Philip Morris International is developing products with the potential to reduce the risk of smoking-related diseases by heating tobacco and avoiding combustion. The resulting aerosol has a less complex chemical composition and contains more water than conventional cigarette smoke.

It has been determined that the trapping and extraction procedure used for conventional cigarettes, defined in the International Standard ISO 4387, is not suitable for the high water content present in such heated tobacco aerosols. Errors occur because of water loss during the opening of the Cambridge filter pad holder to remove the filter as well as the manual handling of the filter, and because the plastic housing of the filter may absorb water. This results in inaccurate values for the water content, and erroneous and overestimated values for Nicotine Free Dry Particulate Matter (NFDPM).

A modified Cambridge filter holder and extraction equipment and methodology has been developed that involves the use of a metallic filter pad holder which supports *in situ* extraction from the Cambridge filter pad. Investigation has shown that this approach delivers more accurate water measurements for aerosols with high water content.

This equipment and methodology has also been used to collect the aerosol generated by conventional cigarettes and it was found that, for such aerosols, the water yield is larger than that generated by the ISO standard 4387, and that the difference in water yield between the two methods increases as the smoking regime intensity increases. However, for conventional cigarettes an impact on NFDPM values is only observed under intense smoking regimes.

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Puff-by-puff analysis of mainstream smoke constituents of non-LIP and LIP-cigarettes (2)

As presented at the CORESTA Congress 2012 a puff-by-puff profile of LIP cigarettes shows differences in CO and O_2 between a puff taken on the banded area and a puff on the band spacing. The cigarettes for the investigation of CO and O_2 have been smoked according to ISO 3308 with a puff volume of 35 ml.

In continuation of the study presented in 2012 effects were studied for larger puff volumes. Cigarettes have been produced with different base paper parameters and bands with different diffusion capacities. Cigarette papers with permeabilities in a range of 50-125 CU and burn additive levels between 1-2% have been used for the investigation of the base paper parameters and band diffusion capacities vary between 0.05-0.15 cm/s. A puff-by-puff profile of the sample cigarettes with fully blocked filter ventilation was taken by simulating the puff volume of 55 ml of the Canadian Intense smoking regime. Changes in mainstream smoke yields of CO, O_2, tar and nicotine will be presented.

A single channel smoking machine (Borgwaldt RM1), a mass analyser (Airsense Compact), a GC-FID and GC-TCD were used for the determination of mainstream smoke yields.

This study compares smoke yields of non-LIP and LIP cigarettes with different base paper parameters (e.g. permeability, burn additives) and different band diffusion capacities.

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Analytical method to model human mouth-level transfer of ingredients from Swedish pouched snus

In parallel with the human study[1] a laboratory *in vitro* assay was conducted with three media: artificial saliva, water and 1% ß-cyclodextrin in water, using two snus products that were indistinguishable apart from the flavour application rate. To determine an analytical method to model human mouth-level transfer, the transfer rates for each ingredient (menthol, 1,8-cineole and nicotine) were compared using extraction times of 5, 10 or 30 minutes with 30 mL of each of the preheated (370 °C) extraction solvents.

For measuring residual flavours on exposed snus pouches, preliminary extraction tests highlighted quantitative salivary effects with some users. Dichloromethane/Methanol (1:1 v/v) was found to lead to an efficient extraction >95% for all three targeted compounds.

With an extraction time of 30 minutes no artificial media provided a good correlation with the human data. The transfer rates of all media vs. human data set was highest for nicotine (78% to 89%) than 1,8-cineole (ranging from 44% to 69%) and menthol (25% to 60%); for both application rates.

At shorter contact times (10 and 5 minutes) flavour transfer rates for water and artificial saliva were similar but significantly higher for ß-cyclodextrin. Nicotine transfer rates remained highest and overestimated transfer. The 1% ß-cyclodextrin in water enhanced organic solvent extraction (both dichloromethane and dichloromethane/methanol). This media also exhibited higher transfer rates for all contact times (30, 10 and 5 minutes) for the three compounds studied.

An extraction time of 5 minutes into artificial saliva or water gave the best fit to the human data set for all three compounds. The relative extraction of the three constituents reflected the water solubility. In the human data, saliva access may limit upper transfer rates. As was seen in the human study, application rates did not appear to affect transfer rates in any media.

[1] Human mouth-level transfer rate of menthol, 1,8 cineol and nicotine from Swedish pouched snus. Oral session - CORESTA 2013.

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Determination of chlorinated dioxins/furans in mainstream cigarette smoke using gas chromatography-tandem mass spectrometry

Chlorinated dioxins and furans have been cited by the United States Food and Drug Administration (FDA) as harmful and potentially harmful constituents (HPHC) in tobacco smoke. It is the only constituent on the FDA list which is not a specific compound rather it refers to a sub-group of compounds consisting of polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). By convention they are usually referred to by the generic term 'dioxins'. They are anthropogenic chemicals and are formed from combustion/incineration and manufacturing processes. The World Health Organisation (WHO) have defined 17 dioxin congeners as significantly toxic and adopted a system of reporting the total toxic equivalence (TEQ) based on the mass of each component multiplied by a toxic equivalence factor (TEF). The US Environmental Protection Agency (EPA) has set a threshold for safe dioxin exposure at 0.7 pg TEQ/kg body weight per day.

This presentation will demonstrate a clean-up procedure using the Supelco Dioxin Prep System, developed by Maeoka *et al.*[1], followed by analysis using gas chromatography-tandem mass spectrometry (GC-MS/MS). Dioxins were not detected (<4.3 pg TEQ/cig) in Kentucky and CORESTA reference cigarettes under ISO 3308 and Health Canada Intense (HCI) smoking conditions. Transfer rates were evaluated by spiking 500 pg of each component into the tobacco rod and collecting the smoke condensate on a Cambridge filter (CF) pad. Reproducible measurements were obtained with recoveries of 4% to 11% (ISO) and 9 to 27% (HCI). Recoveries of the ^{13}C isotope labelled internal standards were between 74.5% and 125.7%.

[1] Maeoka M., Inoue I., Shimono H., Morita N., 2002, Quick Pretreatment Prep Study for Dioxin Analysis. *11th Symposium on Japan Environmental Chemistry* (June 3-5), 521- 532.

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From seed to smoke: N-nitrosonornicotine (NNN) level in smoke is significantly reduced in experimental Burley isolines bred to have stable low nornicotine content

N-nitrosonornicotine (NNN) is one of several tobacco specific nitrosamines (TSNAs) identified in cured tobacco leaf and smoke. Previous work has demonstrated that by stabilizing a low level of the precursor nornicotine, the level of NNN in cured leaf can also be reduced. Through backcross breeding, novel genetics have been introduced into the Burley cultivar TN90 (TN90e). This change results in a 75% reduction in the overall nornicotine level in the harvested leaf compared to the conventional TN90 LC cultivar. To evaluate the impact of the reduced nornicotine on TSNA levels in leaf and in smoke, we conducted a field study using the two isolines. The study was a split-plot design where the two isolines were grown under three nitrogen (N) fertilization regimes (0, 224 and 448 kg/ha). The main plot was nitrogen rate and sub-plot was genotype. The study was conducted at two locations, Blackstone, Virginia, USA and Glade Spring, Virginia, USA, with three and five replications respectively. In this study both nornicotine and NNN in cured leaf were reduced by 85% in TN90e as compared to the TN90 LC control. To conduct smoke studies, leaf from upper stalk position tobacco was de-stemmed and lamina was cut to produce hand-made cigarettes. From the Blackstone location, all three replications of each genotype and N rate were evaluated (18 total). From the Glade Spring location, only three of the five replications were selected for each genotype and N rate (18 total). Cigarettes were smoked under ISO conditions and NNN and total particulate matter (TPM) were measured. While main plot interactions were observed, statistically significant NNN reductions for the TN90e cigarettes were found across N treatments. For example at the Glade Spring location, the 0N TN90e showed a 60% reduction in smoke NNN (ng/mg TPM) while the 224 and 448 kg/ha treatments showed 75 and 82% reductions, respectively. Results from this replicated study demonstrate that genetics to reduce nornicotine content result in lower NNN in both cured leaf and smoke.

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Search for low polar non-volatile components characterising tobacco leaves using liquid chromatography / atmospheric pressure chemical ionisation mass spectrometer (LC/APCI-MS)

To discriminate the quality of tobacco growing areas, chemometrics methodology has been applied by using gas chromatography mass spectrometry (GC/MS) or by liquid chromatography electro spray ionisation mass spectrometer (LC/ESI-MS). However, these techniques have limitations in detecting low polar non-volatile components due to their ionisation processes. For this reason, atmospheric pressure chemical ionisation (LC/APCI-MS) was used in this study to determine this group of components in tobacco leaf.

Separation of components was performed under the following conditions: column: Excelpak SIL-C18 5CTM (250 mm × 4.6 mm I.D.); mobile phase A: acetonitrile, mobile phase B: acetone; flow rate: 1.0 mL/min; gradient condition: A 100% at 0 min, A 30% at 10 min, A 20% at 30 min, A 0% at 40 min, and A 0% holds until 55 min; column temperature: 25ºC. The detection of components was achieved by APCI/MS configured at the following conditions: capillary voltage: 4000 V; Corona current: 10 mA; drying gas flow: 5 mL/min; drying gas temperature: 350°C; fragmentation voltage: 200 V; nebulizer pressure: 60 psi; vaporiser temperature: 500°C.

More than 100 components in tobacco leaf, such as solanesol derivatives, chlorophyll metabolites, triacylglycerols, and steroid derivatives were detected within 55 minutes at a single run. Data analysis occurred by applying principal component analysis (PCA) to assess the degree in variety separation and canonical discriminant analysis to narrow down the crucial components. From the subsequent statistical analysis, we concluded that solanesyl esters and solanachromene were specific components to flue-cured Virginia, chlorophyll metabolites to Oriental, while Burley did not include specific low polar non-volatile components other than free solanesol.

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Optimisation of bacterial urinary mutagenicity test for assessment of exposure to cigarette smoke

Urinary mutagenicity is widely recognised as a useful biomarker for assessment of mutagen exposure level in humans. In this study, we optimised several parameters affecting the activity of urinary mutagenicity using a highly sensitive mutation test (microsuspension assay) instead of the conventional Ames test for assessment of exposure to smoke. First of all, we chose YG1024 as a highly sensitive strain from three strains of *Salmonella typhimurium* (TA98, TA100, YG1024) using representative mutation substances of cigarette smoke, such as Benzo[a]pyrene, 2-Aminonaphthalene, 2-amino-3-methyl-9H-pyrido[2,3-b]indole(MeAaC) and cigarette smoke condensate (CSC). And we established several kinds of test conditions such as number of bacteria, concentration of metabolic activation system and incubation time for the most sensitive reaction. Also, we optimised the preparation condition of urinary extracts and this method showed more than 90% recovery value. When we compared the urinary mutagenicity between the smokers and non-smokers, it showed a significant difference according to level of smoking and these results were significantly correlated with smoke exposure biomarkers such as nicotine metabolites and acrolein.

In conclusion, the optimised highly sensitive mutation test to measure the urinary mutagenicity of smokers may be useful in the clinical evaluation of less harmful tobacco products developed in the future.

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Combustion characteristics for specifications of low ignition propensity (LIP) cigarettes using computational fluid dynamics

Low ignition propensity (LIP) cigarettes have become a hot topic in tobacco science after the fire safe laws were tightened in USA, 2004. Today, for most countries, LIP cigarettes are developed consistently. Also, these products have become commercially available.

LIP cigarettes are required to extinguish in the banded areas of the rod paper using permeability reducing substances. For smoker satisfaction, however, it is desirable that a cigarette does not extinguish if left to smoulder.

Two general types of LIP cigarette tests are ASTM test and FASE test. They are limited for the combustion characteristics of LIP cigarettes. In this study, the combustion characteristics of LIP cigarettes were predicted with the specifications of the band substances using computational fluid dynamics. Numerical simulations of chemical species, temperature distributions, and combustion reactions were done in STAR-CCM+ 7.06.

The model was applied to smoulder a LIP cigarette under different band specifications. The results show that the model is capable of reproducing the major features of a LIP cigarette during smouldering. The results show the significant effect of band width, distance between bands, and amount of substances.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 13

Benzo[a]pyrene analysis by HPLC/FLD

Benzo[a]pyrene (BaP) has received much attention in reduced risk discussions. Our objective was to develop an accurate and precise method to measure BaP by HPLC/FLD. Previous methods suggested excitation at 294 nm and measurement at 404 nm. In tobacco samples that contained fire-cured product there was a peak that eluted near the internal standard BaP-d12. This interference was minimized by using 364 nm for excitation and measurement at 404 nm. Calibration curves for BaP and BaP-d12/BaP ratio responses for both 294 and 364 excitation had a linear fit with R^2's of greater than 0.99. 294 nm excitation provided much more sensitivity than 364 excitation with similar precision. The protocol was to extract 1 g sample with 40 ml methanol for 30 min; 20 mL of extraction solution dried under N_2 stream; dissolved in 1 mL methanol; filtered through a 0.2 µ membrane filter; and injected 10 µL on to a Zorbax Eclipse PAH 2.1 x 150 mm column with 3.5 µm particle size and eluted with acetonitrile/water. Drying the extracts resulted in much greater precision than not completely drying the samples. In fire-cured tobaccos the drying step may be omitted and after filtering the extract, direct injection could be done. CRP smokeless tobacco samples 1 and 4 with low BaP were best analyzed at 294 nm, whereas CRP samples 2 and 3 which contain some fire-cured tobacco were analyzed at 364 nm. These samples contained levels of BaP from 0.65 to 62.8 ng g^{-1}. Because of the 10-fold difference in samples, two different calibration curves were used; one for samples between 0.0 and 5 ng g^{-1} and one for samples between 5 and 300 ng g^{-1}. Fire-cured tobacco had BaP levels over 250 ng g^{-1}. BaP in mainstream smoke was determined at 364 nm excitation.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 05

GC-MS analysis of e-liquids taken from e-cigarettes and e-liquids (e-juice) before use in e-cigarettes

Concerns have been expressed about the purity and safety of the liquids (e-liquids) used in electronic cigarettes [Goniewicz *et al*., Tobacco Control 2013 March 6 (Epub ahead of print)]. Moreover, Bahl *et al*. [Reproductive Toxicology 34 (2012) 529-537] have reported that some e-liquids were found to be much more cytotoxic than others. Since only *in vitro* cytotoxicity assays were used, no chemical identifications were reported. Therefore, toxicity (or lack thereof) of the e-cigarette cannot be fully assessed without a more in depth knowledge of the toxicants present in the e-liquid. To this end, e-liquids were taken from purchased containers obtained from the vendors or extracted from the cartomizers of e-cigarettes. After dilution of the neat e-liquids, they were analyzed by gas-chromatography-mass spectrometry using a DB-5 column and the following chromatography conditions. Oven initial temp: 50 °C; initial time: 2.00 min; Ramp rate: 2°/min; Oven final temperature: 300 °C; Oven final time: 23.00 min. Injector temperature: 297 °C; split injection with 10:1 split ratio. Our results showed that public information e-liquid composition provided by vendors on their websites was not always complete. For example, in one case we found undeclared triacetin, diacetin, and monoacetin, which probably came from use of triacetin as a flavour carrier, along with an undeclared flavour component, which has been tentatively identified as caryophyllene. Our results indicate that vendors of e-liquids need to take more care in ensuring the purities of the compounds they use and in ensuring that they have their e-liquids analyzed before publishing information on their composition.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 34

Routine and detailed analyses of materials other than tobacco fillers used to fabricate filtered cigars

Filtered cigars and similar cigar-like products continue to be a growing market. They are also attracting increased regulatory scrutiny. However, there continues to be a dearth of information in the scientific literature about the composition of the tobacco blends, ingredients applied to the tobacco blends, and non-tobacco materials used to manufacture these products. Consequently, we began a research program in 2010 to provide more detailed information to the scientific community. Our initial research into filtered cigars focused on the tobacco blend, ingredients applied to the tobacco, and wrappers taken from product (CORESTA Congress 2010, SSPT 16; CORESTA Congress 2012, SSPT 11). More recently, we have investigated the composition and properties of the two types of reconstituted tobacco wrappers (before use with products) used for filtered cigars, flavored and unflavored filters, papers and adhesives. We have used a combination of GC/MS analyses (with and without derivatization reagents), routine tobacco chemistries, and physical methods to characterize the components and filtered cigars made from them. We will also provide examples of how different combinations of fabrication materials other than tobacco can affect the hedonic aspects of the filtered cigars.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 12

Optimisation of headspace sampling using solid-phase microextraction (SPME) for volatile organic acids in different tobacco types

Different solid-phase microextraction (SPME) materials were investigated to optimise headspace sampling of volatile organic acids from tobacco samples. Experimental parameters such as sampling temperature, pH, and SPME fibres were optimised to achieve maximum and selective adsorption of volatile organic acids.

Three types of SPME fibres: PDMS (polydimethylsiloxane), PA (polyacrylate) and PDMS/DVB (polydimethylsiloxane/divinylbenzene) were investigated to determine the selectivity and adsorption efficiency.

A variety of tobacco samples, such as flue-cured, Burley and Oriental were used in this study.

The effect of these parameters was often dominated by the physical and chemical nature (volatility, polarity) of target compounds. PA (polyacrylate) fibre was shown to be the most efficient at extracting 10 selected volatile organic acids. The operation parameters were optimised as follows: adsorption temperature 80 °C, adsorption time 30 min, desorption temperature 240 °C, desorption time 3 min.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 33

The correlation between physical tipping paper parameters and different perforation methods and its influence on cigarette smoke properties

Within the last decade huge efforts have been made in research and development of various filter ventilation methods in order to achieve specific smoke deliveries and to control the sensory quality of cigarette smoke. Nowadays, the originally widespread offline tipping paper perforation by means of pulsed laser radiation or electrostatic discharge is progressively complemented by online-laser perforation directly inside the cigarette machine. This poses a challenge for both tipping paper manufacturers and the cigarette industry to gain profound knowledge on the features of the individual perforation techniques and their impact on smoke characteristics. The first approach was to investigate the dynamic smoke flow underneath perforated tipping paper which provided a fundamental understanding of the effect of diluted cigarette smoke on the human sense of taste. Physical and geometrical parameters of different types of perforation served as the basis for the second step, to reveal the existence of quasi-diffusion effects co-determining filter ventilation processes with the emphasis to optimise the carbon monoxide (CO) / tar ratios of smoke yields. The objective of this study is to reveal the contribution of variable tipping paper parameters to the quasi-diffusive regulation of the basic smoke deliveries and to the physiological perception of the cigarette taste. This is realised by applying a numerically derived quasi-diffusion model on specific tipping paper properties on one hand and by carrying out a survey with a professional smoker panel on the other hand. The findings complete the topic of perforation comparison and open the potential to consider the most suitable tipping paper design for tailor-made cigarette solutions.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 16

Study on the application of nano-iron oxide to reduce CO in the production of reconstituted tobacco sheets

Nanoparticle iron oxides were prepared by an innovative Sol-Gel method and their surface properties including specific surface area, particle diameter distribution, crystal face diffraction were characterised by employing BET surface area analyser, X-ray diffraction (XRD) and transmission electronic microscopy (TEM). Nanoparticle iron oxides of different sizes were prepared at different baking temperatures and were added to the tobacco sheet for smoking analysis. The research results showed total particle matter (TPM) and carbon monoxide (CO) were reduced by the addition of nanoparticle iron oxides. The baking temperature of the nanoparticle iron oxides had a significant impact on the particle diameters. When the use rate of iron oxides was 6%, the maximum CO reduction was achieved. When nanoparticle iron oxide particles of the size of 10 nm were added to the tobacco sheets, TPM and CO were reduced by 47.84% and 24.4%, respectively. The crystal face analysis of the burned ashes from the tobacco sheets by TEM showed very interesting new peaks compared with the standard iron oxide crystal card. The research effectively demonstrated some evidence for the first time in the tobacco sheet processing industry that nanoparticle iron oxide particles reduced CO yields. More research needs to be carried out to further understand the mechanisms of how the CO and TPM were reduced by the addition of the nanoparticle iron oxides.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 36

A novel auto-antibody test for the detection of pre-neoplastic lung lesions

Atypical adenomatous hyperplasia (AAH) and squamous cell dysplasia (SCD) are pre-neoplastic lesions which are reported to be associated with the development of malignant lesions in the lungs [Alaa, 2011]. Traditionally, detection of AAH and SCD has been achieved by imaging and bronchoscopy, but sensitivity remains unsatisfactory. In this study, we utilised the ability of the immune system to identify lesion specific proteins for detection of AAH and SCD. Previous work has shown that serum auto-antibody panels as biomarkers for the detection of malignant lung lesions [Zhong, 2006] have been successfully applied.

Following informed consent and Internal Review Board approval, AAH and SCD tissue was surgically removed from six patients of Chinese descent (3 AAH and 3 SCD) with corresponding serum samples. A cDNA library was generated from total RNA extracted from the tissues and incorporated into a T7 bacteriophage vector and biopanned to enhance the selection of tumour-associated proteins. Following selection, candidate phage clones underwent two-colour fluorescent protein microarray construction.

Microarray slides were tested with 50 AAH and 50 SCD patient serum samples along with 100 control serum samples as a training group for statistical classifier development. AAH-associated phage proteins were used to develop a classifier with 92.3% sensitivity and 90.2% specificity in distinguishing AAH samples. SCD-associated markers generated a classifier with 98.3% sensitivity and 95.6% specificity in distinguishing SCD samples. These were further validated in an independent double-blinded sample population consisting of 100 AAH, 100 SCD and 200 control serum samples. 82% sensitivity and 70% specificity was achieved in the detection of AAH samples and 86% sensitivity and 78% specificity was achieved in the detection of SCD samples.

Both diagnostic values reached much greater accuracies than any other current biomarkers for AAH or SCD and hence could be a useful tool to assess lung cancer risk in human populations.

References: Alaa M *et al.*, Lung Cancer 2011; 72 : 303–308
Zhong L *et al.*, J Thorac Oncol. 2006; 1(6):513-9

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

Chemical characterisation of e-device aerosols

Electronic devices such as e-cigarettes deliver nicotine without the combustion of tobacco and are increasingly used by global consumers. Typically, the liquid formulation of an e-cigarette is composed of nicotine, excipients (propylene glycol and/or glycerol), water and flavours, and is vaporised and inhaled by the user. Analytical methods used to measure constituents of tobacco smoke can be adapted for the testing of aerosols produced by e-cigarettes, but this may not always be appropriate and may impact the quality of the data. This is because there are large differences in the chemical composition and complexity of mainstream smoke and an e-cigarette aerosol.

The product test parameters such as puff volume, puff profile, frequency and duration of each puff, should be defined to better reflect consumer use. This will result in the development of more representative sampling regimes, the establishment of robust standardised procedures (including the test environment and materials used) for the collection of the aerosol and the optimisation of analytical techniques to provide adequate selectivity of chemical identification and sensitivity of measurement.

Initial investigations indicate a need to approach e-cigarette sample collection very differently when contrasted with traditional combustible techniques. We will present our experiences and demonstrate the need to segregate combustible and non-combustible sampling to limit the risk of cross-contamination with combustible vapour phase compounds such as isoprene and toluene, and flavour components such as menthol. We will provide an overview of our current GC-MS based techniques used to chemically evaluate e-cigarette aerosols and highlight their limitations. Existing vapour phase techniques suffer from low sensitivity as a result of limited sample volume introduction direct from a Tedlar bag. On-going work continues to explore alternative instrumentation in the form of Thermal Desorption GC-TOFMS and GC-HRTOFMS.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 15

Application of an Omics approach and soft ionisation of the apolar fraction of tobacco leaf

Tobacco leaf is highly complex, containing thousands of different components that range in molecular weight and polarity. Some components can be used to characterise and differentiate leaf types using analytical techniques and data processing. In CORESTA 2012, an Omics approach was applied to the characterisation and comparison of tobacco leaf types. Also discussed was the non-targeted analysis of the low molecular weight fraction from three leaf types (Burley, flue-cured and Oriental) for the identification of potential marker components using analytical techniques and data processing.

The objective of the current work is to apply the Omics approach for characterising flue-cured tobacco leaves from the view point of their origins. In this approach, low molecular weight apolar extracts were analysed by GC-MS. The GC-MS data were processed using deconvolution software (AMDIS, Agilent Technologies) prior to analysis in multivariate software (MPP, Agilent Technologies). As a result, tobacco leaves from different origins were differentiated and six potential marker components were extracted; five potential marker components were identified by library research based on EI spectra but one alkane compound was not. For identification, supersonic molecular beam ionisation mass spectroscopy (SMB-MS) was coupled to the GC separation. SMB is a cold EI technique, giving fragmentation and molecular weight information simultaneously. GC-SMB-MS for the identification of the alkane compound will be also discussed in detail.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 53

Comparison of the variability in smoke yield data when smoking cigarette brands onto a 44 mm Cambridge filter pad with a rotary smoking machine

The outline of this study was to evaluate the variation of Tar, Nicotine and CO when smoking low yield cigarette brands with a common and a modified Cambridge filter pad holder.

Several low yield cigarettes as well as cigarettes with target yields up to 10 mg/cig have been included in this study. All test pieces are fire-safe cigarette (FSC) compliant cigarettes. Smoking was done with a Borgwaldt RM20H smoking machine according to ISO 4381 and the HCI-Regime. As a reference the Kentucky 1R5F was included.

Two different types of Cambridge filter holders were used. Firstly a conventional filter holder for filter pads with a diameter of 92 mm; secondly a modified filter holder for Cambridge filters with a diameter of 44 mm. The only difference was in the diameter of the Cambridge filter, while the construction of the filter holder stayed the same.

The volume of solvent used for extracting the Cambridge filter was changed when using the 44 mm Cambridge filter pad:
- 44 mm Cambridge filter with 20 ml solvent (isopropanol)
- 92 mm Cambridge filter with 50 ml solvent (isopropanol)

The study was composed of five replicates on each sample with ISO Regime as well as with the HCI-Regime, using both types of filter holders and Cambridge filters with different diameters. Results concerning yields with a special focus on the variation on the analysis data will be shown.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 46

Impact of using a metal sheet as an "alternative substrate for ISO 12863" on SE performance

The aim of this study was to demonstrate the impact on SE performance when using different material than filter paper as a possible substrate. NIST proposed to perform these tests on a thin metal sheet (0,2 mm) with one layer of filter paper. As the ISO 12863 allows the use of an alternative substrate, all tests have been conducted on Whatman No. 2 and LIPCan filter papers. NIST proposed to use a metal sheet of type "AISI 302" with one layer of substrate paper. To see if this proposal would be in contradiction with the aims of the regulators, we also included an old design cigarette without any LIP technology on the cigarette paper. The regulators consider the old design cigarettes as dangerous in relation to fires caused by these cigarettes. Two different cigarette / test pieces have been chosen for this study. A conventional FSC brand taken from the Austrian market and the CORESTA Monitor CM7 as a non-LIP cigarette. A clear study plan describes the different steps of testing. For each sample we performed three replicates. When the proposed metal plate is used for testing, the results should be compared and be in line with results from existing test methods with ten layers of substrate paper. The final conclusions would show the impact, when using a metal sheet as an alternative substrate instead of ten layers of filter paper. Does a metal plate really match the results from tests with substrate paper? The conclusion will also show that a non-LIP cigarette will have a percentage of self-extinguishment when performing these tests on a metal plate. When testing the CORESTA Monitor CM7 cigarette without LIP bands on ten layers of filter paper it is confirmed that these test pieces will not self-extinguish and will burn the whole tobacco rod. The metal plate could not be an alternative for testing the self-extinguishment performance of a cigarette.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 09

Determination of volatiles and semi volatiles in cigarette smoke using GC-MS

The method discussed here is developed to meet US-FDA regulatory requirements in reporting harmful and potentially harmful constituents (HPHC) in smoke.

Currently, there are two separate methods available in literature for the analysis of volatiles and semi volatiles in cigarette smoke using GC-MS. Also, there is no method available for analysing all the 12 volatiles and semi volatiles in cigarette smoke in a single run using GC-MS. The analytes of interest studied were vinyl chloride, 1,3-butadiene, ethylene oxide, isoprene, propylene oxide, acrylonitrile, vinyl acetate, benzene, toluene, ethyl benzene, styrene and quinoline.

In this context, a very simple and rapid method has been developed for analysing all the 12 volatiles and semi volatiles in cigarette smoke using GC-MS.

In the method, the analytes are collected by passing the mainstream smoke through a Cambridge filter pad and into cryogenic traps containing methanol. The pad is extracted with methanol after spiking with internal standard solution. The impinger extracts are also spiked with internal standard solution. These impingers and pad extracts are injected onto a GC/MS for further analysis and quantification.

This method has been validated for various standard parameters i.e., linearity, limit of detection, limit of quantification, repeatability, reproducibility and recovery. Excellent linearity with a correlation coefficient of >0.99 has been obtained over a wide range of concentrations for all the analytes and the recoveries varied from 76% to 106%. The limit of detection is in the range of 0.02 µg/cig. to 2.80 µg/cig.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 08

Quantification of 17 polycyclic aromatic hydrocarbons in mainstream smoke by GC-MS/MS

Polycyclic aromatic hydrocarbons (PAHs) comprise a large group of chemical compounds that are known as potential cancer-causing agents. Hundreds of individual PAHs are formed due to partial combustion or incomplete pyrolysis of tobacco.

17 PAHs are part of the 93 harmful and potentially harmful constituents (HPHC) list released by the US FDA and Section 904(a)(3) of the Tobacco Control Act, which requires each tobacco product manufacturer or importer or an agent to begin reporting all the 93 HPHC to the US FDA.

Currently there is no analytical method that can analyse all the 17 PAHs present in cigarette smoke in a single method. PAHs are present in nanogram level in tobacco smoke, it is required to have an instrument technique that can detect PAHs at very low levels. Gas chromatography coupled with tandem mass spectrometry appears to be a suitable method to analyse PAHs in tobacco smoke.

A simple method has been developed for quantification of 17 PAHs in cigarette mainstream smoke. The PAHs in cigarette smoke are extracted with iso-octane by sonication. The extract is purified by passing the concentrated extract through a SPE (NH2/Silica) cartridge using hexane. The hexane eluent is concentrated and injected into the GC-MS/MS. The method has been validated using standard validation protocols and there is excellent linearity over concentration range from 0.5 ng/cig to 400 ng/cig with correlation coefficient of $\geq$0.99 and recoveries are more than 90% for all 17 PAHs. Limit of quantification ranges from 0.01 ng/cig to 1.50 ng/cig and limit of detection ranges from 0.03 ng/cig to 4.99 ng/cig.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 01

The Spanish tobacco market

Spain's initial contact with tobacco goes back as far as 1492 when Christopher Columbus, in his first trip to America described in his diary that he has seen people using a burning cone called "Tabaco". Seville was the port that received all the legal trade with America and was where tobacco first entered into Europe. Jean Nicot, the French ambassador in Spain, brought the tobacco plant to Catherine of Medicis, the Queen of France, from where the fame of the plant was spread across Europe. The first tobacco factory was established in Seville in 1728 to further trade within Europe. It replaced former factories that had been operating around the city since the beginning of the XVI Century.

In 1636 the first tobacco monopoly in the world was established. It was called "Real Estanco del Tabaco". This monopoly was continued through the centuries, becoming the Compañía Arrendataria de Tabacos (CAT) in the XIX century and Tabacalera S.A. in 1945. Tabacalera was later merged with SEITA after the tobacco monopoly was abolished and the resultant company Altadis SA was later bought by Imperial Tobacco Group.

The Canary Islands have a special tax regime and were never part of the Monopoly so other companies make cigarettes and cigars in the islands, with many small cigar manufacturers existing, especially in the Island of La Palma.

Tobacco is grown in several regions of Spain with Extremadura producing 95% of the total production. Other regions like Andalucia and the Canary Islands also grow a significant amount of tobacco. The traditional Dark Air Cured Tobacco (Tabaco Negro) has been replaced by flue-cured and Burley for its use in American blends. The traditional Spanish Dark Air Cured cigarettes nowadays represent 10% of the market, with American Blend cigarettes being the most popular ones.

Spain is the first market in the world for Cuban cigars, as part of an old tradition of smoking cigars in the country. Current trends, as observed over the last few years, indicate that Spanish consumers are changing from cigarettes to other tobacco products, such as Roll-Your-Own.

The number of tobacco factories in the Spanish Peninsula has been reduced since the 1990s from 13 to 2, one for cigarettes in Logroño and one for cigars in Cantabria.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 30

Application of multidimensional GC in combination with tandem mass spectrometry and accurate mass detection for identification of sulphur compounds in tobacco smoke extract

Tobacco smoke is a highly complex mixture of a wide variety of chemical compounds including sulphur-containing compounds which are present at very low concentrations and have low odour thresholds. From past studies, it is well known that required chromatographic resolution for sulphur compound analysis in complex samples is much higher than conventional 1D GC can provide. At the 2012 CORESTA Congress, the author reported that 1D/2D selectable GC-MS with sulphur chemo luminescence detection (SCD) can significantly improve the resolution. More than 450 sulphur compounds were detected by using this system, however, most sulphur compounds could not be identified. For the identification of trace sulphur compounds, detailed mass information (e.g. molecular ion, accurate mass) is necessary.

In this presentation, 1D/2D selectable GC-Q-TOF-MS/SCD is applied for the identification of trace sulphur compounds in tobacco smoke extract. Recently introduced GC-Q-TOF (Quadrupole-Time-of-Flight)-MS instrumentation allows tandem mass spectrometry (MS/MS) with accurate mass detection. With this system, both Electron impact (EI) and Chemical ionization (CI) can be applied simultaneously. EI mass spectra are used for MS library search, while CI provides molecular ion information.

For the identification of unknown sulphur compounds, MS library search, 2D LRI (Linear Retention Indices), and EI spectra based formula calculations were performed. The results were verified by applying MS/MS on protonated molecular ions obtained in CI mode. Molecular ion information and MS/MS provided complementary information to EI. The results suggest that this hyphenated system is very useful for the identification of trace compounds in complex samples.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 11

Mainstream smoke menthol delivery after using different flavour application methods in filter production

Menthol without a doubt is one the most popular flavours in the cigarette industry. It is applied to the filter, tobacco and even packaging. Indeed, menthol cigarettes almost certainly have mentholated filters which have been enriched with the flavour one way or the other. To keep the customer satisfied with the favoured product the taste of mentholated cigarettes has to be the same whenever the customer picks up another pack. Hence, it is crucial to have a very stable amount of menthol in the filter from the moment of production to transportation and storage all the way to the final analysis on the cigarette machine.

In this study we quantitatively analysed mainstream smoke of a standardised tobacco column attached to a standardised filter containing the same concentration of either encapsulated menthol, directly injected pure menthol crystals or menthol solution in propylene glycol. Smoking was done by the ISO method using a rotary Borgwaldt RM20D smoking machine and menthol analyses were performed on Agilent 5973N GC-MS equipment.

This study also compared different packaging materials used for flavoured filter storage. We used multilayer composite polyamide/polyethylene, pure polypropylene and pure polythethylene bags. The quantity of menthol in different filters was checked 24 h and 48 h post production. Then filters were kept in different packaging in standard warehouse conditions for 90 days and menthol extracted from them every 30 days for comparative analysis.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 38

Methodology and application of a non-targeted metabolome platform for biological samples from smokers and nonsmokers

Metabolomic profiling is suitable for the identification of metabolites that differ significantly between groups such as smokers (S) and nonsmokers (NS). Gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS) is a powerful tool for metabolomic approaches. It combines the separation power of GC with structural information obtained by high resolution mass spectrometry.

Here we developed and validated a sensitive GC-TOF-MS method for analysing the metabolome of human plasma, saliva and urine. Sample preparation includes derivatisation was carried out by methoximation and silylation using methoxylamine hydrochloride and N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA). Metabolite separation was conducted with a non-polar dimethyl polysiloxane column with total run time of 30 minutes. For data analysis, MZmine (peak integration and alignment), and partial least-squares discriminant analysis (PLS-DA; group separation) was applied. This methodology was applied to plasma, saliva and urine samples collected from 25 S and 25 NS kept under strictly controlled dietary conditions. PLS-DA clearly separated the two groups on the basis of the evaluable signals (characterised by their m/z and retention time values). About 40 endogeous metabolites with significantly different levels between S and NS could be identified with high probability. These metabolites are involved in lipid and energy metabolism as well as in oxidative stress and detoxification processes.

In summary, a sensitive and validated GC-TOF-MS based method for non-targeted metabolomic fingerprinting is presented. This methodology is a powerful tool for identification of potential biomarkers of effect.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 05

Cigarette Reference Material: Regulatory requirements, manufacture, and validation of CORESTA Monitor CM7 test pieces

International standards (e.g. ISO 16055) describe the use of monitor test pieces for monitoring the stability of the analytical processes involved when using a cigarette-smoking machine for routine analyses. In particular, monitor test pieces are used to assess whether the analytical process related to the machine-smoking of cigarettes is "in statistical control". Therefore, monitor test pieces represent an important quality control tool for regulatory and industry testing laboratories. This poster describes the steps taken and the challenges faced to produce the CORESTA Monitor N°7 (CM7) to fulfil the regulatory requirements and to reach the performance expectations.

The CORESTA monitor CM7 was produced in June 2010 at the Imperial Tobacco Group factory in Berlin. As the test pieces were produced for process-control purposes only, the product design was chosen to give smoke yields well suited for the determination of TNCO in mainstream smoke. Since the target values for TNCO did not conform to commercial cigarettes, permission by DG SANCO and the German Federal Office of Consumer Protection and Food Safety (BVL) was required prior to its production. In order to avoid any mishandling and misuse of the products, strict principles were followed for the production, packaging, labelling, storage, and distribution.

During production special attention was paid to blend and rod homogeneity, low weight variation, and machine speed. Data from physical measurements (weight, pressure drop and diameter) were recorded in control charts, and ISO 8243 sampling was conducted. For validation purposes, a CORESTA collaborative study was performed for TNCO; results indicate that ISO yields were consistent with expectations.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 49

Influence of conditioning on NFDPM yields of mentholated cigarettes

When comparing NFDPM yields for mentholated and non-mentholated cigarettes between laboratories, mentholated cigarettes tend to be more variable. The following work was done to identify factors during conditioning which may have an impact on NFDPM yields of menthol cigarettes. Factors included in the study design were the air flow rate, the arrangement of cigarettes (horizontally in a flat box and vertically in a plastic cup with holes in the bottom), and the time of conditioning.

The results demonstrated that the total air flow volume to which a cigarette was exposed during conditioning was a main factor. The total air flow volume per cigarette was calculated by using the air flow rate, the open surface area arranged in the box or cup, and the conditioning time. A relationship was found between NFDPM yields and the total air flow volume. These findings suggest that differences in NFDPM yields could be minimised between laboratories by optimising the total air flow volume during the conditioning of mentholated cigarettes.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 29

Analytical multi-compound method for the determination of selected volatile analytes in mainstream cigarette smoke by GC-MS: Method development and validation

In 2012, an enlarged list of "Harmful and Potentially Harmful Constituents" (HPHC) in tobacco products and tobacco smoke was established and published by the US FDA[1]. Additional to the listed compounds, the FDA submitted a list with references about quantification methods published in the past. However, only selected validation parameters have been reported so far.

Due to the need to generate reliable analytical results in a short time frame, an analytical multi compound method for the quantification of volatile organic compounds in cigarette smoke mentioned in the HPHC list, such as ethyl benzene, furan, vinyl chloride, ethylene oxide, and propylene oxide was developed and validated following international guidelines. The new multi compound method is based on the same sampling as reported for the determination of selected volatile Hoffmann Analytes presented during the CORESTA Congress 2010, but by applying a different separation column[2]. Calculation of results was carried out using deuterated internal standards.

The cigarette samples selected and smoked for this study ranging from 1-36 mg NFDPM per cigarette. The regression coefficients of the applied calibration curves for each compound were calculated better than 0.99.

In this presentation, further validation parameters, e.g. precision, repeatability, and recovery will be discussed. The performance of the new method has been evaluated by comparing obtained results with results coming from laboratories applying different methodologies.

[1] The established list is available on the Internet (under the Regulatory Information heading) at http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/default.htm.
[2] Otte S., Intorp M.; Alternative Analytical Methods for the Determination of Selected Volatile Hoffmann Analytes in Mainstream Cigarette Smoke by GC-MS: Method Development and Validation; CORESTA Congress Presentation SSPT 11; 2010

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 20

Isolation, screening, identification and application of a bacterium strain with high lutein-degrading and β-ionone-producing abilities from the surface of tobacco leaves

In the culture medium with lutein as the sole source of carbon, the bacterium strains with lutein-degrading and ß-ionone-producing abilities were isolated from the surface of tobacco leaves. After primary and further screening, a strain, JBBM09-12, with a higher ability of lutein-degrading and ß-ionone-producing was obtained and identified as *Pantoea agglomerans*, whose rate of lutein-degrading reached 77.38% with the ß-ionone yield of 785.23 mg/L and the colony diameter of 4.8 mm. The microbial agent prepared from JBBM09-12 was used in the fermentation of tobacco leaves, the content of lutein in tobacco leaves decreased by 23.30%, while the content of ß-ionone in tobacco leaves increased by 7.1 times after fermentation, which provided a reference for the production of quality tobacco leaves by means of microbial fermentation.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. IG 01

Sustainability in leaf tobacco production

Sustainability in the leaf production sector of the industry aims at long-term farming. As such, it not only embraces all aspects of optimising yield, quality and profitability of the crop, but also of conserving the environment and improving quality of life for farmers, their workers and surrounding communities. Sustainability is not a new concept and has been a part of the philosophy and practice of agriculture since the start of settled farming. In recent years, however, it has been receiving heightened attention because of increasing concerns for the environment and security of agricultural products attributed to burgeoning population growth on the one hand and loss of arable land by erosion, industrialisation and accelerated climate change on the other. At the same time, there is a greater insistence by the public that agricultural products should be produced ethically, taking into account environmental and sociological issues, and that they comply with strict standards of integrity in terms of quality, traceability and purity. The industry is responding vigorously to these challenges to long-term farming. For most members, it is a critical part of their business policy. In support of this, sustainability has been a major topic at meetings of the CORESTA Agronomy/Phytopathology Study Groups for some time now, culminating in the setting up of a Task Force which is actively identifying the principal issues associated with sustainability in the leaf production sector with a view to publishing appropriate guidelines for ensuring its long-term future.

This presentation introduces the subject of sustainability in the leaf production sector, defines its key elements and illustrates how some of these are being implemented in practice.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 14

Development of a LC-GC-ECD method for the analysis of organochlorine pesticide residues in tobacco and tobacco products

A new on-line LC-GC-ECD method for the determination of residues of 16 organochlorine pesticides in tobacco and tobacco products was developed, which employed a switching valve to completely separate capillary pre-column and analytical column during the solvent evaporation period. With the developed method, the LC solvent could be completely evacuated through the pre-column, no solvent was transferred into the GC column or detector, large volume injection was achieved and the sensitivity of the method was improved as well. Furthermore, on-column injection was adopted to reduce the loss of volatile component, and a gel permeation chromatography (GPC) column was used to eliminate the non-volatile components in samples, which reduced the pollution of the pre-column. The developed online LC-GC coupling method possessed the advantages of loop type and on-column interfaces, while it also overcame the shortcomings of the two interfaces. Sample pretreatment was performed by ultrasonic extraction with ethyl acetate-hexane (v/v=1:1) and then solid-phase extraction (SPE) with florisil as adsorbent. Within the concentration of 1-100 ng/mL, the method exhibited good linear relations with the limits of detection from 0.58 to 1.42 µg/kg and good repeatability with RSDs of 1.89-5.44%. The recoveries were 73.49-108.2%, 87.14-106.4% and 88.06-111.1% at the addition rates of 100, 30 and 10 µg/kg, respectively. The developed method was successfully applied in the analysis of trace organochlorine pesticide residues in tobacco and tobacco products.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 11

Research on dynamic changes of cut tobacco physical and chemical properties during the over-drying process

In order to investigate the dynamic changes of cut tobacco physical and chemical properties during the over-drying process, the moisture content, the filling values, the microstructure, the routine chemical components, the polyphenols content and the sensory quality were studied for cut tobacco during the over-drying process. The results showed that the physical properties significantly changed when the moisture content of cut tobacco during the over-drying process was 11.50% which was the critical point of the physical properties (the moisture content). The chemical properties and the sensory quality showed significant change also when the moisture content was 8.00% with the physical properties variation. The moisture content of 8.00% was the critical point of intrinsic quality of cut tobacco. The ratio of tissue vacuity in the microstructure of cut tobacco increased significantly when the moisture content was 6.00%, which was the critical point of microstructure of cut tobacco. And the processability of cut tobacco decreased significantly. Comprehensive evaluation was that 8.00% was the critical point of moisture content considering the sensory quality.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 01

Quantification of total alkaloids as nicotine in tobacco by continuous flow analyser to replace CRM 35 / ISO 15152:2003 method

ISO 15152:2003 describes analysis of total alkaloids as nicotine (TNA) in tobacco using continuous flow analyser. It is based on the measurement of the colour complex generated by the reaction of cyanogen chloride and nicotine alkaloids. Cyanogen chloride is generated *in situ* by reactions involving chloramine-T and potassium cyanide (LD 50 of 5 ppm), a known toxic chemical.

In the titled method, toxic potassium cyanide is replaced by a non-toxic chemical potassium thiocyanate (LD 50 of 854 ppm) and chloramine-T has been replaced by a novel stable reagent sodium dichloroisocynurate. Various parameters i.e. the concentration and pH of reagents, reaction conditions to form a colour complex, buffer solution, size of sample and length of reaction coil were optimised to obtain results equivalent with CRM 35 / ISO 15152 method in both aqueous and acetic acid (5%) extract.

The titled method has been validated as per standard validation protocols i.e. limit of detection, limit of quantification, recovery, precision, accuracy, repeatability, reproducibility and ruggedness. Minimum recoveries of 93% were obtained with linear regression coefficient of 0.9999. "r" and "R" studies on the samples has been conducted with nicotine concentration range 0.6 to 5.6%. Various tobacco grades with different nicotine concentrations were analysed simultaneously using both methods in water and acetic acid (5%) medium and maximum difference in the results between the two methods was ±0.10 units.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 07

Assessing the exposure to 'smoke' from electronic cigarettes by biomarkers

Electronic cigarettes (e-cigs) significantly differ from conventional cigarettes (CCs) in terms of the composition of the 'smoke' as well as in how they are used (smoking topography). The determination of biomarkers of exposure (biomonitoring) constitutes a suitable way of assessing the exposure, irrespective of the product used. A key point in biological monitoring is the selection of appropriate biomarkers, which should not only provide specific information on the exposure dose, but also on some pharmacokinetic data, including the exposure route. Biomonitoring data for e-cigs are rare and mostly comprise the uptake of nicotine. Apart from nicotine, the vapour/aerosol released from e-cigs contains excipients (most frequently 1,2-propylene glycol and glycerol) as major constituents, flavouring agents, and toxicants such as aldehydes, HCN, benzene, TSNA, 1,3-butadiene and acrylonitrile. E-cigs-derived smoke concentrations of toxicants are usually >100-fold lower than that of CCs and can, therefore, be assessed by biomonitoring only under strictly controlled study conditions with extremely sensitive analytical methods. Biomarkers for excipients and flavouring agents released from e-cigs are available and could be applied in human studies. Approaches for assessing the exposure to these constituents of e-cigs-derived vapour/smoke will be presented and the application of exposure data in risk evaluation will be discussed.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 41

Activation of transcription factors in human bronchial epithelial cells exposed to aqueous extracts of mainstream cigarette smoke *in vitro*

Transcription factors are recognised as important components of signalling cascades controlling all types of normal cellular processes as well as response to external stimulus. Activation of transcription factors can alter the expression of specific genes.

The present study aimed to identify transcription factors activated by aqueous extracts of mainstream cigarette smoke (MCS) *in vitro* and assess cigarette smoke from different experimentally-designed cigarettes on transcription factor activation.

Ten transcription factors were selected by pathway analysis from comprehensive gene expression profile in NCI-H292 cells exposed to MCS. Luciferase reporter assays for each transcription factor were developed with two human bronchial epithelial cells, NCI-H292 and BEAS-2B, to assess the activation of these transcription factors. Activation of each transcription factor by aqueous extracts of MCS from Kentucky Reference 3R4F cigarettes (3R4F) showed the same tendency in both cells. However, BEAS-2B showed higher reactivity than NCI-H292. Nuclear factor erythroid 2-related factor 2 (Nrf2) was the most activated transcription factor by MCS from 3R4F under the same condition.

The luciferase reporter assay for Nrf2 was utilised to assess cigarette smoke from different experimentally-designed cigarettes. Charcoal-filtered cigarette reduced Nrf2 activation in comparison with non-charcoal filtered cigarette. Furthermore, aqueous extracts of MCS from the flue-cured cigarette (FC) activated Nrf2 more strongly than those from the Burley cigarette (BLY).

In summary, the results suggest that Nrf2 is activated by both particulate and gas/vapor phase in MCS, and more so in FC than in BLY.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 18

Denormalisation, smoking rates and the way ahead for tobacco product regulation

The primary objective of most international tobacco control initiatives is to reduce smoking rates. Addressing the European Parliament on 25 February 2013, the Irish Minister for Health, James Reilly said: "*…The overall prevalence rates for Ireland are more or less similar to the EU average with 29% of Irish adults being current smokers. This is simply not acceptable.*"

Data confirm smoking rates in Ireland to be at 29% of the population[1] and this figure has remained unchanged since 2002. According to the latest available data reported by the Organisation for Economic Co-operation and Development (OECD), Greece and Ireland have the highest smoking rates in the European Union, whereas Sweden has the lowest – at just 12.5% for men and 14.3% for women in 2011. To put this in context, the EU average is currently 28%.

Rates of decline in smoking rates between 1990 and 2010 were significantly greater in Sweden, Denmark and Norway, all countries where smokeless tobacco remains freely available. In each, the rate of decline over a 20 year period was over 45% from 1990 levels. The lowest rate of decline was in Ireland – less than 3% lower than it was in 1990. Of those countries reported by OECD, only Russia has a worse record then Ireland.

The efficacy of tobacco regulatory policy can be judged by its effect on smoking rates. This paper will investigate some facts behind the OECD report and consider what impact initiatives such as smoking bans, bans on tobacco display and other initiatives have had. The data show consistently the decline in smoking rates has been levelling off for the last ten years in most developed countries, including in Ireland, Australia, Canada and the UK where these measures have been in force for much of that time.

On the other hand, in those countries where there is an attractive alternative to smoking, smoking rates are still declining consistently. In Sweden and Norway, not only are the smoking rates already low, but the current rates of decline in smoking look set to continue.

Put in the simplest terms the data suggests that the WHO's FCTC 'denormalisation experiment' will not deliver on its central promise to "…progressively reduce the prevalence and exposure from tobacco use". Analysis of smoking rates in Europe over the last 20 years suggest that it will only be possible to reduce smoking rates significantly by presenting the consumer with a choice, including the availability of smokeless tobacco and electronic cigarettes.

[1] OECD Factbook 2013, Smoking: http://dx.doi.org/10.1787/factbook-2013-en

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 28

Quantitative analysis of organic compounds in gas phase of mainstream cigarette smoke with on-line atmospheric pressure chemical ionisation tandem mass spectrometry

In order to measure selected organic compounds in cigarette smoke which might possess potential risks to human health and the environment, a tandem mass spectrometry method was developed and applied in the on-line analysis of mainstream cigarette smoke (MSS). After passing through a Cambridge filter, the gas phase of MSS was directed into the APCI ion source via the inlet end of the mass spectrometer. A commercial single-port smoking machine and APCI ion source were modified to achieve on-line single puff or puff-by-puff analysis of MSS, and seven organic compounds in gas phase of MSS were qualitatively and quantitatively analysed. The results showed that the calibration curves of the seven compounds exhibited good linear relations (R^2>0.99) with the limits of detection from 0.016 to 2.05 ng/mL and the relative standard deviations (RSDs) from 2.63% to 8.89%. This method is simple in operation and features fast response, high sensitivity and good repeatability, and it is suitable for on-line analysis of organic compounds in MSS.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 35

The effect of smoke components on the photodegradability of cellulose acetate filters

A current and continued interest in the environmental impact of materials used in consumer products has resulted in the need for an extensive understanding of the degradability of cellulose acetate tow used in cigarette filters.

Cellulose acetate tow is subjected to a number of environmental forces leading to degradation through a variety of mechanisms such as photo degradation, biodegradation, chemical degradation, dispersion, and disintegration.

Previous work has shown that the incorporation of photoactive materials in cellulose acetate can significantly increase the rate of photo degradation. Most of the previous studies were conducted with un-smoked filters. In this study, the effect of filtered smoke components on the degradation of cigarette filters was investigated. The degradation of smoked versus unsmoked cigarette filters was examined under two testing conditions, an accelerated test method using bench top weatherometers and a rooftop weathering test method. Samples were tested that contained either one of two photodegradation enhancing additives or no additive at all. The effect of the plug wrap on filter degradation was also examined by testing samples with and without plug wrap.

Results indicate that filtered smoke components do not interfere with the natural degradation of conventional cellulose acetate. Furthermore, these smoke components do not reduce the enhancement in photodegradation obtained by incorporating active additives into cellulose acetate fibres.

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The effect of puff profile and volume on the yields of e-cigarettes

The increase in use of e-cigarettes has led to interest in testing these products to study the yields of various compounds. E-cigarettes normally operate by heating an element to vaporise a solution of flavours and nicotine so that some of these chemicals are released into the air steam and delivered to the user. The operation of an e-cigarette relies on the device sensing the start of a puff to begin the heating process to deliver nicotine and/or flavour to the user. It might be expected therefore that the puff profile could affect the performance of an e-cigarette in a different manner to a standard product as different puff profiles will produce different rates of flow increase that may be easier for the device to sense. At the moment no standard smoking regime or series of regimes exist for the machine smoking of e-cigarettes. The effect of puff profile and volume on the yields of particulate matter, nicotine and tobacco specific nitrosamines will be presented. The efficiency of a Cambridge filter pad for trapping nicotine in the vapour generated by e-cigarettes has also been studied. Data for a CM7 monitor cigarette will be given for comparison. Standard puff duration of 2 seconds was used for all the testing. Puff volumes in the range 35 to 55 ml and three puff profiles - a standard ISO profile, a square profile and a triangular profile - have been studied.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 59

Multiple point in time evaluation of commercial and reference cigarette products for abbreviated HPHC yield for mainstream smoke and filler

United States statutory requirements under The Family Smoking Prevention and Tobacco Control Act require that the FDA establish a list of all constituents identified by the FDA as harmful or potentially harmful (HPHC), that the FDA establish regulatory requirements for testing and reporting of smoke constituents by brand and sub-brand, and that the FDA publish the list of HPHC in tobacco products in a format that is understandable to a lay person.

It has been demonstrated through collaborative studies that testing of single batch reference products with standardized methods may have a spread in mainstream smoke yields of approximately 20% for 'tar', nicotine, and carbon monoxide and greater than 50% for water when comparing results from different laboratories[1].

In anticipation of possible regulatory reporting for commercial products, what spread in data might be expected in results for multiple batches of production cigarettes tested over time with standardized and non-standardized methods was of interest in our work.

With this in mind, an investigation was undertaken to characterize mean smoke and filler HPHC yields for selected products. Also of interest was the determination of relative differences in yield between ISO and intense smoking regimes and the determination of relative variability in yield between repeated testing of single batches of cigarettes versus testing of multiple batches for a given set of cigarettes.

Cigarettes with a range of construction variables and 'tar' yields were tested. Results presented will include mean, temporal variability, and batch to batch variability comparisons within and across products.

[1] Collaborative Study of CORESTA Monitor #6 (CM6) for the Determination of Test Piece Weight, TPM, Water, Nicotine, NFDPM, Carbon Monoxide and Puff Count Obtained Under Mainstream "ISO" and "Intense" Smoking Regimes, 2010

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 04

Tobacco reference materials: uses and limitations

In the framework of the growing regulations of tobacco products, interest is expressed in wider characterisation in terms of contents and emissions. This leads to the need for analytical methods providing accurate, reliable and consistent data. This is of major importance within each laboratory and among all laboratories when product comparisons and / or proposals of ceilings arise.

For this purpose, it is the usual practice of laboratories to refer to data obtained on reference materials or monitor test pieces. Such materials are useful for method development and validation, calibration and quality assurance. However, tobacco reference materials may present some limitations.

This poster presents an overview of the definitions, specifications and uses of reference materials as described by ISO; the European co-operation for Accreditation & Eurolab & Eurachem; the International Laboratory Accreditation Cooperation; the Labnetwork and the US FDA.

In this context, the suitability of the following tobacco reference materials is assessed: CORESTA Monitor test piece (CM7), Kentucky reference products (3R4F and 1R5F), CORESTA Reference Products (Snus CRP1, moist snuff CRP2, dry snuff CRP3, loose leaf chewing tobacco CRP4) and Polish certified tobacco materials (Tobacco leaves INCT-OBTL-5 and INCT-PVTL-6).

It has been shown that tobacco product references cannot be strictly considered as reference materials, especially for trueness because certified values are not available.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 15

A modified Ames methodology for the assessment of mainstream cigarette smoke genotoxicity using an aerosol-based exposure system

To date most toxicological testing of cigarettes has been performed on the particulate phase of cigarette smoke using standard genotoxic and cytotoxic methods, which include the AMES reverse mutation assay, neutral red uptake, mouse lymphoma and micronucleus assays. However, traditional test methods are based on a particulate test material and under submerged conditions and are not suitable for the testing of aerosols; including cigarette smoke. As a result there is a requirement for new methodologies which facilitate the testing of aerosols *in vitro*.

In this study we have modified the Ames reverse mutation assay, using a spread plate methodology, to allow exposure to a cigarette smoke aerosol at an air-agar interface (AAI). The methodology was evaluated using cigarette smoke generated from 3R4F reference cigarettes on a Vitrocell® VC 10 Smoking Robot. Four strains of *S. typhimurium* and one strain of *E. coli* were tested individually on 6 independent occasions in the presence of S-9. A dose-related increase in revertant numbers was observed in strains TA98, TA100, YG1024 and YG1042 up to mean fold increases of 5.6, 1.7, 24.8 and 5.5-fold, respectively. *E. coli* strain WP2 *uvrA* pKM101 was unresponsive at all concentrations tested. To enable us to accurately quantify dose, we measured deposited particulate mass using Quartz Crystal Microbalance technology *in situ* of exposure.

In conclusion, we have modified the traditional Ames reverse mutation assay using an aerosol-based exposure system for the assessment of cigarette smoke toxicology. Furthermore, this method is not restricted to the testing of whole smoke and could be applied to the testing of other gases, mixtures or aerosols.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 54

Air flow, turbulence and smoke yields. The unexpected consequences of machine design.

An important but little understood factor in the determination of mainstream smoke yield is the impact of air flows during the smoking process. Although the ambient air velocities surrounding cigarettes in an analytical smoking machine during the smoking process are defined in ISO3308, and in turn referenced by the Health Canada Intense method, the specification lacks detail concerning vectors and stability of air flow. These are considered to contribute to both the absolute yields obtained during smoking but also to the repeatability of measurements. A series of experiments were undertaken to understand the origins of the air consumed during smoking, where the smoke generated goes and how seemingly simple changes within the smoke hood can change yields. In particular user exposure to smoke, as evidenced by CO exposure, was examined and it was found that the user is not exposed (less than 1 ppm CO measured compared with OSHA PEL based on an 8 h TWA 50 ppm or the ACGIH TLV of 29 ppm) provided overall extraction was maintained. It was observed that adding a barrier for environmental tobacco smoke (ETS) has the unexpected consequence of increasing yield variability. This can be explained by examining the detailed path whereby air impinges on the smoked cigarette. Some methods of controlling air flow were investigated including the use of various “air straightening” systems which were used to reduce smoke hood edge effects, reduce turbulence in the smoke path and how these efforts changed yields and variability of yields.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 06

Lighter life, temperature and initial CO yields

Coil lighters used to initiate smoke runs can be operated at different surface temperatures whether deliberately or unwillingly. This surface temperature is shown to be related to the pre-light time and the age of the lighter in terms of in use cycles and in use temperature. Lighter surface temperature decreases with repeated use and this is accelerated as the initial surface temperature is increased. It is possible to approximate the surface temperature of the lighter coil by reference to the number of lighting cycles employed and the pre light time. Using different lighter surface temperatures, achieved by altering the pre-light time, it is shown that different initial CO yields are achieved when smoking monitor test pieces under ISO conditions but that these differences are not statistically significant when smoking under a Health Canada Intense (HCI) regime. It is concluded that by understanding this effect modifications can be made to lighting temperature and this should be a consideration in maintaining consistent CO yields.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 40

Micronucleus induction by fresh whole smoke and DMSO extracts from three conventional tobacco products

The present study was initiated to investigate and compare the genotoxic effects of cigarette smoke condensate extracted in DMSO and fresh whole smoke from three conventional tobacco products by means of the *in vitro* micronucleus assay (IVM). The IVM was conducted using V79 Chinese hamster lung fibroblasts exposed to both DMSO extracts of condensate and fresh whole smoke from the Kentucky Reference Cigarette 3R4F (American blend), a Burley only tobacco test piece and the CORESTA monitor test piece CM7 (Virginia lamina only). Both exposure to DMSO extracts and whole smoke exposure were conducted using a 24 well multi titer plate (MTP) format either with cells grown directly on the collagen coated wells or in MTPs equipped with cells grown on microporous inserts. Whole smoke exposure of the latter was realised under air liquid interface conditions using the Bt020 Whole Smoke Exposure Apparatus. Smoking conditions for both approaches (condensate generation and whole smoke generation) were according to ISO.

Regarding the genotoxic potential in the IVM assay, a clear ranking could be shown for the different condensate extracts following the order CM7 > 3R4F > Burley. Regarding the MN induction by whole smoke, differences between the three tobacco products could also be detected when calculated on a % whole smoke basis. Differences between the results obtained with condensate and with fresh whole smoke are discussed.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 24

Influence of gaseous phase components from mainstream cigarette smoke on the propensity of smoke particulate matter to generate hydrogen peroxide

Generation of hydrogen peroxide (H_2O_2) by chemicals in mainstream cigarette smoke is of prime interest for the estimation of the pro-oxidant activity of the smoke. Since most of phenolic compounds (potential H_2O_2 precursors) reside in the particulate phase of the cigarette smoke, the total particulate matter (TPM) generates a larger amount of hydrogen peroxide compared to the gas phase of the smoke. To date there are no data in the literature which reveal the influence of the gas phase of cigarette smoke on the ability of the particulate phase to generate H_2O_2. The key part of the H_2O_2 generation mechanism involves the formation of superoxide species from appropriate semiquinone reactants in particulates. These superoxide intermediates undergo spontaneous dismutation to afford a limited amount of hydrogen peroxide. Conversely, catalysis by superoxide dismutase (SOD) results in a significantly enhanced H_2O_2 yield. It is possible that the smoke gas phase reduces the SOD activity, thus inhibiting H_2O_2 formation. To investigate this mechanism, we used commercially sourced cigarettes (BAT Russia; with TPM delivery at 11.0 ± 0.5 mg), which were smoked by a A14 single-port machine (Borgwaldt KC GmbH), while the gas and the particulate phases of the smoke were separated by Cambridge filter pads. The developed chemiluminescence assay for H_2O_2 in experimental samples utilised luminol as the chemiluminophore and horseradish peroxidase (HRP) (Dia-M, 400 units/mg) as the catalyst. The sensitivity of such an assay enabled measuring as little as 0.5 ng/ml of H_2O_2 in the sample. We found that the gaseous phase smoke significantly affects H_2O_2 generation by the TPM (thus, gas-phase condensate of the smoke collected from eight cigarettes caused more than a two-fold decrease of the TPM-derived H_2O_2 level).

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 51

Chemiluminescence approach to monitor efficiency of cigarette filters

It is known that fresh cigarette smoke is at an excited state and this is reflected by the light emission or chemiluminescence. Chemiluminescence of the cigarette smoke can be measured from the mainstream smoke emitted from cigarette filters. In this work, we used this principle to estimate the cigarette filter's efficiency towards attenuation of volatile oxidants formed in the cigarette smoke. For this purpose, comparison of the chemiluminescence intensities from the tobacco side (linking to the filter when the filter was removed) and the mouth end of the cigarette filter was made. The results showed that chemiluminescence emission derived from the filter side depended on the amount of tar retained but more importantly, on its ability to react with ambient oxygen to form oxidation and oxidising products of molecular and free-radical nature. The observed difference in the chemiluminescence intensities from the tobacco and the mouth sides of the cigarette filter also depended on cigarette design (type of filter and its ingredients such as activated carbon) based on different commercial cigarettes tested. This technique enables comparative analysis of the filter retention efficiency towards smoke-borne oxidants by different cigarette brands and allows rapid screening of potential adsorbent materials according to this property.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 19

Human mouth-level transfer rate of menthol, 1.8-cineole and nicotine from Swedish pouched snus

To facilitate the development of a transfer model which can be used to estimate human exposure to flavour and tobacco constituents, a human *in vivo* study involving 198 Swedish active snus users was conducted. All subjects agreed to participate in the study. Two snus products (X and Y) manufactured by Skruf Snus AB were selected for the study which were indistinguishable apart from the flavour application rate (%). Participants were divided into two groups (X: n = 98, Y: n = 100) and required to consume three individual pouches for 30 minutes per pouch.

An in-house extraction method[1] was developed to extract relevant flavour and tobacco constituents from test pouches: Menthol, 1,8-cineole (Eucalyptol) and Nicotine were quantified from dichloromethane/methanol extracts prepared from used and unused pouches. Analysis of Product X test pouches based on the total amount extracted from used snus pouches revealed an average mouth-level transfer rate for menthol, 1,8 cineole and nicotine at 9 (SD ± 14), 25 (SD ± 13) and 22% (SD ± 15), respectively. Analysis of Product Y test pouches demonstrated an average mouth level transfer rate of 13 (SD ± 5), 25 (SD ± 8) and 23% (SD ± 10) for menthol, 1,8-cineole and nicotine, respectively. Intra-human variability for mouth-level exposure of the three replicate pouches was consistent: respectively 98%, 87%, and 89% of the users exhibit an analytical variability below 20% in menthol, 1,8 cineole and nicotine respectively for Product X (99%, 88% and 93% for Product Y). The mouth-level transfer of menthol and 1,8-cineole was positively correlated to their respective water solubilities of 490 mg/L and 3500 mg/L; however an upper transfer limit may be reached due to limited access to saliva. It was concluded that whilst water solubility may be an important factor in the mouth-level transfer of flavour constituents evaluated, the initial flavour application rate is not.

To complete the transfer model, results with an alternative *in vitro* extraction media (artificial saliva, water and 1% ß-cyclodextrin in water) will be presented.

[1] Analytical method to model human mouth-level transfer. Poster session - CORESTA 2013.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 08

Smoking Behaviour System: methodology for validation

A typical human smoking cycle includes distinct physical activities. Firstly, smokers puff on the smoking article and the smoke is drawn into the mouth where it may be held for a short period of time, defined as the mouth hold. Many smokers then go on to inhale the smoke, where they draw the smoke into their lungs. This requires additional air to be drawn into the respiratory tract. At the end of the inhalation phase a small breath hold occurs, after which the smoker then exhales the smoke, before resuming normal breathing. The Smoking Behaviour System (SBS) has been developed to measure flow and duration characteristics of the typical smoking cycle, which may consist of puffing, mouth hold, post puff inhalation, breath hold and post puff exhalation. The SBS is a novel system with regards to its capability to capture the complete smoking cycle. It compromises of a Smoking Analyser 7 (SA7) head that measures the puffing topography and optical obscuration from particles and a heated Breathe In Breathe Out (BIBO) head that measures the respiratory topography and optical obscuration from exhaled particles. The system was calibrated for flow, and validated in terms of system to system and day to day variation for volume. The SA7 and BIBO have been tested to operate between 0 and 7 $L.min^{-1}$, and 0 and 50 $L.min^{-1}$ respectively, and met design specification of precision of ±5%. These flows are typically encountered during the human smoking cycle. The results provide confidence in the robustness of the system for use in field studies. These data will be presented and discussed in addition to the characterisation of the complete smoking cycle using the SBS.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 47

LIP cigarettes: Proposal for an alternative sampling design

Low ignition propensity cigarettes are intended to increase the chances of self-extinguishment under laboratory conditions following the protocol detailed in either ISO 12863:2010 test method or ASTM E2187-09. The safety requirements state that a cigarette batch is certified to have passed the test when 75% or greater of the cigarettes, coming from a sample size of 40 cigarettes, self-extinguish before burning to the tipping paper. However, the key point is to know the link between the percentage of compliant products obtained with the sample (40 cigarettes) and the genuine percentage in the entire production batch. In other words if a batch had a percentage of compliant cigarettes of 75% then, what would have been the probability with a sample of 40 cigarettes to observe less than 75% of self-extinguishment?

To answer this question, the statistical properties of the sampling design used to certify the cigarettes was investigated, and the sampling theory was approached to determine if the application of a statistically equivalent alternative sampling design was possible. From the understanding of the sampling theory consequences, an improved alternative sampling design can be proposed. This alternative has the benefit of reducing the mean testing time to certify the products without any loss on the statistical performance.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 17

Some key points to assess LIP regulations

Lower ignition propensity (LIP) cigarettes are designed to extinguish more quickly than conventional cigarettes with the intention not to cause accidental fires when left unattended. LIP regulations have so far been introduced in several countries. The performance of LIP cigarettes is tested under standardised laboratory conditions, following the protocols given in either the ASTM E2187-09[1] or ISO 12863:2010[2]. Limited statistical data on cigarette related fires from different countries are regularly published. In order to assess the impact of existing LIP regulation, these figures have to be used and interpreted carefully. For example, some reports refer to the total number of cigarette-related fires as an indicator to assess the effectiveness of LIP regulation while others refer to the number of fatalities caused by cigarette-related fires. Also, relevant confounding factors are rarely taken into account when interpreting the statistical data (e.g. public fire education, smoke alarms and cigarette consumption). Moreover, conclusions do not often take into consideration any long-term trends or seasonal effects and are based on a short period of time.

In this poster, the important factors that should be taken into account and the statistical techniques to apply for a rigorous impact assessment are discussed.

[1] ASTM E2187-09 Standard Test Method for Measuring the Ignition Strength of Cigarettes
[2] Standard test method for assessing the ignition propensity of cigarettes, ISO 12863:2010

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 23

Insights from initial analysis for harmful and potentially harmful constituents (HPHC) in cigarette and smokeless tobacco products

A total of 20 commercial cigarette and 16 commercial smokeless tobacco products were initially assayed for 96 HPHC (Draft HPHC list, Federal Register, August 12, 2011) prior to draft guidance by FDA (March 30, 2012). The FDA Draft Guidance specified a reduced list of HPHC with more replicates than initially tested. Three contract laboratories were used to complete all testing. The same lots of commercial product were used for all testing. Cigarettes were tested using both the ISO and Canadian intense smoking regimens. In general, the levels of HPHC constituents in the commercial products tested were consistent with levels previously reported in the literature. Approximately 40% of HPHC measured for cigarettes and 35% of HPHC measured for smokeless tobacco products were not found or were below the limit of quantification. Average relative standard deviations among replicates can vary widely for constituent measurements above LOQ. Comparison of initial HPHC results with results from additional replicates for both cigarettes and smokeless tobacco products demonstrated statistically significant differences for most HPHC (i.e. temporal variability within a laboratory) despite each assay being validated within a laboratory and the assay being present on their ISO 17025 accreditation.

These differences demonstrate the need for standardized methods between laboratories with defined repeatability and reproducibility determined by inter-laboratory collaborative testing for each HPHC using certified reference standards. These differences also mean that simple conventional comparisons, such as two-sample t-tests are inappropriate for comparing products tested at different points in time from the same laboratory or from different laboratories.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 56

Dose-effect relationship between main precursors and delivery of hydrocyanic acid in cigarette smoke and influencing factors of the same

With a home-made cigarette smoking simulator of rapid heating, the source of hydrocyanic acid (HCN) in cigarette smoke was studied. The effects of tobacco matrix, pyrolysis temperature, oxygen content in atmosphere, flow rate and heating rate on the yield of HCN from the main precursors were investigated, and the dose-effect relationship between HCN delivery and precursors was determined. The results showed that the main precursors were protein, proline and asparagine amide, their contribution rates to the HCN in cigarette smoke were 85.13%, 3.55% and 1.04%, respectively. Pyrolysis temperature, oxygen content in atmosphere and heating rate significantly influenced HCN delivery, while the effect of flow rate of carrier gas was slight. However, an oxygen-enriched atmosphere, low heating rate and low temperature could inhibit the formation of HCN.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 50

Research on the burning temperature distributions and variation law of cigarette during a puff

The peripheral solid-phase temperature of cigarettes smoked according to the standard machine smoking regimens was studied by a FLIR-SC660 infrared thermal imager. The results showed that: (1) the highest solid phase temperature was positively correlated with the density of the cigarette rod. Linear regression equation between the highest transient combustion temperature and density of the cigarette rod accorded with TTM=9.30d-1219.10, R2=0.8693. The highest transient burning temperature in solid phase was observed after 0.5 seconds by puffing, which is closely related to the changing rule of the proportion above 850 °C. (2) the experimental results showed that the proportion at lower than 300 °C was 34.06%; between 300 °C~800 °C was 62.84%, higher than 800 °C was 3.10% respectively during a puff. (3) compared with the situation of puffing 0 second, the proportion of the various burning temperature zones below 550 °C showed a downtrend. The decrease amplitude in 400~500 °C was maximum, about -32.87%. The decrease amplitude in 500~550 °C was minimum, about -6.80%. The proportion of various burning temperatures from 550 to 700 °C showed a rising trend. The rising amplitude was 191.36% in temperature zone of 650~700 °C. When the burning temperature was higher than 700 °C, the proportion of various temperature zones rose to the maximum at first, and decreased subsequently. The temperature zone higher than 850 °C rose to 6000.00% at 0.594 second, decreased to 0% at 1.683 second, then decreased to -100.00% when puffing finished.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 07

Smoking puff ratio of cigarettes and its determination method

The ratio between cut tobacco burned at puffing stage and cut tobacco burned during the whole smoking process of a cigarette was defined as smoking puff ratio (SPR), and a method for determining the SPR of a cigarette was proposed. Cigarettes were smoked by a smoking machine at different puffing intervals, the regression model between the increase of puff number and the reduction of smouldering time was established based on puff number and puffing duration. Then the SPR of cigarettes under given smoking conditions could be calculated with the regression model, smouldering time and puff number. The SPRs of cigarette samples made of different auxiliary materials and cut tobacco were determined, and the results showed that the SPRs of cigarettes made of the same auxiliary materials and different cut tobacco were basically constant. SPR was obviously decreased by the ventilation of the filter, however, it increased rapidly with the decrease of puffing interval. The proposed method for SPR determination is simple and convenient, which requires no additional instrument other than a smoking machine and no extra measurements other than cigarette smoking. The finding that SPR is determined mainly by cigarette auxiliary materials is helpful for the design of cigarettes with low deliveries of harmful components.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 45

Alternate test substrate for ASTM test method E2187-09

The ASTM test method E2187-09 "Standard Test Method for Measuring the Ignition Strength of Cigarettes" lists Whatman #2 filter paper as the substrate to use in testing the ignition propensity of cigarettes. Tests are performed on 15, 10, and 3 layers of filter paper. As cigarette ignition propensity regulations are expanding worldwide, there is a need to include other filter papers or an alternate substrate. The target would be a substrate that is uniform, reproducible, can be easily sourced, low cost, and reusable, if possible. NIST tested and is proposing to use a stainless steel grade 302 sheet with one layer of filter paper. Test results at NIST yielded results close to those obtained on 10 layers of Whatman #2 filter paper. This presentation will discuss properties of grade 302 stainless steel plate. Test cigarettes with different levels of self-extinguishments were also tested according to the ASTM test method on 10 layers of Whatman #2 filter paper and also tested using the same method but substituting the 10 layers of filter paper with one sheet of stainless steel covered by one layer of filter paper. Findings will be reviewed and compared.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 31

Retention distribution patterns of nicotine and phenolic compounds in filters

The retention distribution patterns of nicotine and seven phenolic compounds in cigarette filters were studied. The nicotine and seven phenolic compounds retained in each segment were quantitatively analysed after the filters were transversely and longitudinally concentrically cut into segments by a precision laser cutting device. After correction, the concentration distribution data were processed by polynomial fitting and interpolation analysis, and the concentration distribution patterns of nicotine and phenolic compounds in filter were obtained. The retention characteristics of nicotine and phenolic compounds in common acetate filter under ISO and Canada smoking regimes were compared. The effects of filter structure changes on the filtration efficiencies and retention distribution patterns of phenolic compounds under ISO smoking regime were investigated. The results indicated that compared to nicotine, acetate filter showed high retention selectivity to phenolic compounds, and this selectivity was due to the interaction of gaseous phenolic compounds with filter material. The filter structure obviously influenced the filtration efficiencies and distribution patterns of phenolic compounds, and different phenolic compounds had their own retention distribution characteristics.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 44

Control or monitoring of the LIP testing process: The fitness for purpose of the LIP standard products

Lower Ignition Propensity (LIP) is a product safety standard intended to increase the probability that a cigarette will self-extinguish under strictly controlled laboratory conditions. LIP compliance indicates that the probability that a cigarette taken from the population will self-extinguish under the conditions of the test is ≥87.5%. Test validity is ensured by adhering to the rigorous requirements concerning the environment, apparatus and materials, coupled with periodic testing of a control, reference and/or monitor whose characteristics have been established. These materials have different functions dependent upon the experimental context. For example, a control displays the characteristic of interest under the conditions of the test; a reference provides a basis for comparison with an unknown sample, and a monitor is used to assess the temporal stability of the analytical process. When using a reference or monitor it must be demonstrated that the analytical process being evaluated is measuring a homogenous characteristic. This may be established when it is notionally possible to obtain an unlimited range of finite values. However, when LIP is the characteristic of interest, the population from which samples are derived is considered heterogeneous by means of a mathematical construct in which the population contains both compliant and non-LIP compliant cigarettes. Statistical modelling demonstrates that a range of Full Length Burn (FLB) rates will be measured irrespective of the target FLB rate. Several target FLB rates have been modelled supporting the conclusion that the NIST standard is an appropriate control to ensure test validity due to the low probabilities of finding this non-LIP compliant and a 0% FLB rate. Given the rigorous conditions prescribed within the analytical methods it is uncertain what additional value is offered by the use of a reference or monitor when the aim is to ensure test validity.

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Arterial stiffness – noninvasive assessment of smoking-related vascular changes and cardiovascular disease risk

Arterial stiffness, as measured along the length of the aorta, has been shown to be highly predictive of cardiovascular disease, morbidity and mortality[1]. The speed of the pressure wave travelling from the heart along the aorta (aortic pulse wave velocity or aPWV) is a direct measure of the aortic stiffness. Over the past decade, noninvasive methods to measure aPWV have become widely available and accepted[2]. In addition, noninvasive measurement and analysis of the central arterial pressure waveform (PWA) allows the assessment of changes in cardiac and vascular function, including vascular reserve, arterial wave reflections, and structural (elastin and collagen) alterations in arteries. All of these changes have been associated with tobacco smoking[3,4]. Ethnic differences in response to smoking have also been identified through measurements of aPWV and analysis of the central pressure waveform[5]. Chronic obstructive pulmonary disease (COPD) has also been linked to increases in arterial stiffness, and changes in aPWV have been used to assess impact of drug treatments on COPD[6]. In addition, measurement of aPWV and PWA allow the assessment of the reversibility of deleterious vascular effects of tobacco smoking. This presentation will review the various relationships between arterial stiffness and the cardiac and vascular effects of tobacco smoking. The latest noninvasive methodologies are widely being used in pharmaceutical trials. The basis for such measurements, along with a discussion of the validation, efficacy, and operation of the most commonly used systems, will also be presented.

[1] Vlachopoulos *et al., J Am Coll Cardiol.* 2010; 55:1318–1327.
[2] Laurent S *et al., Eur Heart J.* 2006; 27:2588–2605.
[3] Enevoldsen *et al., Journal of Biomechanics* 2011; 44:1209–1211.
[4] Doonan *et al.,* PLoS ONE 2011; 6(10): e26151.
[5] Lemogoum *et al., J. Hypertens* 2011; 24:683–689.
[6] Rubin *et al.*, presented at Annual Congress of American Thoracic Society, 2013

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 57

Effects of pyrolysis conditions on the formation reaction of 4-aminobiphenyl in non-isothermal pyrolysis of tobacco

4-Aminobiphenyl (4-ABP) is a polycyclic aromatic amine in cigarette smoke. Previous reports indicate that proteinaceous compounds (e.g. amino acids, peptides and proteins) in tobacco can highly contribute to the yield of 4-ABP measured in smoke. In a presentation given at the CORESTA Congress 2012, it was proposed that aromatic ring formation between low-molecular-weight amine compounds and alkyl-substituted benzenes was a major formation reaction for 4-ABP during cigarette combustion. However, it still remains unclear to which extent different pyrolysis conditions can influence the formation of 4-ABP from tobacco. The aim of this study was to clarify the relationship between experimental conditions and 4-ABP yield under non-isothermal pyrolysis of tobacco.

Burley tobacco (BLY) and flue-cured tobacco (FC) were non-isothermally pyrolysed under different pyrolysis conditions by changing the O_2 concentration in the carrier gas N_2, the flow rate of the carrier gas and the target temperature. 4-ABP in total particulate matter was determined by gas chromatography/mass spectrometry after solid phase extraction and derivatisation.

The yield of 4-ABP from BLY decreased by increasing the O_2 concentration in the carrier gas N_2 or by increasing the flow rate of N_2, from ambient temperature to 800 °C. However, these conditions did not significantly change the yield of 4-ABP when pyrolysing FC. The results suggest that the formation of 4-ABP from nitrogenous components in BLY are affected by either O_2 in the atmosphere or the gas flow in the heated layer. Regarding the investigation of the target temperature under N_2 flow, the profile obtained for BLY and FC was similar.

In the presentation, reaction kinetics of the formation of 4-ABP from BLY will also be discussed.

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

Determination of nicotine and total nitrogen in tobacco leaves by HPLC-CLND

A new method for determining the nicotine content in tobacco leaves with HPLC-CLND was established, and the content of total nitrogen was also estimated. HPLC conditions were as follows: Sun Fire C_{18} column (50 mm × 2.1 mm, 2.5 µm), mobile phase (A) water-TFA (100:0.1, m/m), mobile phase (B) methanol-TFA (100:0.1, m/m), gradient elution at a flow rate of 0.2 mL•min^{-1} and the column temperature of 30 °C. The linear range for nicotine was 0.32-2.88 mg•mL^{-1} (r=0.9998) with the precision RSD of 2.1% (n=6), the average recovery of 96.7% and the RSD of 3.8% (n=6). CLND detecting conditions were: detection wavelength 255 nm, flame temperature 1050 °C, the flow rates of argon and oxygen 2 and 6 mL•min^{-1}, respectively. Comparing with other methods, the established method was specific, simple and efficient, and total nitrogen content could be estimated while determining nicotine content. This method and its determination results could completely meet the requirements of determination of nicotine and total nitrogen in tobacco leaves.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 37

Good relationship between saliva cotinine kinetics and plasma cotinine kinetics after smoking one cigarette

This study investigated the relationship between plasma and saliva cotinine kinetics after smoking one cigarette and the relationship between cotinine kinetics and estimated nicotine intake, which was calculated as mouth level exposure (MLE) of nicotine, from smoking two test cigarettes with different nicotine yields. This study was conducted in sixteen healthy adult Japanese smokers, who did not have null nor reduced-activity alleles of CYP2A6, with a quasi-randomised crossover design of smoking a low-tar cigarette or a high-tar cigarette. Saliva cotinine showed similar concentration profiles to plasma cotinine, and all of the calculated pharmacokinetic parameters of cotinine showed the same values in plasma and saliva. The Cmax and AUC of cotinine showed almost the same dose-responsiveness to the estimated MLE of nicotine between plasma and saliva, but the tmax and t1/2 of cotinine were not affected by the estimated MLE of nicotine in either plasma or saliva. The results show that saliva cotinine kinetics reflects plasma cotinine kinetics, and measurement of saliva cotinine concentration gives the same information as plasma cotinine on the nicotine intake. Thus, saliva cotinine would be a good and less-invasive exposure marker of cigarette smoke, reflecting the plasma cotinine concentration and kinetics.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 17

Study of PAMAM grafted silica gels for selectively reducing harmful compounds in cigarette mainstream smoke

This study is the first report of PAMAM (Polyamidoamine) grafted silica gels adopted as selective absorbents added to the cigarette filter. Preparation, characterisation and application of PAMAM grafted silica gels are discussed in this paper. PAMAM grafted silica gels were prepared according with the method Thomalia reported, and their surface chemistry were studied by FT-IR and Elemental Analysis (E.A). Analysing of cigarette smoke components showed that there were no obvious effects observed with silica gels addition. Yields of harmful compounds in cigarette smoke showed a relevance between selectivity of harmful compounds to surface properties of PAMAM grafted silica gels. HCN, phenol and crotonaldehyde were selectively reduced by 33.3%, 65.3% and 32.8% respectively, which depends on the terminal groups of PAMAM moieties. Organic residues and fragments decomposed from PAMAM grafted silica gels would bring risks to smoking. The analysis of organic residues and stability of PAMAM grafted silica gels were taken to estimate their risks to smoking, which showed that these modified silica gels still hold high stability and safety under smoking conditions. It was suspected that PAMAM grafted silica gels could be adopted as new, high safety and stability absorbents applied in filters for selectively removing harmful compounds in cigarette smoke. More studies are required to reveal how the selectivity of harmful compounds would be controlled by surface properties of PAMAM grafted silica gels, and which would lead to new absorbents with high selectivity for harmful compounds.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 10

A prediction model for effects of atmospheric pressure on deliveries of tar and carbon monoxide in mainstream smoke based on ventilation rate of cigarettes

The deliveries of components in mainstream cigarette smoke determined under various atmospheric pressures are quite different, especially for tar and carbon monoxide. Multiple smoke analysis laboratories of China are located in high altitude areas, wherein atmospheric pressures are below 86 kPa, which results in lower detection results for tar and carbon monoxide. To modify the effect of atmospheric pressure on the detection results of tar and carbon monoxide in mainstream smoke, a new approach to modelling the relationship between the deliveries of mainstream smoke components and atmospheric pressure was developed based on the ventilation rate of cigarette. Cigarette samples with different tar deliveries (3-14 mg) were used to verify the accuracy of prediction models and satisfactory results were achieved. Results indicate that the filter ventilation rate of an unburned cigarette was inversely proportional to the square root of atmospheric pressure and the ventilation rate of cigarette paper was inversely proportional to atmospheric pressure. With the drop of atmospheric pressure, the ventilation rate of cigarettes increased gradually, which resulted in the lower detection results of tar and carbon monoxide deliveries. Taking cigarette ventilation rate as a variable, the effect of atmospheric pressure on the tar deliveries of cigarette samples with different tar deliveries could be reasonably interpreted. The prediction model was proved to be effective for revising the tar and carbon monoxide deliveries obtained under different atmospheric pressures. Comparing with the results determined under standard atmospheric pressure, the revised results obtained with the prediction model were lower for cigarettes with un-perforated tipping paper, while they were higher for cigarettes with the ventilation rate of above 60%. This work provided a reference for further investigating the mechanism of effect of atmospheric pressure on deliveries of components in mainstream cigarette smoke and establishing a precise model for cigarette burning.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. STPOST 12

Application of modified activated carbon fibre in reduction of carbonyl and phenolic compounds in mainstream cigarette smoke

The activated carbon fibres (ACF), with a proprietary surface modified by the solution of palladium chloride ($PdCl_2$), cuprous chloride (CuCl) and graphite oxide (GO) separately, were analysed by scanning electron microscope (SEM) and X-ray photoelectron spectroscopy (XPS). The changes in the physical and chemical properties of the ACF surface after modification were characterised in terms of specific surface area, pore distribution and functional group content. The deliveries of tar, carbonyl and phenolic compounds in mainstream smoke of cigarettes with a filter containing ACF were determined. The results showed that: firstly, the absorption characters of ACF were modified by $PdCl_2$, CuCl or GO and were significantly improved with the changes of specific surface area, pore structure and surface chemical property. Secondly, the delivery of tar in mainstream smoke of all samples with an ACF-containing filter decreased to some extent. The ACF treated by $PdCl_2$/CuCl (Pd^{2+}/Cu^{+}-ACF) possessed a significant selective removal efficiency for phenolic compounds, the reduction rates for tar, phenol and catechol were 8.3%, 22.7% and 35.7%, respectively compared to the control; while the selective removal effect of Pd^{2+}/Cu^{+}-ACF on carbonyl compounds was not obvious. Thirdly, ACF treated by GO (GO-ACF) presented a significant selective filtration efficiency for carbonyl and phenolic compounds, the reduction rates for tar, phenol, catechol and crotonaldehyde were 8.3%, 28.3%, 18.0% and 33.4%, respectively, compared with the control. Fourthly, the reduction rates of carbonyl and phenolic compounds were independent of the specific surface area, and they were mainly affected by the changes in chemical properties of the ACF surface.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Seville, 2013, abstr. ST 52

Evaluation of tobacco stem granules in cigarette products to reduce harmful components of mainstream smoke

The development of production technology for biomass stem granules has provided a viable new application for the utilisation of waste tobacco stems. In this paper, the effects of stem granules blending proportions in cigarette products on conventional chemical compositions, deliveries of harmful components, heavy metals of cigarette mainstream smoke and cigarette sensory quality were studied. The results showed that adding stem granules can significantly reduce the deliveries of tar, nicotine, total particulate matter (TPM), CO, HCN, NNK, NH3, B[a]P, phenol, crotonaldehyde, Cr, Ni, Cd, Pb etc. in cigarette smoke. By increasing the proportion of added stem granules, hazard index (*H*) has been significantly reduced. Meanwhile, there is a good negative correlation between the deliveries of harmful components in mainstream smoke, evaluated hazard index (*H*) and the proportion of added stem granules in cigarette products. Within the ranges studied, stem granules in cigarette products have no obvious effect on sensory quality. When the proportion of added stem granules was 8%, the deliveries of tar, nicotine, Cr, Ni, Cd, Pb and Hg in cigarette mainstream smoke were decreased by 32.37%, 32.37%, 27.81%, 17.39%, 52.55%, 27.81% and 14.41%, respectively, and the hazard index (*H*) was decreased by 40.48% compared to the control check, which indicated that stem granules added into cigarette products could effectively reduce health risks of smoking.

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